applied biosystems

VeriFiler™ Plus PCR Amplification Kit

USER GUIDE

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Revision C.0

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

ThermoFisher SCIENTIFIC

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

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

Revision history: Pub. No. MAN0017493

Revision	Date	Description
C.0	10 August 2020	Add validation experiments for the updated formulation. Add information about the D10S2148 marker.
B.0	17 October 2018	Add new chapter: Chapter 6, “Experiments and results”. Minor edits.
A.0	24 May 2018	New document.

Contents

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™ VeriFiler™ Plus PCR Amplification Kit is a 6-dye, short tandem repeat (STR) multiplex assay for the amplification of specific loci in human genomic DNA.

The kit amplifies:

- 23 autosomal STR loci: D3S1358, vWA, D16S539, CSF1PO, D6S1043, D8S1179, D21S11, D18S51, D5S818, D2S441, D19S433, FGA, D10S1248, D22S1045, D1S1656, D13S317, D7S820, Penta E, Penta D, TH01, D12S391, D2S1338, and TPOX
- Two internal quality control markers (IQCS and IQCL)
- One insertion/deletion polymorphic marker on the Y chromosome (Y indel)
- Amelogenin (sex determining marker)

Internal quality control system for PCR

The VeriFiler™ Plus PCR Amplification Kit is an STR kit that includes an internal quality control (IQC) system for PCR. The IQC system has two synthetic targets, one low molecular weight and one high molecular weight, that are amplified with the sample. The behavior of the IQC target peaks can be used to evaluate the success of the PCR and indicate sample quality.

Validated DNA amounts and PCR cycles

The kit is validated for use with 500 pg of DNA for 29 PCR cycles. The DNA input volume is 17.5 µL, for a total reaction volume of 25 µL.

About the primers

The VeriFiler™ Plus PCR Amplification Kit primers are manufactured to maximize the assay signal-to-noise ratio and simplify the interpretation of results.

Table 1 compares the VeriFiler™ Plus kit primers to the VeriFiler™ Express PCR Amplification Kit, GlobalFiler™ PCR Amplification Kit, GlobalFiler™ Express PCR Amplification Kit, and NGM Detect™ PCR Amplification Kit. The VeriFiler™ Plus kit primers do not contain any mobility modifiers.

Table 2 lists the dyes that are used in the kit.

Table 3 provides the following information about the loci amplified by the kit:

- Locus designation
- Chromosome location
- Alleles included in the VeriFiler™ Plus Allelic Ladder
- Dye label
- AmpFℓSTR™ DNA Control 007 genotype

Changes to the primers in the updated formulation

Artifacts, caused by primers amplifying non-human DNA, were reported by a small number of laboratories with the original formulation of the VeriFiler™ Plus kit. After internal investigations, we determined that a few primers caused artifact peaks when DNA from certain bacterial species was present. Accordingly, we extended primers for the D6S1043, D16S539, Penta E, D18S51, and CSF1PO markers by 1–2 nucleotides and leveraged the design of the IQCL marker from the NGM Detect™ kit and the GlobalFiler™ IQC kit. The primer change for the IQCL marker causes the marker to size ~15 bp greater in the updated formulation than in the original formulation. We did not make any other changes to the VeriFiler™ Plus kit formulation, protocols, or workflow.

The updated formulation of the VeriFiler™ Plus kit was revalidated with internal and external testing. Other than mitigating the artifact issue, the performance of the updated formulation is fully comparable to that of the original formulation. In Chapter 6, “Experiments and results”, all data from the revalidation studies are labeled with "updated formulation".

Primer comparison

Use Table 1 to compare the VeriFiler™ Plus kit to the four kits listed in the table. Do not use Table 1 to make comparisons between the other kits; for example, do not compare the VeriFiler™ Express kit to the GlobalFiler™ kit.

Note: A concordance population study was performed and genotypes were compared between the same samples amplified with the VeriFiler™ Plus kit and the VeriFiler™ Express kit and Huaxia™ Platinum™ kit. For more information, see "Concordance studies" on page 109.

Table 1 VeriFiler™ Plus kit primer comparison

Marker	VeriFiler™ Plus kit compared to...		
	VeriFiler™ Express kit and Huaxia™ Platinum™ kit	GlobalFiler™ kit and GlobalFiler™ Express kit	NGM Detect™ kit
IQCS	Marker not included	Marker not included	Primers are the same
IQCL	Marker not included	Marker not included	Primers are the same
D2S441	1 primer is the same	1 primer is the same	Primers are different
D10S1248	Primers are different	Primers are different	Primers are different
D22S1045[1]	Primers are the same	Primers are the same	Primers are different
D3S1358[1]	Primers are different	Primers are different	Primers are different
D8S1179[1]	Primers are the same	Primers are the same	Primers are different
D19S433[1]	1 primer is the same	1 primer is the same	Primers are different
D5S818	Primers are different	Primers are different	Marker not included
vWA[1]	Primers are the same	Primers are the same	Primers are different
D1S1656	Primers are different	Primers are different	Primers are different
TH01	1 primer is the same	1 primer is the same	Primers are different
D21S11	Primers are the same	Primers are the same	Primers are different
D13S317	1 primer is the same	1 primer is the same	Marker not included
D12S391	Primers are different	Primers are different	Primers are different
D16S539[1]	1 primer is different	1 primer is different	Primers are different
FGA[1]	Primers are the same	Primers are the same	Primers are different
D18S51	1 primer is the same	1 primer is the same	Primers are different
D7S820	Primers are different	Primers are different	Marker not included
D2S1338	Primers are the same	Primers are the same	Primers are different

Table 1 VeriFiler Plus kit primer comparison *(continued)*

Marker	VeriFiler™ Plus kit compared to...		
	VeriFiler™ Express kit and Huaxia™ Platinum™ kit	GlobalFiler™ kit and GlobalFiler™ Express kit	NGM Detect™ kit
CSF1PO	1 primer is the same	1 primer is the same	Marker not included
TPOX	1 primer is the same	1 primer is the same	Marker not included
D6S1043[1]	Primers are different	Marker not included	Marker not included
Penta D[1]	Primers are different	Marker not included	Marker not included
Penta E	1 primer is the same	Marker not included	Marker not included
Y indel	Primers are the same	Primers are the same	Primers are the same
AMEL[1]	Primers are the same	Primers are the same	Primers are the same

[1] The VeriFiler™ Plus kit has one or more additional SNP-specific primers

Dyes used in the kit

Table 2 Dyes used in the VeriFiler™ Plus PCR Amplification Kit

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

Loci amplified by the kit

Table 3 VeriFiler™ Plus kit loci and alleles

Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	DNA Control 007 genotype
IQCS	—	1, 2	6-FAM™	2
D3S1358	3p21.31	9–20		15, 16
vWA	12p13.31	11–24		14, 16
D16S539	16q24.1	5, 8–15		9, 10
CSF1PO	5q33.3-34	6–15		11, 12

Table 3 VeriFiler Plus kit loci and alleles *(continued)*

Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	DNA Control 007 genotype
D6S1043	6q15	7–25	6-FAM™	12, 14
IQCL	—	1, 2		2
Y indel	Yq11.221	1, 2	VIC™	2
Amelogenin	X p22.1–22.3, Y: p11.2	X, Y		X, Y
D8S1179	8q24.13	5–19		12, 13
D21S11	21q11.2–q21	24, 24.2, 25–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38		28, 31
D18S51	18q21.33	7, 9, 10, 10.2, 11–13, 13.2, 14, 14.2, 15–27		12, 15
D5S818	5q21-31	7–18		11
D2S441	2p14	8–11, 11.3, 12–17	TED™	14, 15
D19S433	19q12	5–12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, 19.2		14, 15
FGA	4q28	13–26, 26.2, 27–30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 49.2, 50.2, 51.2		24, 26
D10S1248	10q26.3	8–19		12, 15
D22S1045	22q12.3	8–19	TAZ™	11, 16
D1S1656	1q42.2	9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18, 18.3, 19.3, 20.3		13, 16
D13S317	13q22-31	5–16		11
D7S820	7q11.21-22	6–15		7, 12
Penta E	15q26.2	5–26		7, 12
Penta D	21q22.3	2.2, 3.2, 5–17	SID™	11, 12
TH01	11p15.5	4–9, 9.3, 10–12, 13.3		7, 9.3
D12S391	12p13.2	14–19, 19.3, 20–27		18, 19
D2S1338	2q35-37.1	11–28		20, 23
TPOX	2p23-2per	5–15		8

Standards and controls that are required

For the VeriFiler™ Plus kit, the panel of standards needed for PCR amplification, PCR product sizing, and genotyping are:

- **DNA Control 007**—A positive control for evaluating the efficiency of the amplification step and STR genotyping using the VeriFiler™ Plus Allelic Ladder. DNA Control 007 is present in the kit. See “DNA Control 007” on page 15.
- **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.
- **VeriFiler™ Plus Allelic Ladder**—Developed for accurate characterization of the alleles amplified by the kit. The Allelic Ladder is present in the kit and allows automatic genotyping of most of the reported alleles for the loci in the kit. See Figure 1.

Allelic ladder profile

The allelic ladder profile appears on the next page.

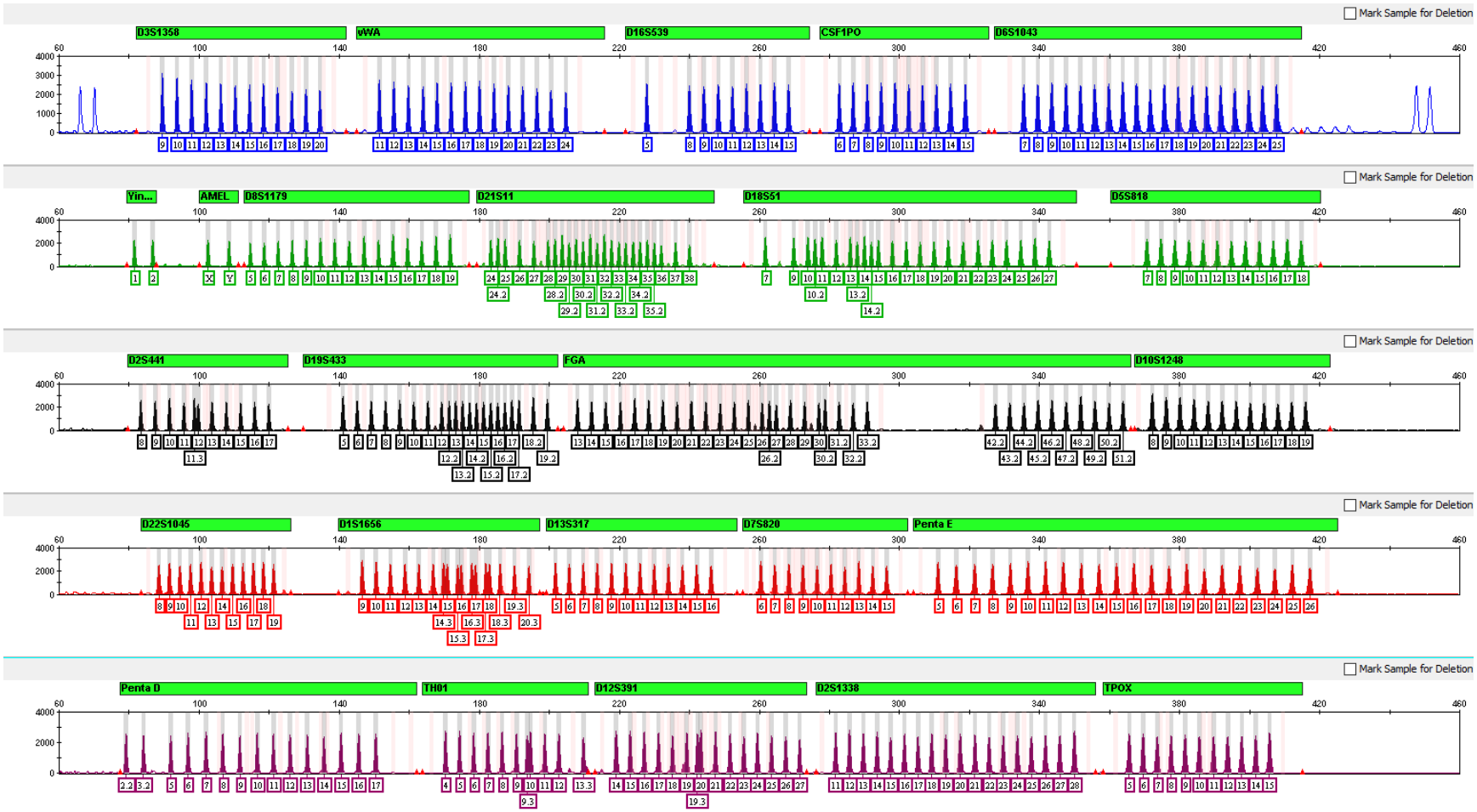


Figure 1 GeneMapper™ *ID-X* Software plot of the VeriFiler™ Plus Allelic Ladder (updated formulation)

The IQCS and IQCL markers are visible as the smallest and largest markers in the FAM™ dye (blue) channel. Each IQC marker has two alleles in the allelic ladder (alleles 1 and 2). However, the IQC markers are not normally analyzed and genotyped because they are primarily intended to be qualitative indicators.

DNA Control 007

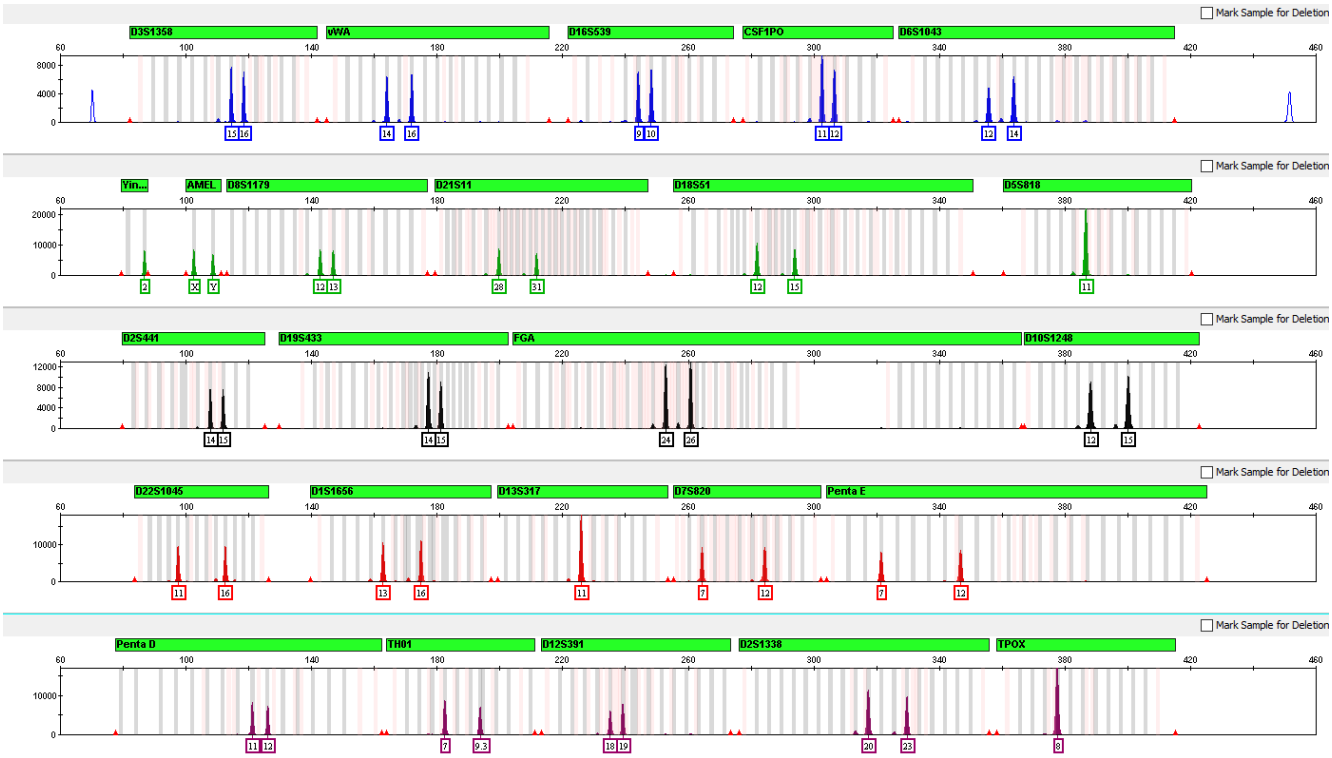


Figure 2 DNA Control 007 (500 pg) amplified with the VeriFiler™ Plus kit and analyzed on an 3500xL Genetic Analyzer (Y-axis scale 0–18,000 RFU) (updated formulation)

The smallest and largest peaks in the FAM™ dye (blue) channel are the IQCS and IQCL markers, respectively.

Contents and storage

The VeriFiler™ Plus kit (Cat. No. A35495) contains sufficient reagents to perform 200 amplifications with a total reaction volume of 25 µL.

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.

Table 4 VeriFiler™ Plus PCR Amplification Kit (Cat. No. A35495; 200 reactions)

Contents	Description	Amount	Storage
VeriFiler™ Plus Master Mix	Contains enzyme, salts, dNTPs, bovine serum albumin, and 0.05% sodium azide in buffer and salt.	2 × 0.50 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).
VeriFiler™ Plus Primer Set	Contains forward and reverse primers to amplify DNA targets.	2 × 0.25 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.
VeriFiler™ Plus Allelic Ladder	Contains amplified alleles. See Figure 1 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 "DNA Control 007" on page 15 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 DNA Control 007, the concentration may differ from the labeled concentration.

Required materials not supplied

See Appendix B, "Materials required but not supplied".

Instruments and software compatibility

Type of instrument or software	Validated models or versions
Thermal cyclers	• ProFlex™ 96-well PCR System (Cat. No. 4484075) • ProFlex™ 2 × 96-well PCR System (Cat. No. 4484076) • ProFlex™ 3 × 32-Well PCR System (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)
Genetic analyzers[1]	• 3500/3500xL Genetic Analyzer with any of the following: – 3500 Series Data Collection Software 1 (Windows™ Vista operating system) and HID Updater 3500 Data Collection Software v2 (Cat. No. 4480670) – 3500 Series Data Collection Software 2 (Windows™ 7 operating system) and HID Updater 3500 Data Collection Software v2 (Cat. No. 4480670) – 3500 Series Data Collection Software 3.1 Upgrade (Windows™ 7 operating system) (Cat. No. A26287) – 3500 Series Data Collection Software 3.1 (Windows™ 7 operating system) (Cat. No. 4475183) – 3500 Series Data Collection Software 4, v4.0.1 software patch (Windows™ 10 operating system) (Cat. No. A40059 or A40376) • 3130/3130*xl* Genetic Analyzer with: – 3130 Data Collection Software v4 (Cat. No. 4475105) or 3130*xl* Data Collection Software-v4 (Cat. No. 4475126), Windows™ 7 operating system – 3130/3730 Data Collection v4 6-Dye Module v1 (Cat. No. 4478404)
Analysis software	Any of the following GeneMapper™ *ID-X* Software versions[2]: • GeneMapper™ *ID-X* Software v1.4 or v1.5; compatible with the Windows™ 7 operating system • GeneMapper™ *ID-X* Software v1.6 or later; compatible with the Windows™ 7 operating system or Windows™ 10 operating system

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

[2] GeneMapper™ *ID-X* Software v1.2–v1.3 can be used to analyze VeriFiler™ Plus PCR Amplification Kit data. However, some genotype quality assessment features of the VeriFiler™ Plus kit are not included in earlier versions of the software. See the Chapter 4, "Analyze data with GeneMapper™ID-X Software" for more details.

IMPORTANT! The VeriFiler™ Plus kit should NOT be used with the following systems:

- 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)

Workflow

Extract DNA, see:

www.thermofisher.com/hid-sampleprep

▼

Quantify DNA

"DNA quantification" on page 19

▼

Perform PCR

"Prepare the amplification kit reactions" on page 23

▼

"Perform PCR" on page 24

▼

Perform electrophoresis

"Set up the 3500/3500xL instruments for electrophoresis (before first use of the kit)" on page 27 or

"Set up the 3130/3130xl instruments for electrophoresis (before first use of the kit)" on page 31

▼

"Prepare samples for electrophoresis" on page 33

▼

Analyze data

"Set up the GeneMapper™ ID-X Software for analysis (before first use of the kit)" on page 37

▼

"Create an analysis method" on page 42

▼

"Create a size standard definition file if needed" on page 51

▼

"Analyze and edit sample files with GeneMapper ID-X Software" on page 53

▼

"Examine or edit a project" on page 54

▼

Chapter 5, "Assess the PCR reaction with the Internal Quality Control System"

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) amplification.
- The amount of sample to use in STR analysis applications.
- The relative quantities of human male and female DNA in a sample (using the Quantifiler™ Trio DNA Quantification Kit). This guides selection of the applicable STR chemistry.
- The DNA quality can be evaluated for:
  - Inhibition level (using any Quantifiler™ kit)
  - DNA degradation level (using the Quantifiler™ Trio DNA Quantification Kit or Quantifiler™ HP DNA Quantification Kit)

  Quality metrics are useful for determining the likelihood of recovery of STR loci with larger amplicon sizes.
- If the sample contains highly degraded DNA. Such samples may require an alternative approach to STR analysis by capillary electrophoresis. The Thermo Fisher Scientific Precision ID NGS System and Panels are 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. See “Related documentation” on page 142 for a list of documents that are related to the Precision ID NGS System and Panels.
- If PCR inhibitors are present in a sample. Such samples 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 capillary electrophoresis instrument ("off-scale" data).

  Off-scale data are a problem because:

  - Quantification (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause a corresponding stutter peak to appear higher in relative intensity, therefore increasing the calculated percent stutter.
  - Multicomponent analysis of off-scale data are not accurate. This inaccuracy results in poor spectral separation ("pull-up").
- False signals of inhibition by the IQC system, although none is present.
- A reduction in the IQCL peak height.
- Incomplete +A nucleotide addition.

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

If too little DNA is added to the PCR reaction, the total number of allele copies added to the PCR is extremely low. 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™ 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 (75–80 bases), to improve the detection of degraded DNA samples. • Uses features that maximize the 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. • Contains an Internal PCR control (IPC) 5′ nuclease assay that amplifies an integrated synthetic DNA sequence. The performance of this assay can be used to assess whether real-time PCR of the sample has been impacted by inhibition.
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

(Optional) Prepare low-TE buffer

We recommend Invitrogen™ Nuclease-Free Water (Cat. No. AM9937) for sample preparation. However, you can also use low-TE buffer. Prepare the low-TE buffer as described in this procedure or use Invitrogen™ TE Buffer (Cat. No. 12090015).

IMPORTANT! Ensure that the low-TE buffer has final concentrations of 10 mM Tris-HCl and 0.1 mM EDTA, pH 8.0. Do not use regular TE buffer because it has a higher EDTA concentration that can chelate Mg2+ ions, therefore reducing PCR amplification efficiency and robustness.

1. Mix:
   - 10 mL of 1 M Tris-HCl, pH 8.0
   - 0.2 mL of 0.5 M EDTA, pH 8.0
   - 990 mL of nuclease-free water

   Note: Adjust the volumes proportionally for specific needs. The final concentration should be 10 mM Tris-HCl and 0.1 mM EDTA, pH 8.0.
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 before first use of the kit. After first use, the reagents are stored at 2–8°C. 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.

IMPORTANT! Use adhesive film for plate sealing to provide a consistent seal across all wells and prevent evaporation. Caps may not provide a consistent seal across all plate wells.

1. Vortex the Master Mix and Primer Set for 3 seconds. Before opening the tubes, remove droplets from the caps by centrifuging the tubes briefly.

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

Component	Volume per reaction
Master Mix	5.0 µL
Primer Set	2.5 µL

   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 7.5 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 as needed:

   Note: We recommend a DNA input amount of 500 pg.

   - If the total sample input amount is >500 pg of DNA, dilute with nuclease-free water or low-TE buffer to achieve a 500-pg input in a 17.5-µL volume.
   - If the total sample input volume is <17.5 µL, bring to volume with nuclease-free water or low-TE buffer to achieve a 17.5-µL input volume.

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

Sample	Add
Negative control	17.5 µL of nuclease-free water or low-TE buffer
Test sample	17.5 µL of DNA[1]

(continued)

Sample	Add
Positive control	Combine, then add to the reaction well or tube: • 5 µL of DNA Control 007 (0.1 ng/µL) • 12.5 µL of nuclease-free water or low-TE buffer

[1] Prepared in step 5 .

7. Mix until the solution is homogenous.
8. Seal the MicroAmp™ Optical 96-Well Reaction Plate with MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical Adhesive Film.
9. Centrifuge the tubes or plate at 3,000 rpm for approximately 30 seconds in a tabletop centrifuge (with plate holders, if using 96-well plates).

Perform PCR

IMPORTANT! This protocol is for casework only. For a direct amplification protocol, see the technical note *Direct Amplification of Reference Samples Using the VeriFiler™ Plus PCR Amplification Kit*.

IMPORTANT! This kit is optimized for use with the thermal cyclers listed in "Instruments and software compatibility" on page 17.

1. Program the thermal cycling conditions.

   IMPORTANT! If you are using the:

   - ProFlex™ PCR System, select **9700 Simulation Mode**.
   - GeneAmp™ PCR System 9700, select the Max ramping mode.
   - Veriti™ Thermal Cycler, set up the method using the **Convert a Method** tool and select **9700 Max Mode**.

   Do not use 9600 emulation mode.

Initial incubation	First stage (2 cycles)		Second stage (27 cycles)		Final extension	Final hold
	Denature	Anneal/extend	Denature	Anneal/extend		
HOLD	CYCLE (29 cycles)				HOLD	HOLD
95°C	96°C	62°C	96°C	59°C	60°C	4°C
1 minute	10 seconds	90 seconds	10 seconds	90 seconds	5 minutes	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 a GeneAmp™ PCR System 9700 *and* 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 Veriti™ Thermal Cycler and the ProFlex™ PCR System 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

The VeriFiler™ Plus PCR Amplification Kit is designed and optimized for casework sample processing (including a pre-amplification extraction and cleanup step) with VeriFiler™ Express PCR Amplification Kit as the partner kit. The VeriFiler™ Express kit is designed and optimized to perform direct amplification of reference samples.

Some laboratories have expressed a preference for a single PCR amplification kit capable of processing both casework and reference samples. To support those laboratories, we have optimized a direct amplification PCR protocol. We performed studies to demonstrate that the VeriFiler™ Plus kit can be used for direct amplification of reference samples. Specifically, we evaluated the performance of the kit using a direct amplification of blood and buccal samples collected on FTA™ cards, NUCLEIC-CARD™ Devices, Bode Buccal DNA Collectors, blood stain cards made out of filter paper, and two types of swabs (Puritan™ Cotton Swabs and 4N6FLOQSwabs™).

Note: The direct amplification uses a different PCR protocol. Perform validation studies before using this kit for direct amplification of single-source samples, or use the VeriFiler™ Express kit. At PCR cycle numbers <29, the IQC peaks may not be detected.

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 the VeriFiler™ Plus 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
3500xL	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.

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 that are generated with this kit. For details on the procedures, see the documents that are listed in “Documentation and support” on page 142.

Genetic Analyzer[1]	Operating system	Data Collection Software	Additional software	Instrument protocols, run modules, and conditions
3500 3500xL	Windows™ Vista	3500 Data Collection Software v1	HID Updater 3500 DC v2 (Cat. No. 4480670)	Set up the following conditions: • Run module: HID36_POP4 (HID36_POP4xl for 3500xL) • Injection conditions[2]: 1.2 kV/15 sec (24 sec for 3500xL) • Run conditions: 13 kV/1,500 sec (13 kV/1,500 sec for 3500xL) • Dye Set J6-T
3500 3500xL	Windows™ 7	3500 Data Collection Software v2	HID Updater 3500 DC v2 (Cat. No. 4480670)	Same as 3500 Data Collection Software v1 listed above

(continued)

Genetic Analyzer[1]	Operating system	Data Collection Software	Additional software	Instrument protocols, run modules, and conditions
3500 3500xL	Windows™ 7	3500 Data Collection Software v3	None	Same as 3500 Data Collection Software v1 listed above
3500 3500xL	Windows™ 10	3500 Data Collection Software v4, v4.0.1 software patch	None	Same as 3500 Data Collection Software v1 listed above

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

[2] Our studies indicate that the injection conditions that are documented generate profiles from 0.5 ng of input DNA with heterozygous peak height averages between 4,000–10,000 RFU (3500 or 3500xL) with no instances of allelic dropout and minimal occurrence of off-scale allele peaks. However, individual CE instrument signal intensities can vary, therefore changes to injection parameters may need to be explored and validated to deliver the best results on your specific system. Large deviations from the recommended injection parameters could affect the performance of the size standard and/or allelic ladder, therefore internal validation studies are recommended.

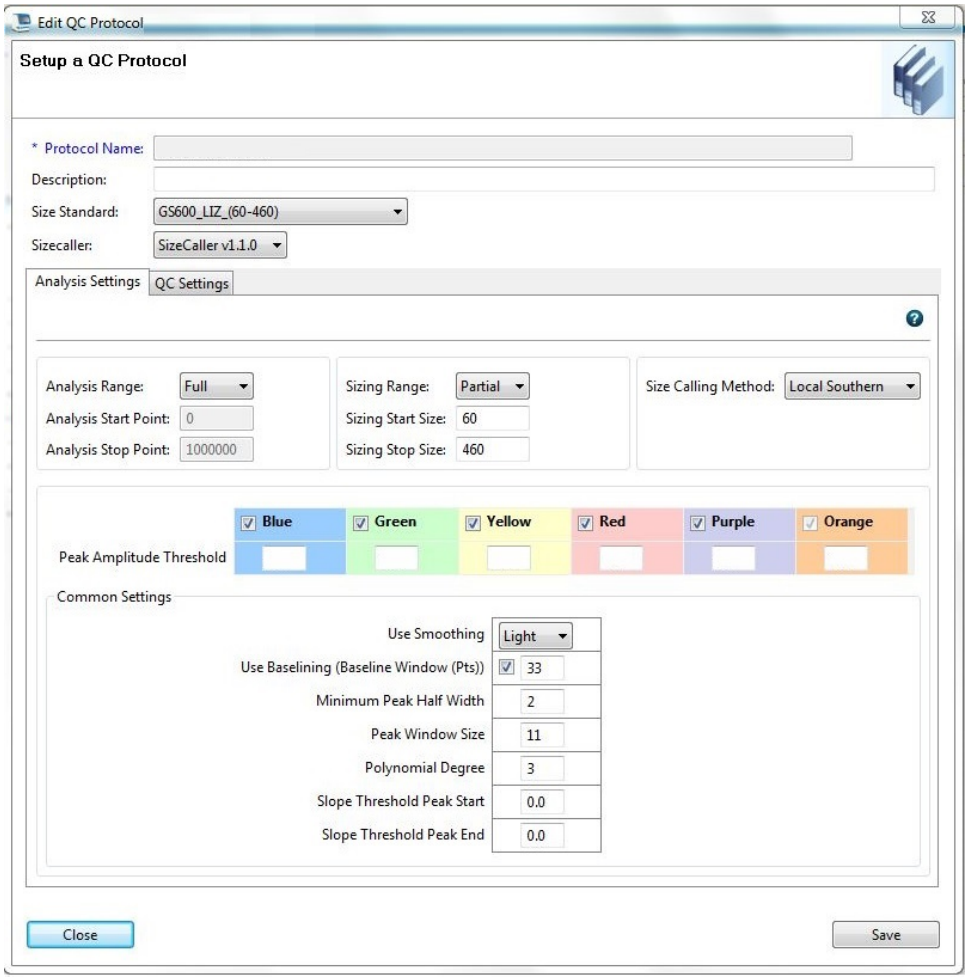
Create a 3500 QC protocol

The VeriFiler™ Plus kit has been validated with data that was analyzed using both the 3rd Order Least Squares method (80–460 base pairs) and the Local Southern method (60–460 base pairs).

1. In the **Library** tab, open the **QC Protocol** window.
2. Create a new QC protocol :
   a. Name the new QC protocol according to your laboratory naming convention.
   b. Set the following parameters:

Parameter	Setting
Size Standard	GS600_LIZ_(60-460)
Size Range Sizing Start Size Sizing Stop Size	Partial 60 bp 460 bp
Size Calling Method	Local Southern or 3rd Order Least Squares method
After checking the "Use Baselining" box: Baseline Window Pts.	33
Peak Window Size	11

c. Click **Save**.



3. Add the QC protocol to the HID assay.

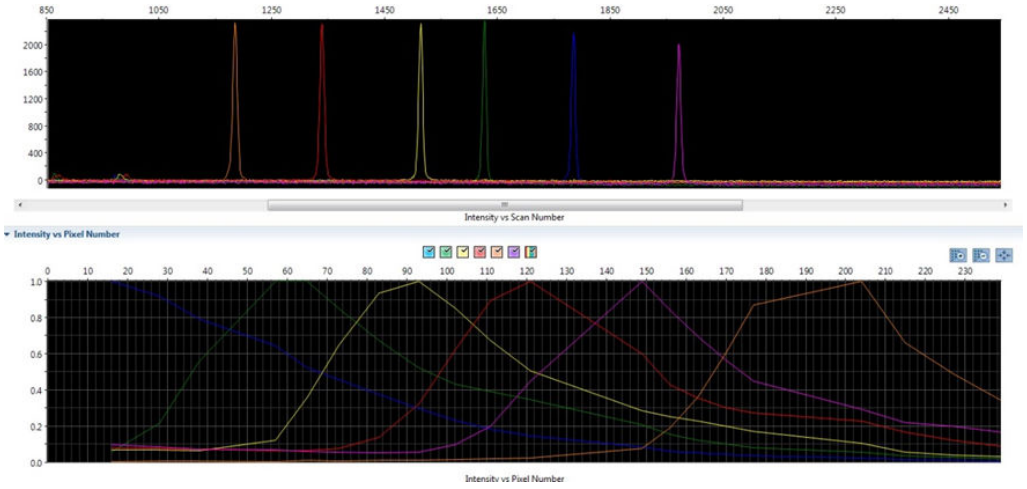
Perform spectral calibration

Perform a spectral calibration using the DS-37 (Dye set J6-T, 6-dye) Matrix Standard Kit (J6-T Dye Set) (Cat. No. A31234).

Note: If it does not already exist, you must create a J6-T Dye Set in the Data Collection Software before running a spectral calibration. For instructions on creating a new dye set, see the "Create a New Dye Set" section of the

3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v2 User Guide (Cat. No. 4476988). Use the J6 template to set up the J6-T dye set.

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 142.

Genetic analyzer[1]	Operating system	Data Collection Software	Additional software	Run modules and conditions
3130	Windows™ 7	Data Collection Software v4	3130/3730 DC v4 6-Dye Module v1	Set up the following conditions: • HIDFragmentAnalysis36_POP4_1 • Injection conditions[2]: 3 kV/4 sec • Run conditions: 13 kV/1,600 sec • Dye Set J6-T
3130*xl*				Set up the following conditions: • HIDFragmentAnalysis36_POP4_1 • Injection conditions[2]: 3 kV/6 sec • Run conditions: 13 kV/1,600 sec • Dye Set J6-T

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

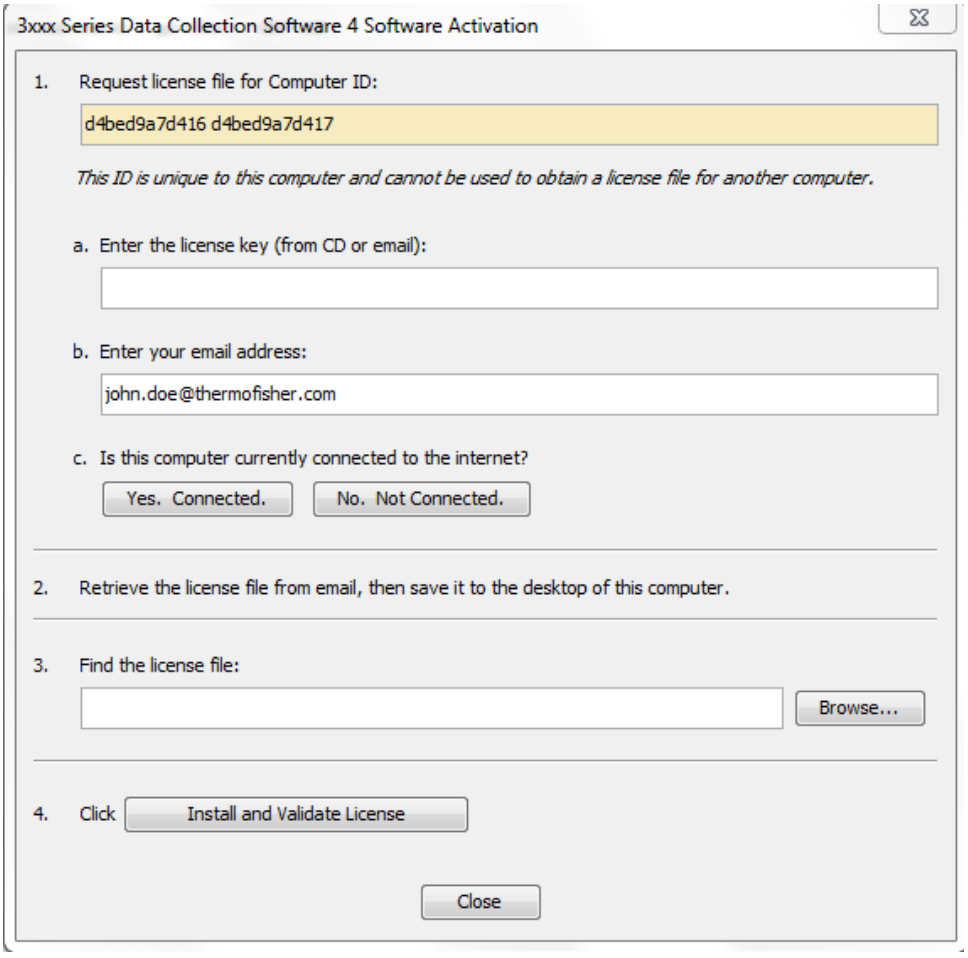
[2] Our studies indicate that the injection conditions that are documented generate profiles from 0.5 ng of input DNA with heterozygous peak height averages between 2,000– 4,000 RFU (3130 or 3130*xl*) with no instances of allelic dropout and minimal occurrence of off-scale allele peaks. However, individual CE instrument signal intensities can vary, therefore changes to injection parameters may need to be explored and validated to deliver the best results on your specific system. Large deviations from the recommended injection parameters could affect the performance of the size standard and/or allelic ladder, therefore internal validation studies are recommended.

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.

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



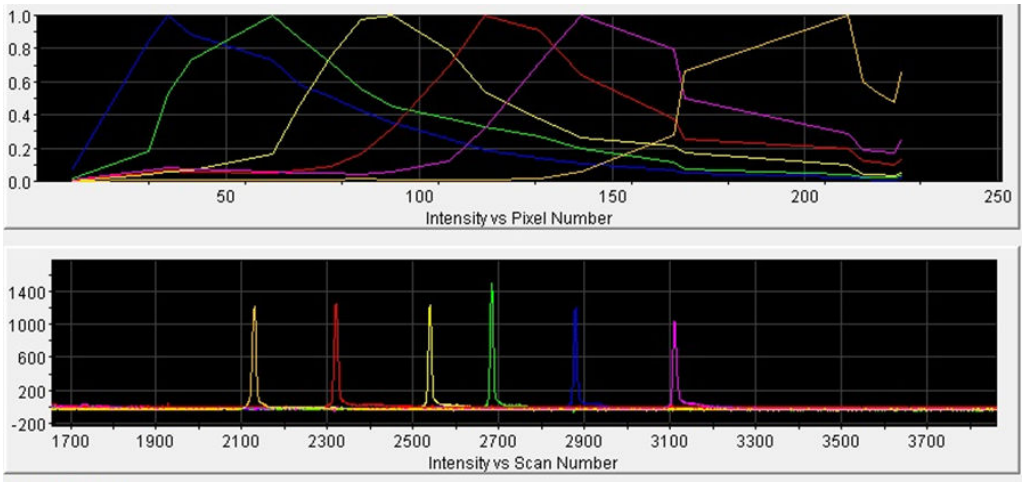
5. Request the software license file by performing steps 1a, 1b, and 1c as listed on the activation screen. The license file will be emailed to you.
6. Obtain the software license file from your email.
7. Make a copy of the software license file and keep it in a safe location.
8. Copy the software license 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-37 (Dye set J6-T, 6-dye) Matrix Standard Kit (J6-T Dye Set) (Cat. No. A31234).

The following figure is an example of a passing 6-dye spectral calibration.



Prepare samples for electrophoresis

This procedure applies to the 3130/3130*xl* Genetic Analyzer and 3500/3500xL Genetic Analyzer.

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 VeriFiler™ Plus 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 the 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 analyze data that is generated using the VeriFiler™ Plus kit. 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.

Earlier versions of GeneMapper™ *ID-X* Software (v1.2 and above) are capable of analyzing data that is generated using the VeriFiler™ Plus kit. However, some quality assessment tools are not fully functional on versions earlier than v1.4. Details on

the data assessment functionality added to specific versions of GeneMapper™ *ID-X* Software are found in the release notes for each version. To summarize:

- In versions earlier than v1.4, samples show a red flag for the CGQ if the Y-indel peak is missing, for example, with a female DNA sample. This occurs because the absence of the Y-indel peak triggers the Allele Number Rule flag.

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 37

▼

"(If needed) Download newer versions of panel, bin, and stutter files" on page 38

▼

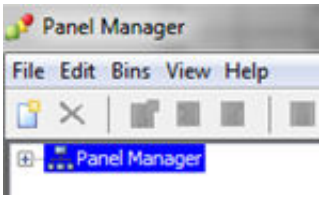
"Import panels, bins, and marker stutter" on page 38

▼

"(Optional) Define custom table or plot settings" on page 42

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

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.

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

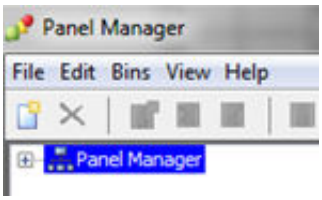
1. Go to **www.thermofisher.com/GMIDXsoftware**.
2. If the file versions listed are newer than the versions on your computer, download the file **VeriFiler Plus Analysis Files**.

   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:

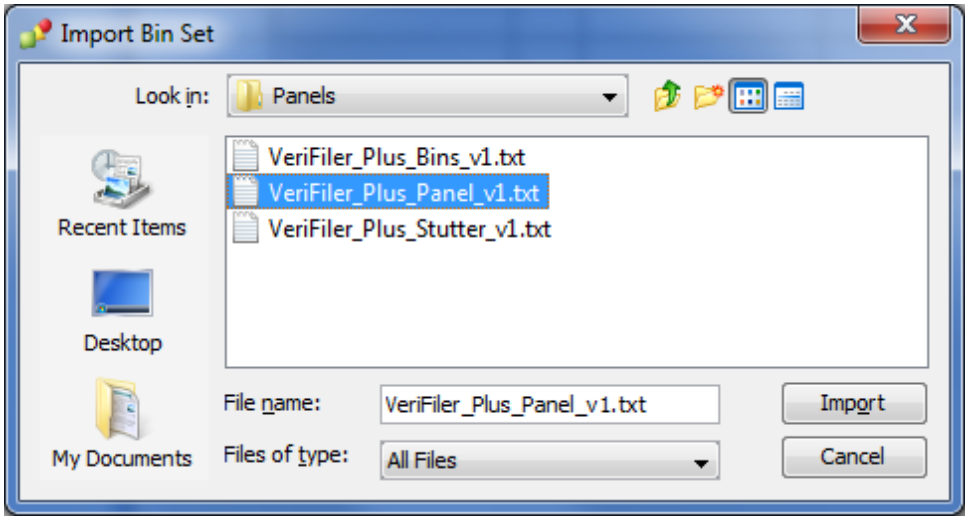
1. Start the GeneMapper™ *ID-X* Software, then log in with the appropriate user name and password.
2. Select **Tools** ▸ **Panel Manager**.
3. Find, then open the folder containing the panels, bins, and marker stutter:



   a. Select **Panel Manager**, then select **File** ▸ **Import Panels** to open the **Import Panels** dialog box.

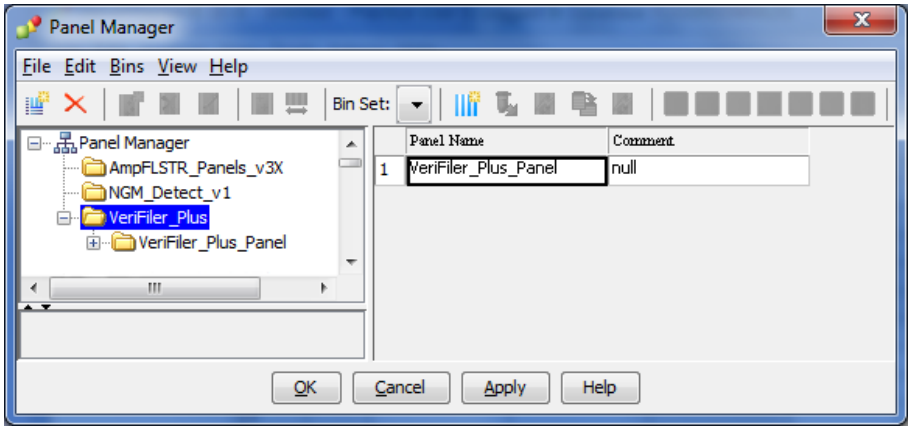
   b. Navigate to, then open the **VeriFiler Plus Analysis Files** folder that you unzipped in the previous procedure.
4. Select **VeriFiler_Plus_Panel.txt**, then click **Import**.

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



5. Import the bins file:

   a. In the **Panel Manager** navigation pane, click the **VeriFiler_Plus** folder to display the **VeriFiler_Plus_Panel**.

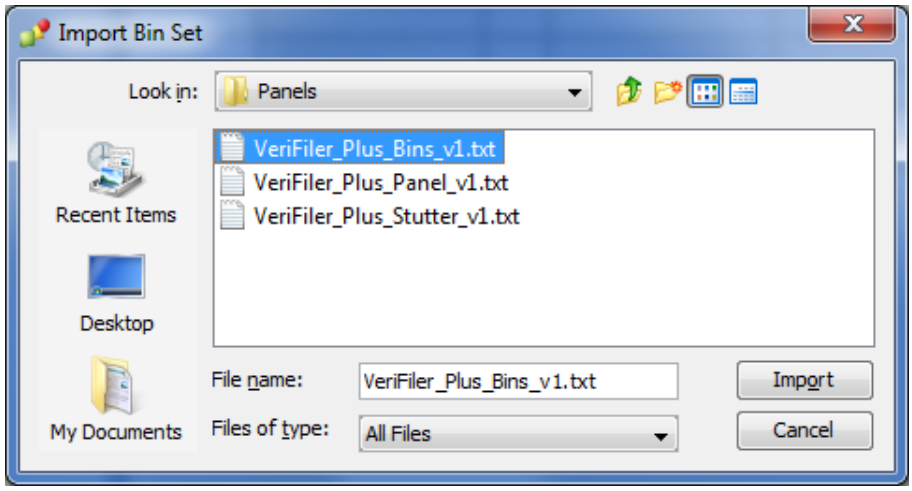


   b. Select **File ▸ Import Bin Set** to open the **Import Bin Set** dialog box.

   c. Navigate to, then open the **VeriFiler Plus Analysis Files** folder.

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

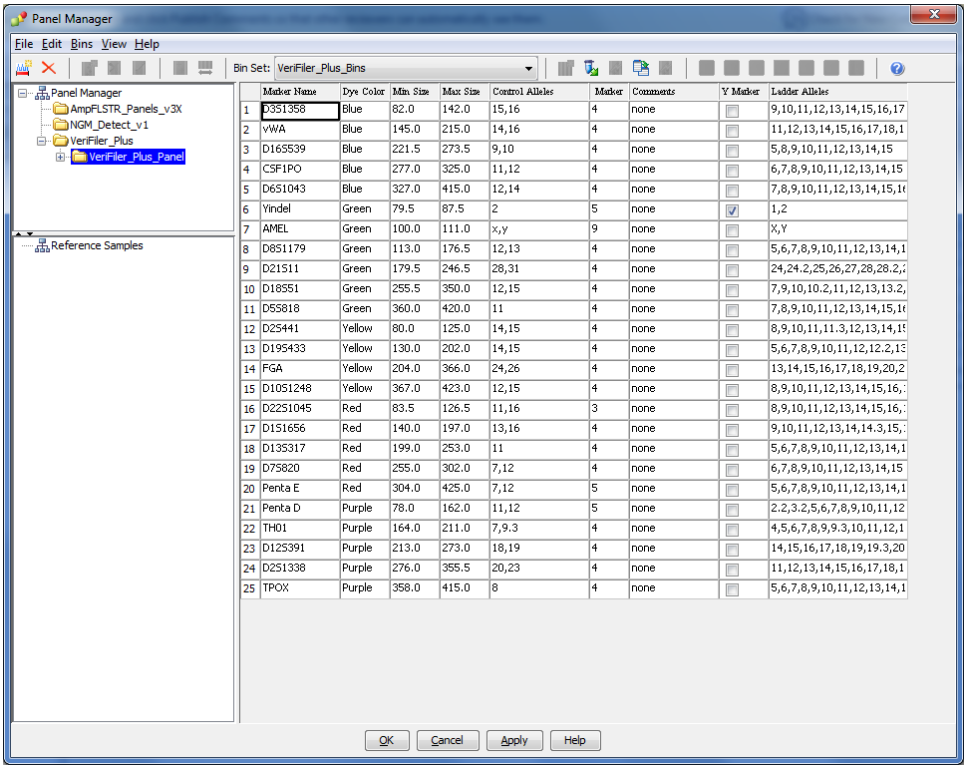
Note: Importing this file associates the bin set with the panels in the **VeriFiler_Plus_Panel** folder.



6. *(Optional)* View information about panels and bins: In the **Panel Manager** navigation pane, click **+** next to the **VeriFiler_Plus** folder, then click **+** next to the **VeriFiler_Plus_Panel** folder to reveal marker-level information.

Note: Select the **Y Marker** checkbox to avoid flagging female samples.

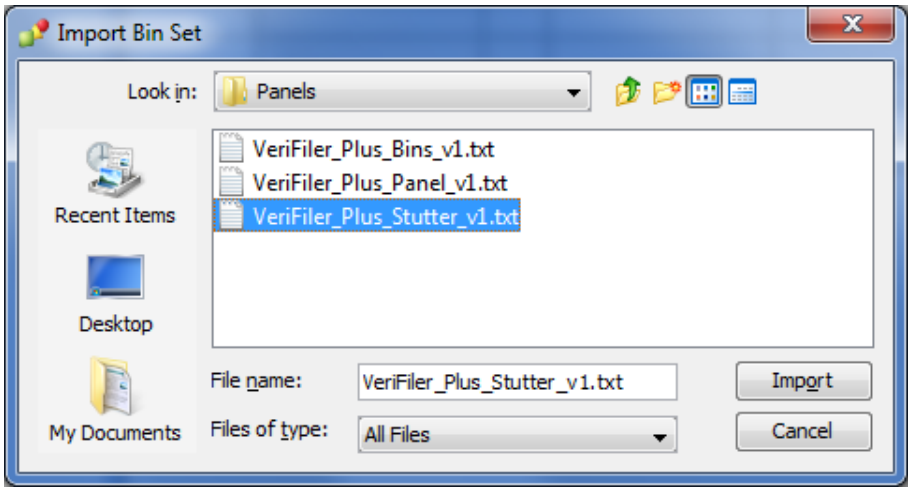
The panel view is shown below.



	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker	Comments	Y Marker	Ladder Alleles
1	D3S1358	Blue	82.0	142.0	15,16	4	none		9,10,11,12,13,14,15,16,17
2	vWA	Blue	145.0	215.0	14,16	4	none		11,12,13,14,15,16,17,18,1
3	D16S539	Blue	221.5	273.5	9,10	4	none		5,8,9,10,11,12,13,14,15
4	CSF1PO	Blue	277.0	325.0	11,12	4	none		6,7,8,9,10,11,12,13,14,15
5	D6S1043	Blue	327.0	415.0	12,14	4	none		7,8,9,10,11,12,13,14,15,1
6	Yindel	Green	79.5	87.5	2	5	none	☑	1,2
7	AMEL	Green	100.0	111.0	x,y	9	none		X,Y
8	D8S1179	Green	113.0	176.5	12,13	4	none		5,6,7,8,9,10,11,12,13,14,1
9	D21S11	Green	179.5	246.5	28,31	4	none		24,24.2,25,26,27,28,28.2,
10	D18S51	Green	255.5	350.0	12,15	4	none		7,9,10,10.2,11,12,13,13.2,
11	D5S818	Green	360.0	420.0	11	4	none		7,8,9,10,11,12,13,14,15,1
12	D2S441	Yellow	80.0	125.0	14,15	4	none		8,9,10,11,11.3,12,13,14,1
13	D19S433	Yellow	130.0	202.0	14,15	4	none		5,6,7,8,9,10,11,12,12.2,1
14	FGA	Yellow	204.0	366.0	24,26	4	none		13,14,15,16,17,18,19,20,2
15	D10S1248	Yellow	367.0	423.0	12,15	4	none		8,9,10,11,12,13,14,15,16,
16	D22S1045	Red	83.5	126.5	11,16	3	none		8,9,10,11,12,13,14,15,16,
17	D1S1656	Red	140.0	197.0	13,16	4	none		9,10,11,12,13,14,14.3,15,
18	D13S317	Red	199.0	253.0	11	4	none		5,6,7,8,9,10,11,12,13,14,1
19	D7S820	Red	255.0	302.0	7,12	4	none		6,7,8,9,10,11,12,13,14,15
20	Penta E	Red	304.0	425.0	7,12	5	none		5,6,7,8,9,10,11,12,13,14,1
21	Penta D	Purple	78.0	162.0	11,12	5	none		2.2,3.2,5,6,7,8,9,10,11,12
22	TH01	Purple	164.0	211.0	7,9.3	4	none		4,5,6,7,8,9,9.3,10,11,12,1
23	D12S391	Purple	213.0	273.0	18,19	4	none		14,15,16,17,18,19,19.3,20
24	D2S1338	Purple	276.0	355.5	20,23	4	none		11,12,13,14,15,16,17,18,1
25	TPOX	Purple	358.0	415.0	8	4	none		5,6,7,8,9,10,11,12,13,14,1

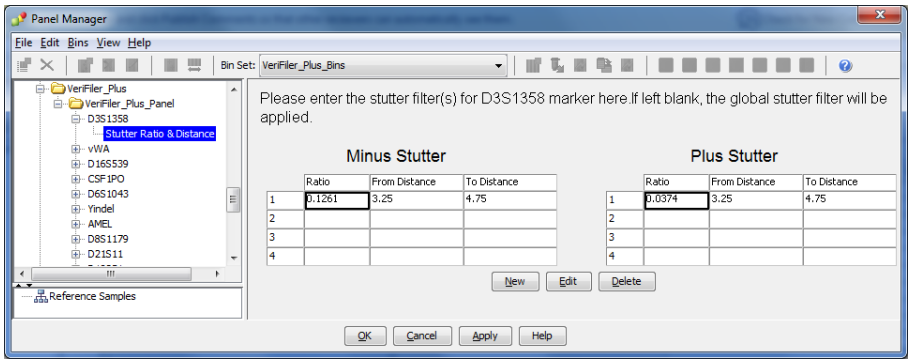
7. Import the stutter file:
   a. Select the **VeriFiler_Plus_Panel** folder in the navigation pane.
   b. Select **File** ▸ **Import Marker Stutter** to open the **Import Marker Stutter** dialog box.
   c. Navigate to, then open the **VeriFiler Plus Analysis Files** folder.
   d. Select **VeriFiler_Plus_Stutter_v1.txt**, then click **Import**.

      Note: Importing this file associates the marker stutter ratio with the bin set in the **VeriFiler_Plus** 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. Click **+** next to the **VeriFiler_Plus** and **VeriFiler_Plus_Panel** folders.

      The list of markers is displayed.
   b. 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

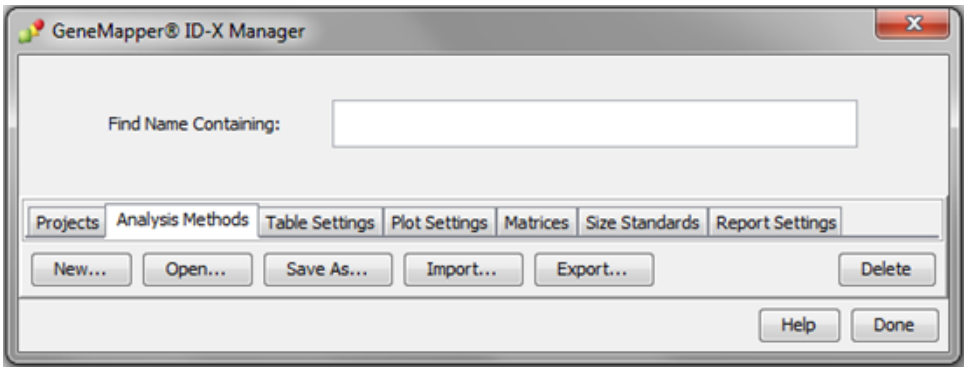
Default views for table and plot settings are provided with the software. For information on defining custom views, see *GeneMapper™ ID-X Software Getting Started Guide— Basic Features*.

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 1.2 is not compatible with analysis methods that are created in earlier versions of software, or with GeneMapper™ Software v3.2.1.

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.

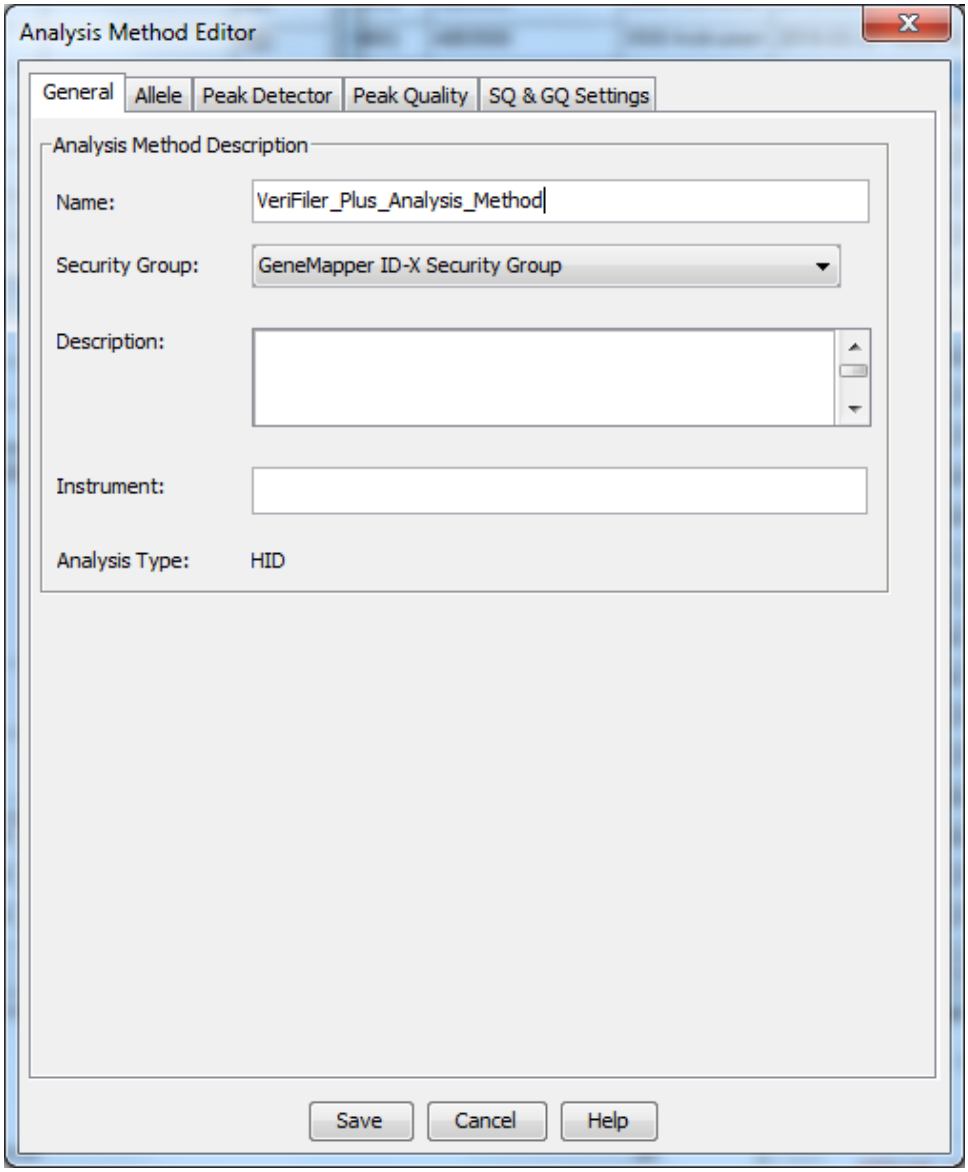
   Note: The **Analysis Method Editor** closes when you save your settings. To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.

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

Enter Analysis Method settings

Enter General tab settings

1. Enter a **Name** and select the **Security Group** appropriate for your software configuration.



2. *(Optional)* Enter a **Description** and **Instrument**.

Enter Allele tab settings

IMPORTANT! Perform appropriate internal validation studies to determine the appropriate settings to use.

1. Select the **VeriFiler_Plus_Bins_v1** bin set.

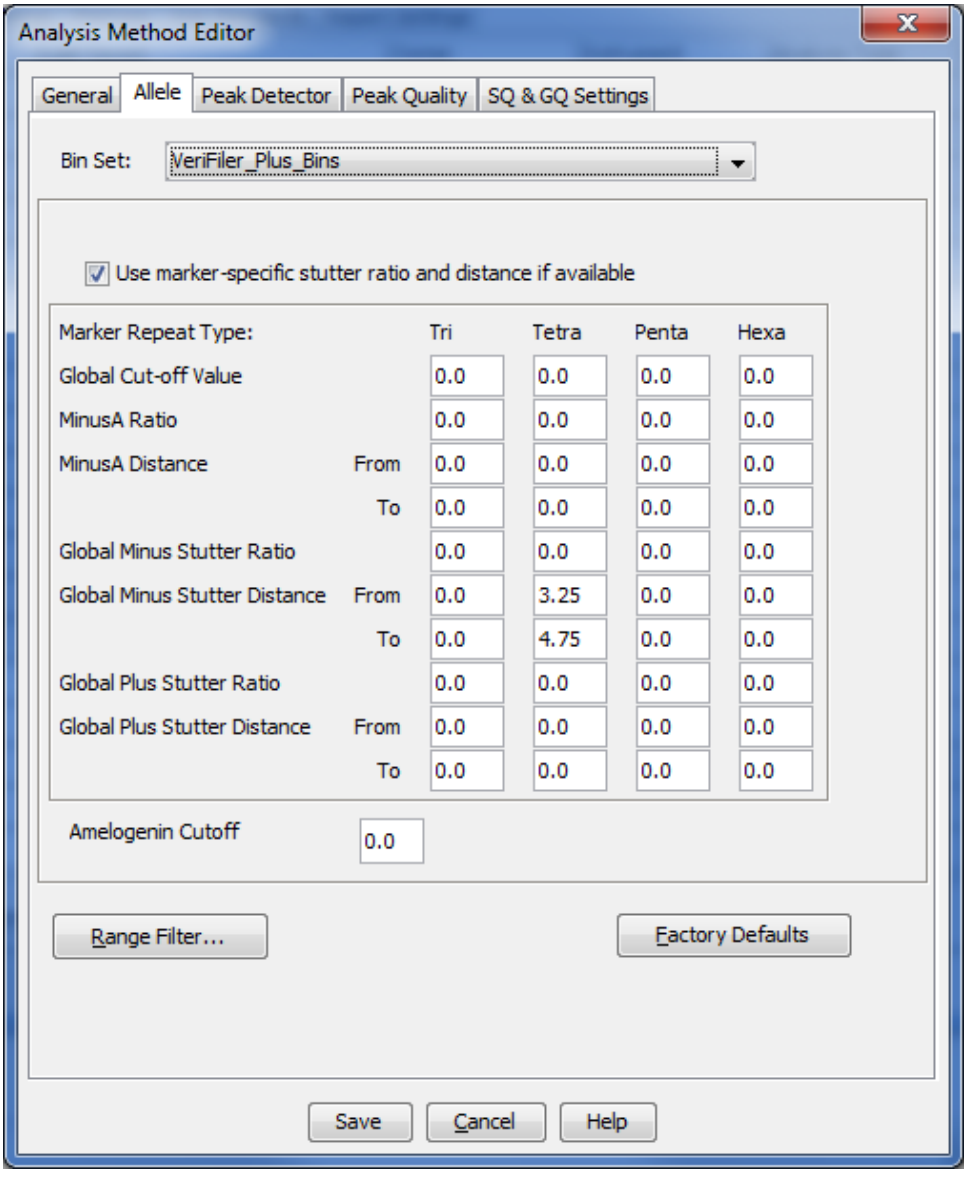


Figure 3 Settings used in developmental validation of the kit

2. *(Optional)* To apply the stutter ratios contained in the **VeriFiler_Plus_Stutter_v1.txt**, select the **Use marker-specific stutter ratio and distance if available** checkbox (selected by default).

3. If you use global stutter filters instead of marker-specific stutters, enter values for Tri-, Tetra-, and Penta-nucleotide repeats.

   Note: Using global stutter filters instead of marker-specific stutters is not typical.

   Note: There are no hexa-nucleotide repeat markers in the VeriFiler™ Plus kit.

4. Enter the appropriate filter settings.

Enter Peak Detector tab settings

Enter the appropriate values:

Field	Values to enter or select	Additional information
Ranges	Enter the values shown in Figure 4. **Note:** The read region for the VeriFiler™ Plus kit is 75–426 bp.	—
Peak Detection	Enter the appropriate settings. **IMPORTANT!** Perform appropriate internal validation studies to determine the appropriate peak amplitude thresholds for interpretation of data.	The software uses the **Peak Amplitude Thresholds** to specify the minimum peak height. 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. **Note:** As shown in Figure 4, there are additional **Peak Detection** parameters, including **Min. Peak Half Width**, **Polynomial Degree**, **Peak Window Size**, and **Slope Threshold**.
Smoothing and Baseline	Enter the values shown in Figure 4.	—

(continued)

Field	Values to enter or select	Additional information
Size Calling Method	Select **Local Southern Method** or **3rd Order Least Squares**, or another method that has been validated during your internal validation studies.	The VeriFiler™ Plus kit has been validated using both the 3rd Order Least Squares sizing method (80–460 bp) and the Local Southern sizing method (60–460 bp). Both sizing methods produced 100% concordance and generated acceptable data. Select alternative sizing methods only after performing the appropriate internal validation studies.
Normalization	*(Optional)* Select the **Normalization** checkbox.	A **Normalization** checkbox is available on this tab in GeneMapper™ *ID-X* Software for use with data that are run on the 3500/3500xL Genetic Analyzer.

IMPORTANT! Both the 3rd Order Least Squares Sizing algorithm and the Local Southern Sizing algorithm have been validated for analysis of VeriFiler™ Plus kit data on 3130- and 3500-series instruments.

Note: The read region for the VeriFiler™ Plus kit is 75–426 bp.

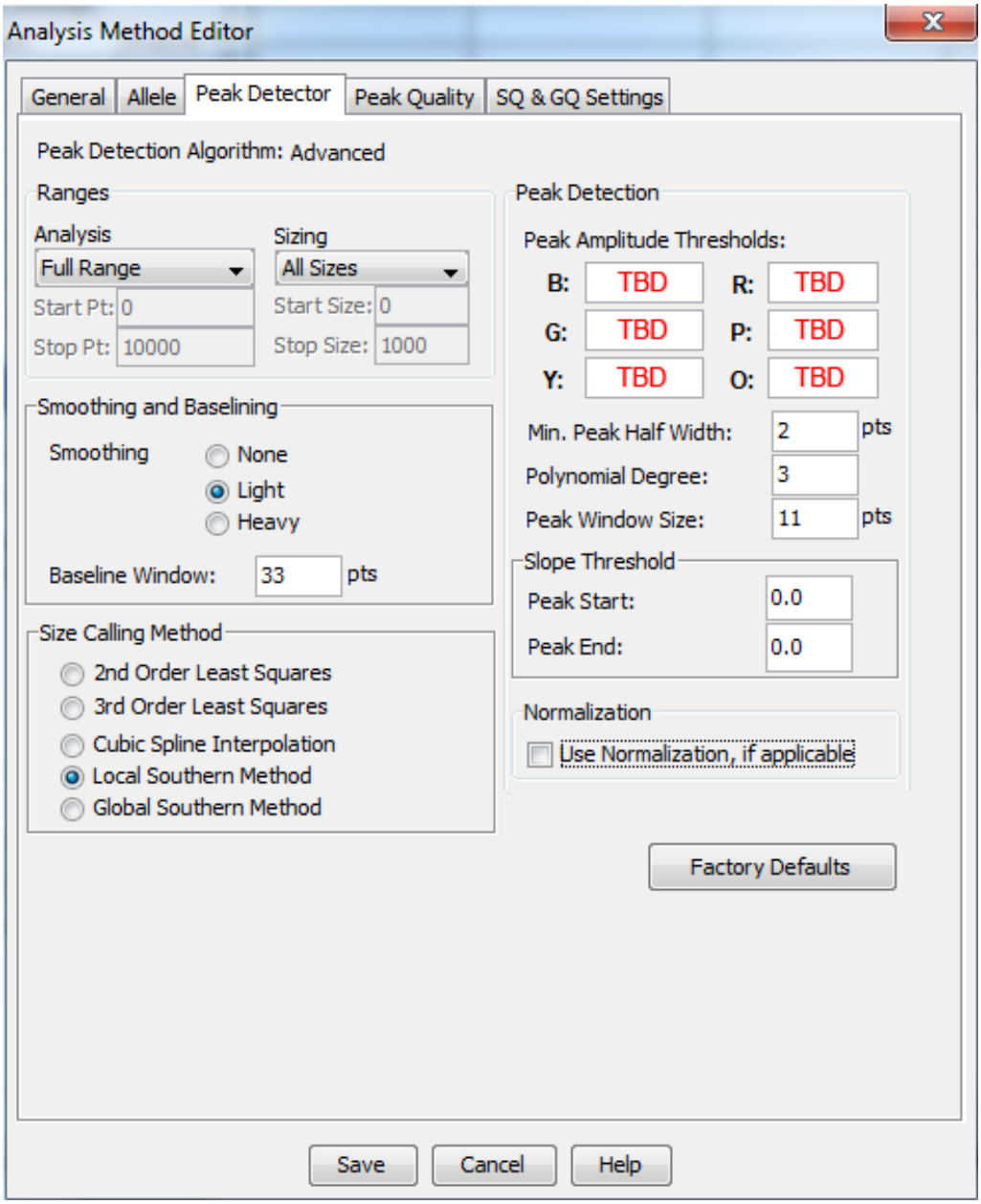


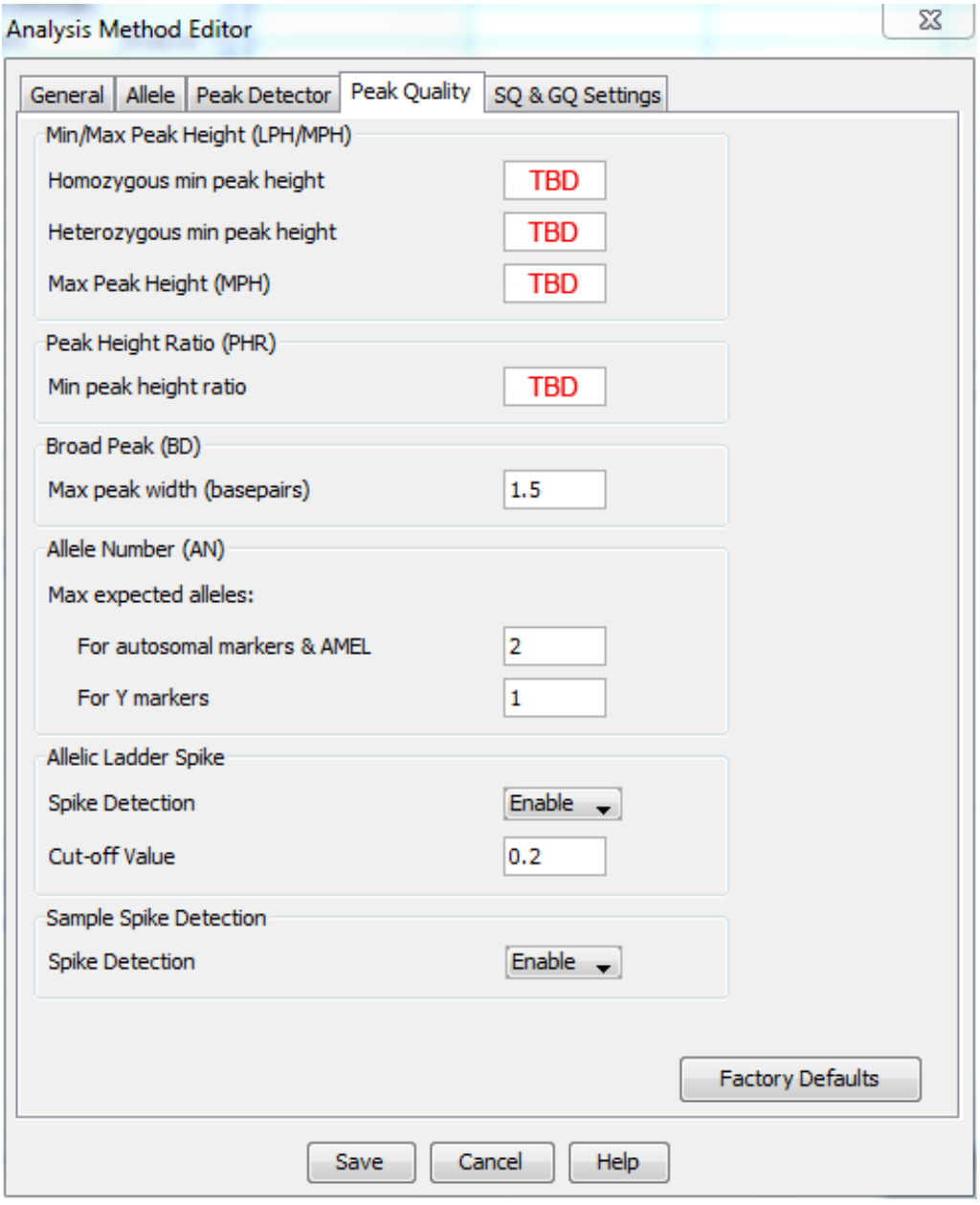
Figure 4 Settings used in developmental validation of the kit

IMPORTANT! *TBD* indicates values to be determined in your laboratory. Laboratories must perform the appropriate internal studies to determine the peak amplitude thresholds for interpreting the VeriFiler™ Plus kit data.

Enter 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.

Enter the following values:

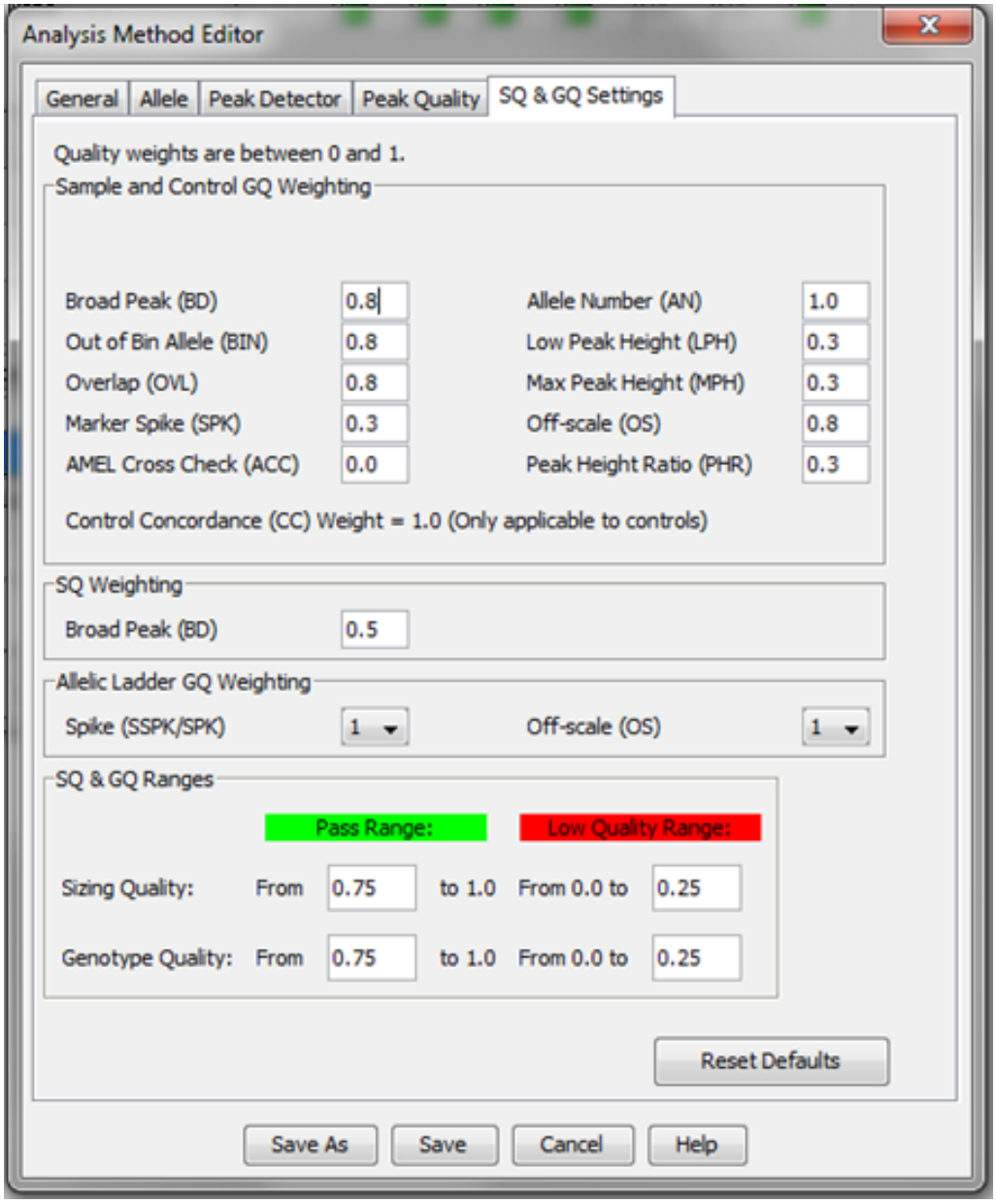


IMPORTANT! *TBD* indicates values to be determined in your laboratory. Laboratories must perform the appropriate internal studies to determine the peak amplitude thresholds for interpreting the VeriFiler™ Plus kit data.

Enter SQ and GQ tab settings

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

Enter the following values:



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

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

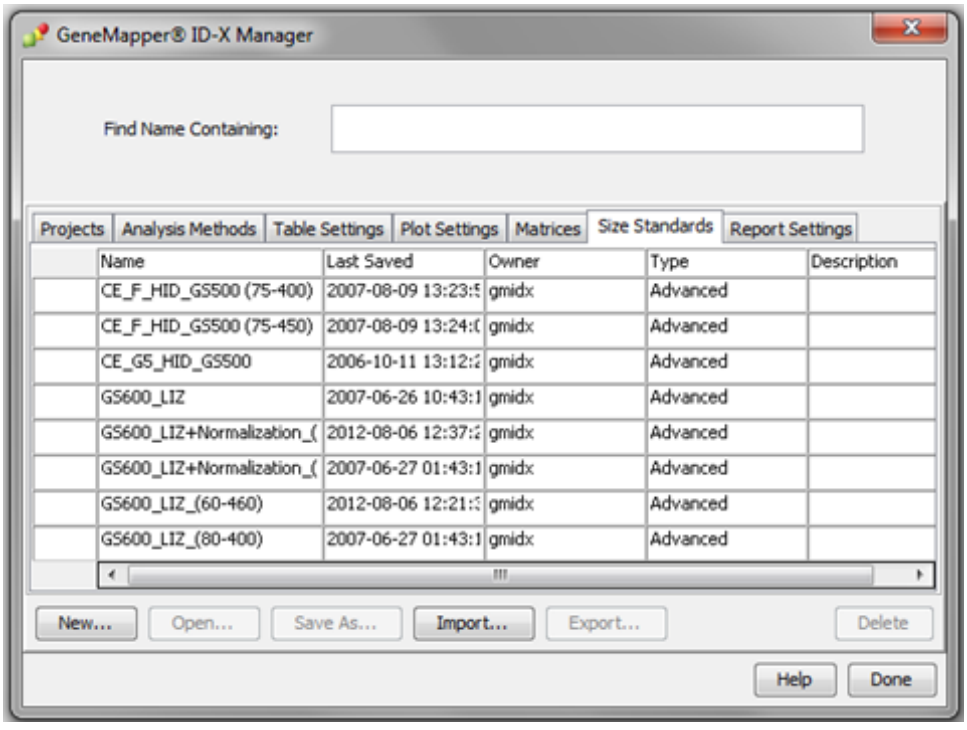
The GS600_LIZ_(60–460) size standard definition that is provided with GeneMapper™ *ID-X* Software and used with the Local Southern size calling method contains the following peaks: 60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, and 460.

This size standard definition has been validated for use with this kit on the genetic analyzers listed in “Instruments and software compatibility” on page 17. If you need to create your own size standard definition, see “Create a size standard definition file” on page 51.

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**.

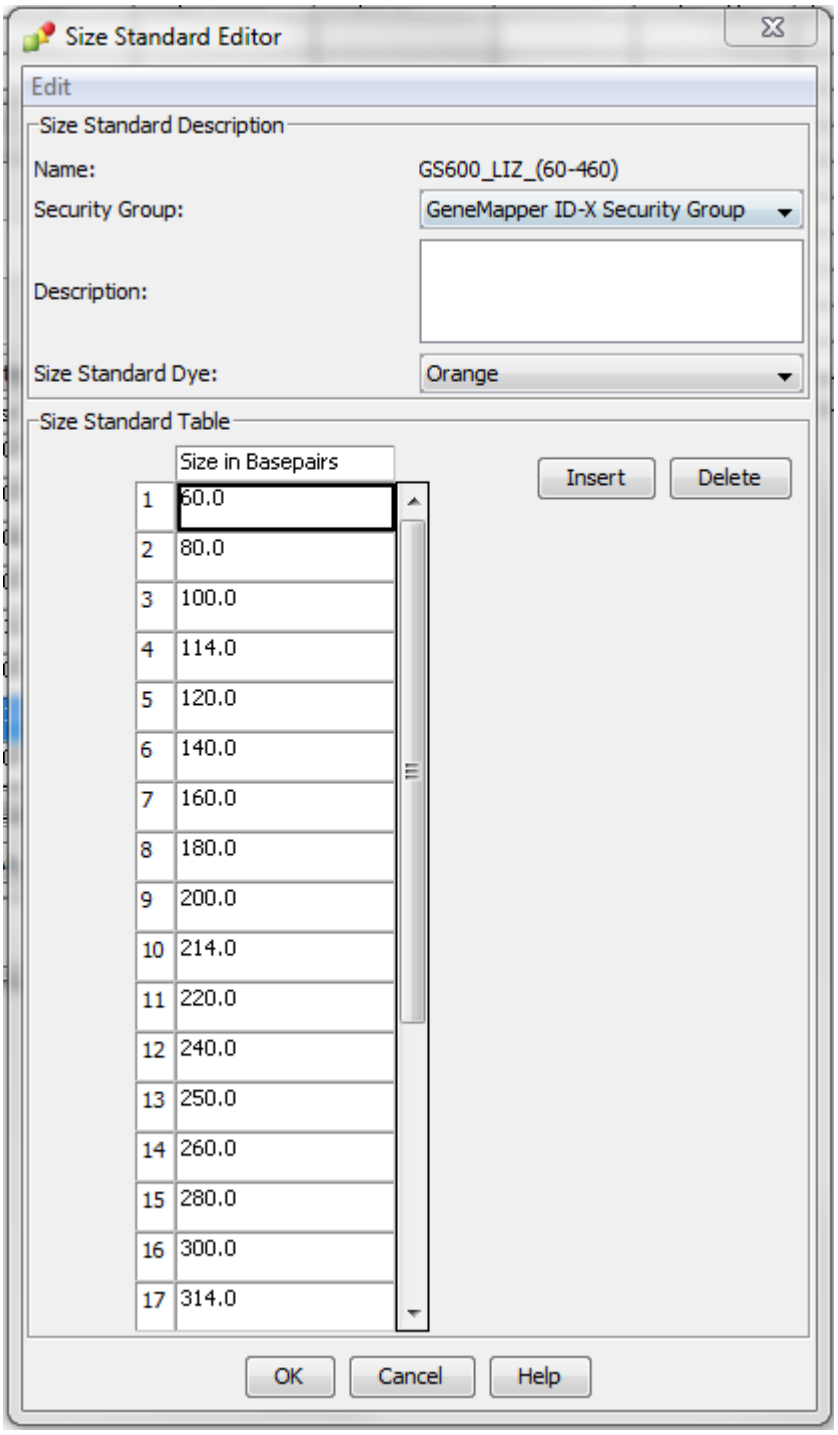


3. Specify settings in the **Size Standard Editor**:
   a. Enter a name as shown in the following figure or enter a new name.

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

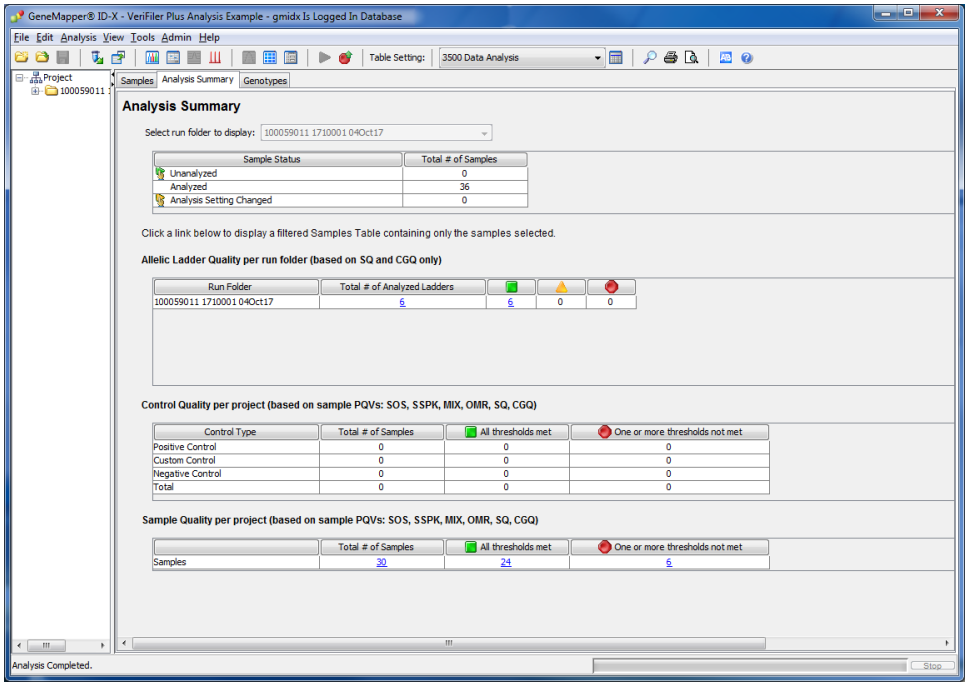
1. In the **Project** window, select **Edit ▸ Add Samples to Project**, then navigate to the disk or directory that contains the sample files.

2. Apply analysis settings to the samples in the project.

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	Select **VeriFiler Plus Analysis Method** (or the name of the analysis method you created).
Panel	Select **VeriFiler_Plus_Panel**.
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 VeriFiler™ Plus kit was originally validated for use 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 VeriFiler™ Plus 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 142 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 Internal Quality Control (IQC) System is a tool that can help you evaluate the PCR reaction and, along with the STR marker data, infer possible sample degradation or inhibition.

The primers for the two IQC markers, IQCS and IQCL, amplify synthetic DNA targets that are included in the primer mix. IQCS is a low molecular weight amplicon, with mobility of 70 nt. IQCL is a higher molecular weight amplicon, with mobility of 451 nt.

Note: The IQC markers enable *qualitative* insight into the sample amplification. By default, the IQC markers are not genotyped during analysis in the GeneMapper™ *ID-X* Software. However, if needed, the VeriFiler™ Plus Allelic Ladder contains two peaks each for the IQCS and IQCL markers (designated 1 and 2) to detect the IQC sequences. Sample reactions contain one peak each for IQCS and IQCL, which corresponds to the larger peak (peak 2) in each IQC marker.

The IQC System allows you to:

- Confirm the success or failure of the PCR reaction, by looking for the presence or absence of the IQCS and IQCL primer peaks on the electropherogram.
- Determine if PCR inhibitors might be present in the PCR reaction, or if the PCR reaction conditions are not optimal, by evaluating the peak heights of IQCS and IQCL.

Evaluate the PCR reaction

To evaluate the PCR performance of the samples, review the peak heights of the IQCS and IQCL. Under ideal PCR conditions, the peak heights of the IQCS and IQCL should be >2,000 RFU using standard injection protocols on the 3500/3500xL Genetic Analyzer (see Table 5). Under suboptimal PCR conditions (for example, moderate inhibition), the height of the IQCL is substantially reduced. Under extremely high inhibition, even the small IQC peak heights are substantially reduced. Note that when high inputs of DNA are amplified (greater than 2 ng) some suppression of the IQCL peak may also be seen.

See Table 5 for outcome scenarios.

Note: The electropherograms that are shown in Figure 5 through Figure 10 are from runs on a 3500xL Genetic Analyzer using standard injection protocols.

Table 5 IQC peak interpretation

Sample DNA profile	IQC peaks	IQC interpretation	Recommended action for re-amplification[1]
		Typically indicates...	
Balanced	Both IQC peaks are >2,000 RFU	PCR performance within the optimal range—no sample degradation or inhibition (see Figure 5)	Re-amplification should not be required. On a case-by-case basis, you can evaluate the profile and determine if re-amplification is required.
Balanced	The IQCS peak height is > 2,000 RFU, while the IQCL peak height is significantly decreased	Mild inhibition (see Figure 6)	Evaluate the profile and determine if additional input would recover alleles without saturating the system.
Ski-slope	The IQCS peak height is near 2,000 RFU or greater, while the IQCL peak is not present	High inhibition (see Figure 7)	Perform a dilution or dilutions of the sample to minimize the inhibitor while still allowing for enough DNA to achieve amplification (for example, 10–20% dilution).
Ski-slope	The IQCS peak height is significantly decreased, while the IQCL peak is not present	Very high inhibition (see Figure 8)	Perform a dilution or dilutions of the sample to minimize the inhibitor while still allowing for enough DNA to achieve amplification (for example, 20–60% dilution).
Ski-slope	Both IQC peaks are >2,000 RFU	Degraded sample DNA (see Figure 9)	Evaluate the profile and determine if additional input would recover alleles without saturating the system.

Table 5 IQC peak interpretation *(continued)*

Sample DNA profile	IQC peaks	IQC interpretation	Recommended action for re-amplification[1]
		Typically indicates...	
No peaks	Both IQC peaks are >2,000 RFU	No DNA or very little sample DNA (see Figure 10)	Check the quantification and normalization calculations. If you have sufficient DNA to generate a profile, increase the amount of DNA in the amplification.
No peaks	No peaks	PCR failure or ultra-high inhibition	Re-amplify the sample at the appropriate target to determine if PCR failure occurred. If DNA is still not detected, perform a dilution or dilutions of the sample to minimize the inhibitor while still allowing for enough DNA to achieve amplification (for example, 20–60% dilution).

[1] The decision to re-amplify the sample should be based on individual laboratory protocols that identify how much information is sufficient for reporting.

Balanced profile

In this example of a balanced profile, the IQC peaks and the DNA profile peaks are balanced, which indicates that PCR has occurred optimally.

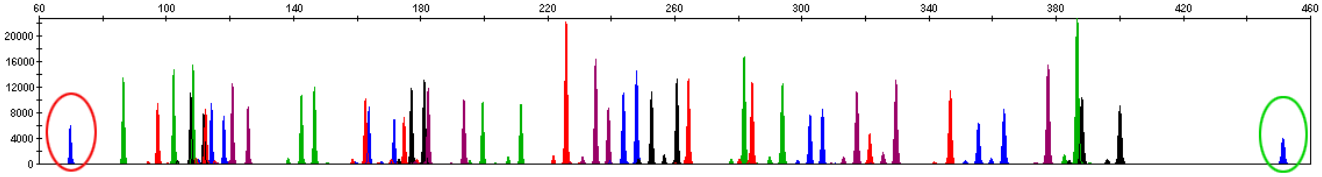


Figure 5 FAM™ dye channel electropherogram that shows IQCS and IQCL peaks with 0.5 ng of DNA (scaled to 22,000 RFU) (updated formulation)

The red circle highlights the IQCS peak and the green circle highlights the IQCL peak.

Ski slope profile with decreased IQCL peak height

Figure 6 shows a significantly lower IQCL peak height. This indicates that the PCR reaction has been compromised by inhibition. Figure 7 shows the complete absence of an IQCL peak, indicating a high level of inhibition.

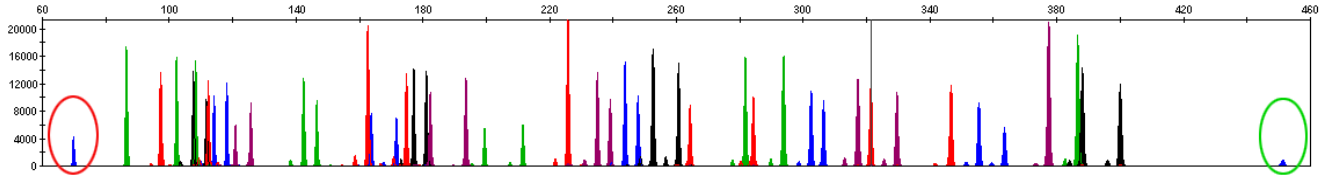


Figure 6 Combined dyes electropherogram for the VeriFiler™ Plus kit in the presence of 150 ng/µL of humic acid (scaled to 22,000 RFU) (updated formulation)

The red circle highlights the IQCS peak (a PCR inhibitor) and the green circle highlights the IQCL peak. While overall peak heights are reduced relative to the uninhibited control sample, only a subtle ski-slope effect is observed.

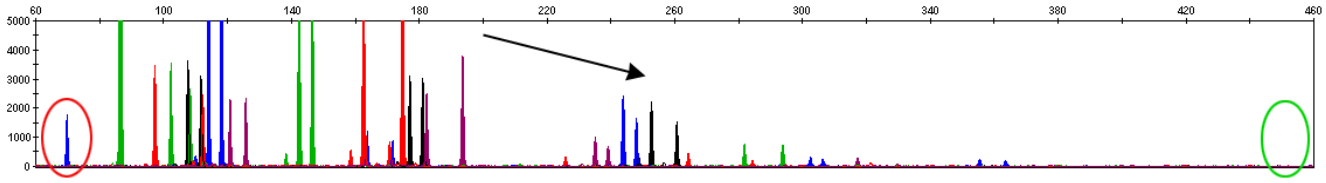


Figure 7 Combined dyes electropherogram for the VeriFiler™ Plus kit in the presence of 350 ng/µL of humic acid (scaled to 5,000 RFU) (updated formulation)

The red circle highlights the IQCS peak; the IQCL peak is absent (green box). The arrow indicates the ski slope peak pattern observed in the DNA profile.

Ski slope profile with IQC peaks

The presence of both IQCS and IQCL peaks >2,000 RFU indicates that PCR has occurred optimally.

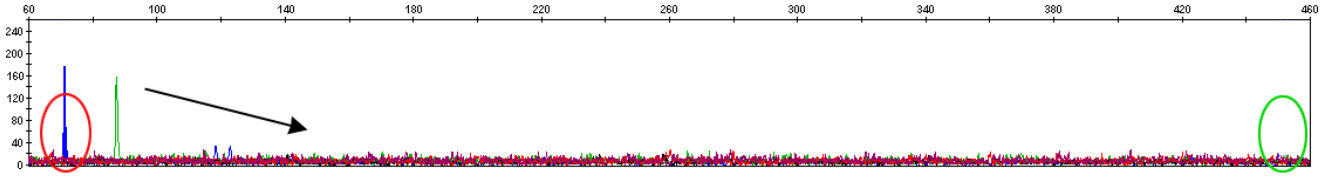


Figure 8 Combined dyes electropherogram for the VeriFiler™ Plus PCR Amplification Kit in the presence of 450 ng/µL of humic acid (updated formulation)

The red circle highlights the IQCS peak; the IQCL peak is absent (green circle). The arrow indicates the ski slope peak pattern observed in the DNA profile. The Y-axis is scaled to 250 RFU to make extremely low peaks visible.

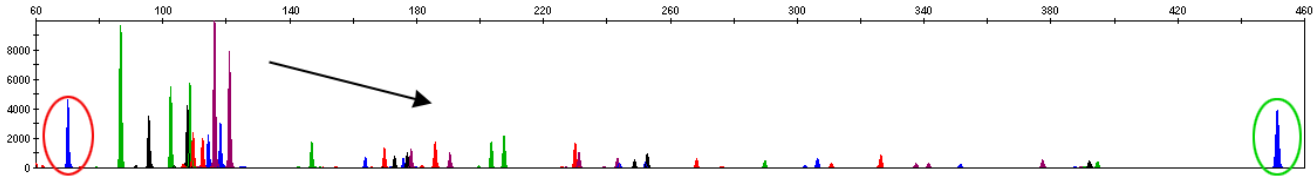


Figure 9 Combined dyes electropherogram from degraded DNA (scaled to 10,000 RFU) (updated formulation)

The red circle highlights the IQCS peak and the green circle highlights the IQCL peak. The arrow indicates the ski slope pattern observed in the DNA profile.

No sample peaks with IQC peaks

There are no DNA profile peaks in Figure 10. However, the presence of both the IQCS and IQCL peaks indicates that normal amplification has occurred in the PCR.

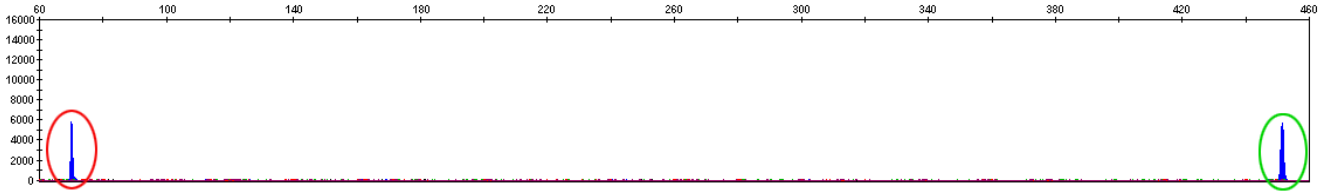


Figure 10 Combined dyes electropherogram that shows IQCS and IQCL peaks with 0 ng of DNA (scaled to 16,000 RFU) (updated formulation)

The red circle highlights the IQCS peak and the green circle highlights the IQCL peak.

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 2016). 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.

Repeated validation experiments using the updated formulation

Minor modifications were made to the VeriFiler™ Plus PCR Amplification Kit primer mix to reduce the incidence of non-specific PCR artifacts that were observed in certain forensic samples with high microbial DNA load. Accordingly, we used the updated formulation to repeat the validation experiments that might be impacted by the primer mix changes, as follows:

- Developmental validation—"PCR components" on page 62
- Extra peaks—"Dye artifact observation" on page 91
- Species Specificity—"Nonhuman studies" on page 95
- Sensitivity—"Sensitivity observation" on page 97
- Stability—"Degraded DNA" on page 98
- Stability—"Effect of inhibitors" on page 99
- Mixture Studies—"Limit of detection of the minor component" on page 104

Because most of the kit components (for example, the master mix) remain unchanged, we did not repeat the remaining validation experiments.

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

Developmental validation studies were performed using the ProFlex™ 96-well PCR System, Veriti™ 96-Well Thermal Cycler, and the GeneAmp™ PCR System 9700 96-Well 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 2016)

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 2016)

SWGDAM guideline 3.9.6

"Criteria for detection of amplified product should be determined based on the platform and/or method. " (SWGDAM, December 2016)

PCR components

We examined the concentration of each component of the kit. The concentration of each component was in the range where data indicated that the amplification met the required performance criteria for specificity, sensitivity, and reproducibility. For example, 0.5 ng of DNA Control 007 was amplified in the presence of varying concentrations of magnesium sulfate, and the results were analyzed on a 3500xL Genetic Analyzer (Figure 11). The performance of the multiplex is most robust within ±20% of the optimal magnesium sulfate concentration.

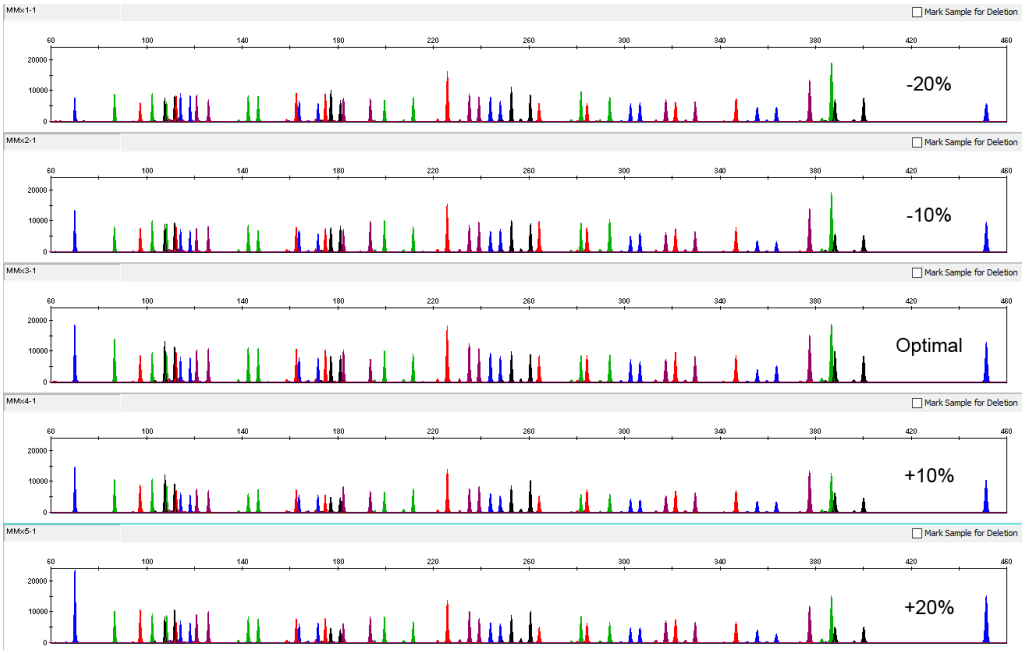


Figure 11 DNA Control 007 (0.5 ng) amplified with the VeriFiler™ Plus kit in the presence of varying concentrations of magnesium sulfate and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0–25,000 RFU) (updated formulation)

Thermal cycling temperatures

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

For example, annealing/extension temperatures of 57, 58, 59, 60, and 61°C were tested using a ProFlex™ PCR System (Figure 12). The PCR products were analyzed using a 3500xL Genetic Analyzer.

Robust profiles were obtained between 57–61°C. 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 recommended.

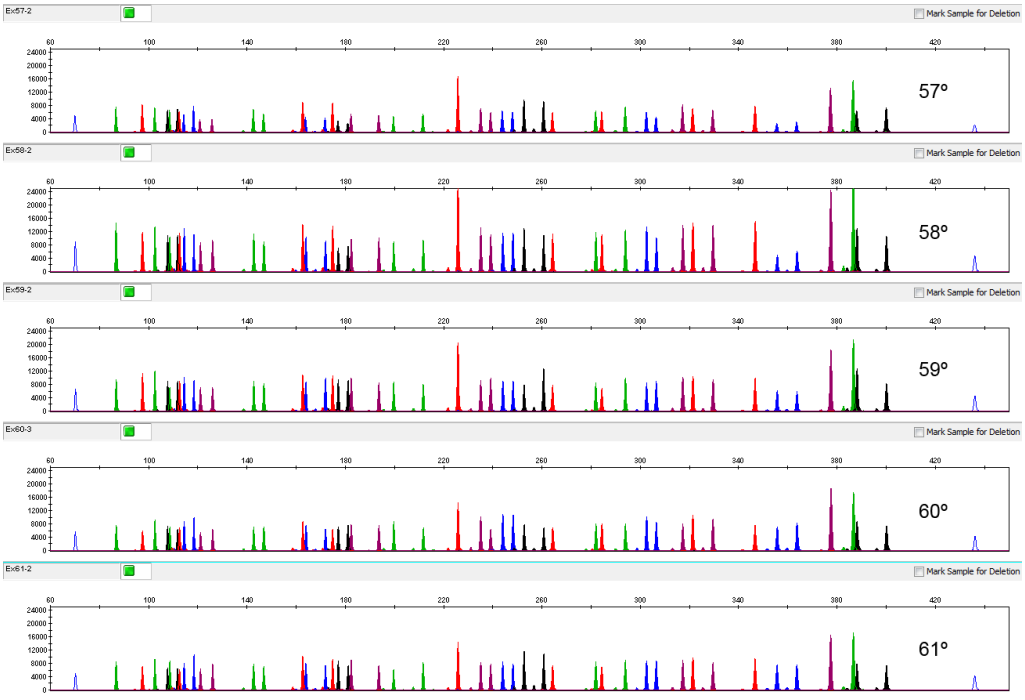


Figure 12 VeriFiler™ Plus kit electropherograms obtained from amplification of 0.5 ng of DNA Control 007 at annealing temperatures of 57, 58, 59, 60, and 61°C, analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0–25,000 RFU)

PCR cycle number

Reactions were amplified for 27, 28, 29, 30, and 31 cycles on the ProFlex™ PCR System using 0.5 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 31 cycles (Figure 13).

Additional experiments were performed not only to optimize for peak heights, peak balance, sensitivity, and so on, but also to minimize the formation of non-target PCR artifacts. The optimized end result is a "touchdown" PCR protocol in which the first two cycles are performed at a slightly higher, more stringent annealing temperature (62°C), followed by 27 cycles with an annealing temperature of 59°C.

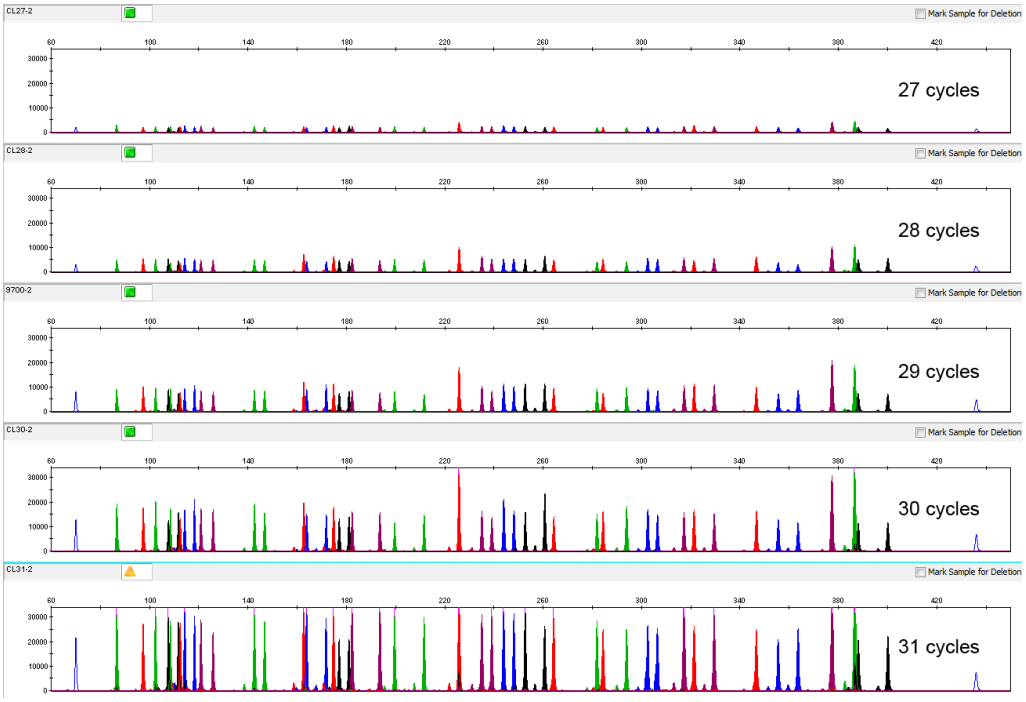


Figure 13 Representative VeriFiler™ Plus kit profiles obtained from amplification of 0.5 ng of DNA Control 007 using 27, 28, 29, 30, and 31 cycles, analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0–35,000 RFU)

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 2016)

Accuracy observation

The accuracy and reproducibility of STR profiles have been determined from various sample types. Important factors include: Sizing accuracy and precision, and the consistency in observed sizing between sample STR alleles and corresponding allelic ladder alleles.

Figure 14, Figure 15, and Figure 16 show the size differences that were observed between sample alleles and allelic ladder alleles on the 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 VeriFiler™ Plus Allelic Ladder. The horizontal 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.

Note: The IQCS and IQCL markers were omitted from this study because they are not used for genotyping.

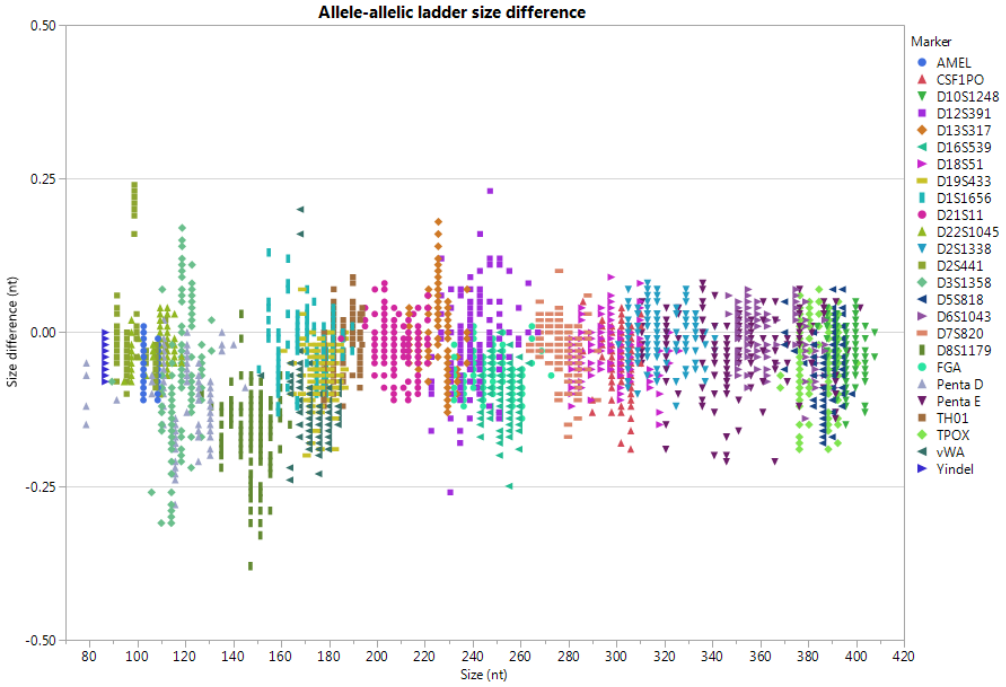


Figure 14 Allele size vs. Allelic Ladder sizing for 84 samples analyzed on a 3130*xl* Genetic Analyzer. Size and ladder sizing for the VeriFiler™ Plus kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.

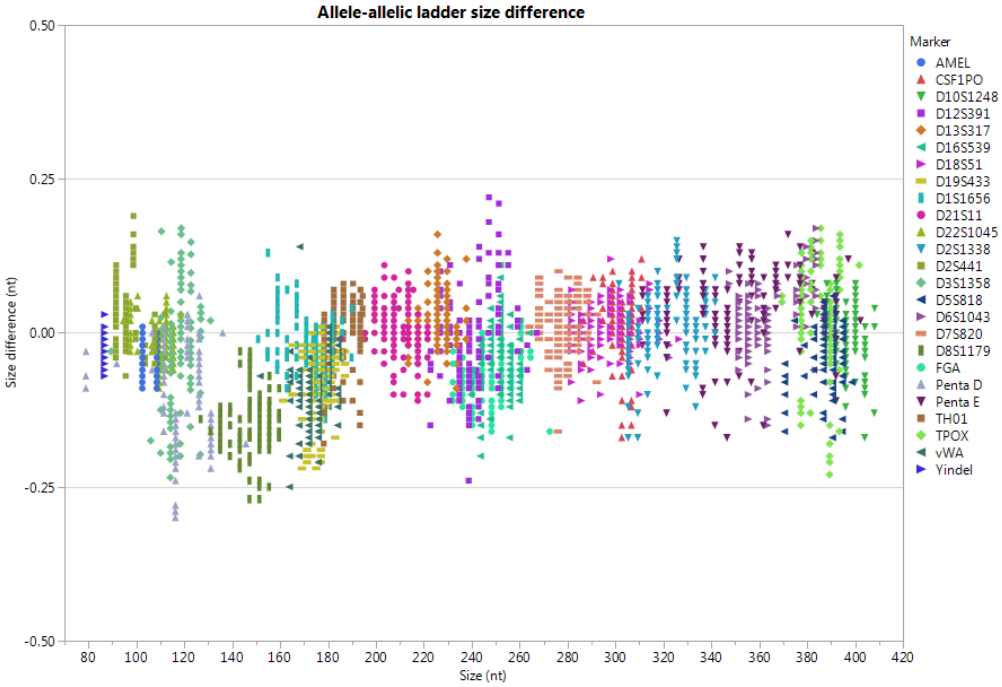


Figure 15 Allele size vs. Allelic Ladder sizing for 84 samples analyzed on a 3500 Genetic Analyzer. Size and ladder sizing for the VeriFiler™ Plus kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.

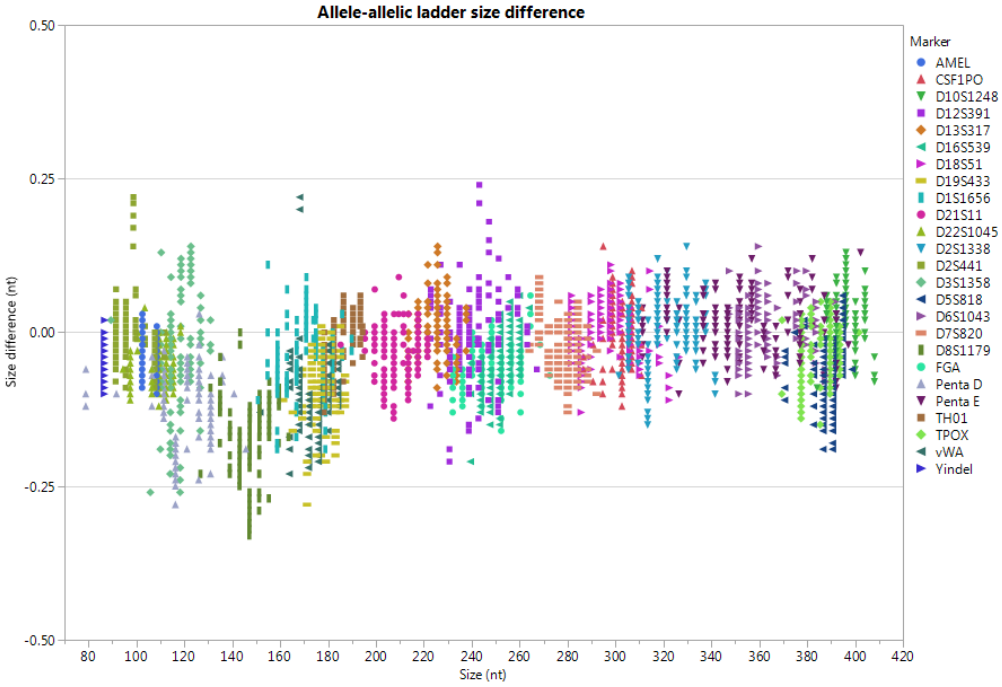


Figure 16 Allele size vs. Allelic Ladder sizing for 84 samples analyzed on a 3500xL Genetic Analyzer. Size and ladder sizing for the VeriFiler™ Plus kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.

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 and size window observation

Table 7 lists typical precision results obtained from multiple runs of the VeriFiler™ Plus 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 is shown in Table 6.

Table 6 Number of repeated injections for each genetic analyzer

Genetic analyzer	Capillaries	Repeated 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 7). 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 65 illustrate the tight clustering of allele sizes obtained on the Applied Biosystems™ genetic analyzers, 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.

The 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 36 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; Mansfield *et al*., 1998).

Note: The IQCS and IQCL markers were omitted from this study because they are not used for genotyping.

Table 7 Precision results of multiple runs of the VeriFiler™ Plus Allelic Ladder

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
D3S1358						
9	89.28–89.33	0.013–0.046	89.48–89.52	0.006–0.039	89.37–89.44	0.010–0.045
10	93.45–93.52	0.012–0.039	93.67–93.70	0.005–0.046	93.55–93.63	0.013–0.039
11	97.62–97.69	0.012–0.042	97.83–97.89	0.006–0.042	97.75–97.79	0.008–0.054
12	101.78–101.86	0.018–0.040	102.00–102.06	0.010–0.040	101.91–101.96	0.010–0.043
13	105.95–105.99	0.014–0.041	106.13–106.20	0.010–0.040	106.04–106.11	0.008–0.034
14	110.07–110.13	0.014–0.043	110.28–110.34	0.013–0.038	110.19–110.26	0.010–0.040
15	114.21–114.26	0.017–0.039	114.41–114.46	0.010–0.039	114.33–114.38	0.005–0.033
16	118.33–118.38	0.016–0.042	118.53–118.58	0.005–0.036	118.45–118.51	0.008–0.043
17	122.45–122.51	0.015–0.050	122.66–122.70	0.006–0.038	122.57–122.63	0.013–0.050
18	126.64–126.70	0.010–0.041	126.85–126.90	0.010–0.044	126.76–126.83	0.005–0.041
19	130.73–130.78	0.016–0.037	130.92–130.97	0.013–0.044	130.84–130.89	0.008–0.044

Table 7 Precision results of multiple runs of the VeriFiler Plus Allelic Ladder *(continued)*

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
20	134.72–134.80	0.008–0.036	134.93–134.98	0.013–0.043	134.83–134.91	0.006–0.044
vWA						
11	151.38–151.45	0.015–0.052	151.76–151.80	0.010–0.045	151.65–151.76	0.019–0.048
12	155.39–155.45	0.021–0.048	155.78–155.83	0.005–0.044	155.69–155.77	0.010–0.049
13	159.46–159.52	0.018–0.049	159.85–159.90	0.010–0.037	159.76–159.83	0.008–0.045
14	163.68–163.74	0.024–0.055	164.07–164.12	0.010–0.041	163.97–164.05	0.010–0.053
15	167.62–167.69	0.026–0.047	168.01–168.06	0.010–0.045	167.93–168.00	0.010–0.050
16	171.66–171.73	0.021–0.049	172.05–172.11	0.010–0.050	171.98–172.06	0.010–0.059
17	175.71–175.77	0.017–0.053	176.10–176.15	0.005–0.046	176.05–176.10	0.013–0.047
18	179.72–179.77	0.014–0.054	180.10–180.18	0.005–0.034	180.03–180.11	0.013–0.060
19	183.76–183.83	0.029–0.054	184.16–184.22	0.008–0.046	184.12–184.17	0.000–0.057
20	187.79–187.87	0.028–0.054	188.20–188.26	0.005–0.056	188.12–188.21	0.015–0.057
21	191.80–191.86	0.023–0.048	192.20–192.26	0.005–0.040	192.13–192.21	0.013–0.067
22	195.83–195.88	0.021–0.055	196.23–196.29	0.006–0.051	196.14–196.24	0.013–0.051
23	199.77–199.84	0.027–0.048	200.20–200.25	0.010–0.046	200.12–200.19	0.010–0.062
24	204.14–204.20	0.027–0.058	204.54–204.60	0.013–0.049	204.48–204.53	0.013–0.057
D16S539						
5	226.98–227.05	0.029–0.055	227.54–227.60	0.008–0.043	227.44–227.50	0.029–0.077
8	239.10–239.18	0.037–0.067	239.67–239.73	0.013–0.043	239.59–239.64	0.034–0.079
9	243.17–243.24	0.029–0.070	243.73–243.79	0.000–0.046	243.60–243.70	0.025–0.090
10	247.21–247.27	0.041–0.078	247.77–247.83	0.013–0.050	247.67–247.75	0.017–0.068
11	251.26–251.33	0.025–0.066	251.84–251.87	0.010–0.045	251.72–251.80	0.026–0.081
12	255.30–255.36	0.043–0.061	255.88–255.93	0.015–0.045	255.76–255.83	0.029–0.075
13	259.33–259.39	0.040–0.074	259.92–259.98	0.008–0.053	259.81–259.88	0.017–0.088
14	263.39–263.45	0.038–0.077	263.96–264.02	0.008–0.057	263.86–263.94	0.021–0.077
15	267.42–267.48	0.037–0.077	268.01–268.07	0.005–0.056	267.89–267.99	0.025–0.094

Table 7 Precision results of multiple runs of the VeriFiler Plus Allelic Ladder *(continued)*

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
CSF1PO						
6	281.95–282.03	0.035–0.077	282.56–282.63	0.010–0.057	282.49–282.58	0.029–0.107
7	285.99–286.07	0.042–0.076	286.58–286.65	0.016–0.045	286.53–286.61	0.024–0.099
8	290.02–290.10	0.047–0.068	290.61–290.69	0.016–0.047	290.57–290.65	0.021–0.090
9	294.04–294.13	0.042–0.071	294.65–294.72	0.008–0.042	294.60–294.69	0.027–0.104
10	298.07–298.15	0.039–0.069	298.68–298.75	0.010–0.050	298.65–298.73	0.028–0.087
11	302.10–302.18	0.033–0.074	302.72–302.79	0.006–0.059	302.66–302.75	0.040–0.081
12	306.12–306.20	0.032–0.075	306.77–306.83	0.013–0.061	306.72–306.79	0.029–0.093
13	310.15–310.23	0.042–0.069	310.78–310.85	0.013–0.054	310.76–310.83	0.024–0.095
14	314.17–314.27	0.045–0.081	314.83–314.89	0.013–0.051	314.78–314.87	0.033–0.091
15	318.19–318.28	0.021–0.072	318.85–318.93	0.010–0.065	318.84–318.91	0.033–0.085
D6S1043						
7	334.73–334.82	0.029–0.074	335.38–335.44	0.006–0.055	335.32–335.38	0.024–0.074
8	338.78–338.87	0.028–0.067	339.43–339.48	0.008–0.055	339.37–339.45	0.028–0.097
9	342.72–342.83	0.023–0.079	343.42–343.48	0.008–0.059	343.32–343.42	0.029–0.087
10	346.78–346.87	0.027–0.071	347.46–347.52	0.008–0.054	347.40–347.48	0.021–0.083
11	350.84–350.93	0.035–0.072	351.52–351.57	0.000–0.049	351.46–351.54	0.025–0.079
12	354.84–354.94	0.047–0.069	355.53–355.61	0.006–0.062	355.47–355.56	0.029–0.076
13	358.88–358.97	0.049–0.085	359.57–359.62	0.008–0.059	359.47–359.57	0.013–0.081
14	362.92–363.01	0.041–0.083	363.61–363.66	0.014–0.060	363.53–363.61	0.013–0.076
15	366.91–367.02	0.037–0.069	367.63–367.69	0.013–0.067	367.55–367.63	0.021–0.085
16	370.90–370.99	0.042–0.065	371.60–371.65	0.010–0.057	371.51–371.60	0.037–0.083
17	374.91–374.99	0.039–0.062	375.63–375.69	0.013–0.060	375.55–375.63	0.019–0.096
18	378.89–378.98	0.026–0.066	379.63–379.70	0.010–0.061	379.55–379.63	0.008–0.089
19	382.92–382.99	0.030–0.088	383.67–383.73	0.008–0.051	383.58–383.67	0.018–0.074
20	386.92–386.99	0.028–0.075	387.69–387.76	0.010–0.061	387.60–387.69	0.026–0.098
21	390.90–390.99	0.029–0.082	391.71–391.76	0.006–0.050	391.62–391.69	0.034–0.091

Table 7 Precision results of multiple runs of the VeriFiler Plus Allelic Ladder *(continued)*

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
22	394.88–394.95	0.021–0.070	395.70–395.76	0.015–0.066	395.57–395.68	0.024–0.081
23	398.85–398.93	0.024–0.082	399.68–399.74	0.008–0.051	399.57–399.68	0.029–0.081
24	402.82–402.91	0.034–0.088	403.70–403.74	0.010–0.057	403.60–403.67	0.027–0.098
25	406.80–406.89	0.035–0.082	407.68–407.75	0.013–0.053	407.57–407.68	0.030–0.085
Y Indel						
1	81.20–81.26	0.019–0.047	81.43–81.47	0.005–0.051	81.33–81.38	0.008–0.036
2	86.50–86.56	0.023–0.041	86.73–86.78	0.008–0.045	86.62–86.70	0.010–0.042
AMEL						
X	102.45–102.50	0.014–0.044	102.53–102.57	0.008–0.034	102.44–102.50	0.013–0.041
Y	108.41–108.46	0.016–0.047	108.52–108.60	0.013–0.032	108.47–108.52	0.008–0.042
D8S1179						
5	114.53–114.58	0.019–0.035	114.64–114.68	0.008–0.038	114.57–114.62	0.005–0.054
6	118.63–118.69	0.014–0.052	118.74–118.79	0.005–0.039	118.67–118.74	0.006–0.044
7	122.74–122.79	0.009–0.040	122.84–122.89	0.006–0.046	122.75–122.83	0.010–0.047
8	126.84–126.89	0.009–0.039	126.94–126.99	0.010–0.047	126.88–126.93	0.005–0.045
9	130.93–131.00	0.008–0.045	131.03–131.08	0.010–0.040	130.97–131.05	0.010–0.042
10	135.02–135.08	0.014–0.041	135.12–135.16	0.010–0.047	135.04–135.11	0.005–0.061
11	139.11–139.16	0.012–0.047	139.19–139.24	0.008–0.058	139.13–139.18	0.000–0.048
12	143.20–143.27	0.015–0.039	143.29–143.34	0.013–0.040	143.23–143.29	0.013–0.047
13	147.39–147.44	0.012–0.035	147.47–147.51	0.010–0.039	147.40–147.47	0.008–0.052
14	151.47–151.53	0.016–0.046	151.52–151.58	0.006–0.039	151.48–151.54	0.010–0.054
15	155.54–155.59	0.010–0.037	155.58–155.64	0.005–0.039	155.54–155.60	0.010–0.048
16	159.61–159.65	0.016–0.032	159.65–159.71	0.010–0.048	159.58–159.65	0.010–0.046
17	163.66–163.72	0.010–0.032	163.70–163.75	0.010–0.041	163.66–163.71	0.006–0.059
18	167.73–167.78	0.015–0.035	167.76–167.82	0.013–0.047	167.72–167.76	0.010–0.045
19	171.79–171.86	0.008–0.027	171.82–171.86	0.010–0.044	171.77–171.83	0.010–0.051

Table 7 Precision results of multiple runs of the VeriFiler Plus Allelic Ladder *(continued)*

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
D21S11						
24	182.85–182.91	0.033–0.079	183.23–183.28	0.006–0.049	183.17–183.25	0.005–0.070
24	184.88–184.94	0.028–0.061	185.26–185.31	0.008–0.056	185.23–185.28	0.013–0.071
25	186.88–186.95	0.037–0.068	187.28–187.32	0.005–0.056	187.23–187.28	0.019–0.062
26	190.94–190.98	0.034–0.076	191.32–191.37	0.008–0.044	191.27–191.33	0.017–0.072
27	194.98–195.04	0.042–0.065	195.38–195.42	0.008–0.047	195.31–195.40	0.014–0.077
28	198.99–199.05	0.035–0.077	199.37–199.43	0.010–0.044	199.34–199.40	0.013–0.067
28	200.99–201.03	0.039–0.061	201.38–201.43	0.010–0.045	201.34–201.39	0.008–0.074
29	203.00–203.05	0.045–0.077	203.39–203.44	0.008–0.052	203.37–203.42	0.005–0.067
29	205.06–205.10	0.042–0.079	205.44–205.50	0.015–0.047	205.40–205.47	0.021–0.078
30	207.05–207.10	0.039–0.074	207.45–207.51	0.013–0.045	207.39–207.47	0.017–0.082
30	209.05–209.11	0.042–0.072	209.45–209.49	0.010–0.043	209.39–209.47	0.021–0.067
31	211.08–211.14	0.032–0.067	211.49–211.55	0.008–0.045	211.43–211.51	0.010–0.072
31	213.08–213.15	0.039–0.073	213.49–213.52	0.010–0.043	213.44–213.50	0.014–0.081
32	215.12–215.17	0.042–0.074	215.52–215.57	0.005–0.040	215.47–215.54	0.010–0.069
32	217.10–217.16	0.050–0.069	217.52–217.56	0.005–0.048	217.44–217.54	0.010–0.069
33	219.14–219.22	0.048–0.075	219.55–219.61	0.006–0.039	219.52–219.59	0.017–0.080
33	221.10–221.16	0.044–0.071	221.50–221.57	0.010–0.050	221.48–221.54	0.020–0.085
34	223.23–223.29	0.035–0.078	223.65–223.69	0.013–0.040	223.59–223.66	0.022–0.075
34	225.17–225.23	0.043–0.074	225.58–225.64	0.010–0.042	225.54–225.61	0.021–0.090
35	227.26–227.30	0.030–0.080	227.66–227.71	0.005–0.046	227.62–227.68	0.024–0.077
35	229.20–229.25	0.042–0.073	229.61–229.67	0.005–0.048	229.57–229.64	0.022–0.076
36	231.20–231.26	0.037–0.082	231.63–231.69	0.010–0.051	231.58–231.65	0.017–0.075
37	235.28–235.34	0.042–0.076	235.71–235.75	0.006–0.051	235.65–235.73	0.030–0.070
38	239.25–239.32	0.040–0.082	239.67–239.75	0.008–0.045	239.62–239.71	0.028–0.072
D18S51						
7	261.02–261.08	0.037–0.075	261.35–261.42	0.005–0.044	261.32–261.38	0.037–0.064

Table 7 Precision results of multiple runs of the VeriFiler Plus Allelic Ladder *(continued)*

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
9	269.17–269.23	0.028–0.068	269.49–269.56	0.010–0.051	269.44–269.51	0.010–0.068
10	273.24–273.30	0.034–0.062	273.56–273.62	0.013–0.045	273.52–273.59	0.013–0.071
10	275.24–275.30	0.034–0.079	275.56–275.61	0.010–0.041	275.51–275.60	0.024–0.060
11	277.31–277.37	0.025–0.063	277.63–277.69	0.010–0.046	277.58–277.65	0.015–0.062
12	281.38–281.45	0.031–0.073	281.70–281.75	0.008–0.056	281.64–281.72	0.015–0.078
13	285.46–285.53	0.037–0.069	285.77–285.81	0.010–0.051	285.69–285.79	0.016–0.063
13	287.48–287.54	0.027–0.075	287.77–287.82	0.017–0.051	287.71–287.79	0.010–0.062
14	289.53–289.60	0.034–0.058	289.82–289.89	0.013–0.044	289.80–289.87	0.013–0.068
14	291.56–291.61	0.032–0.069	291.82–291.89	0.013–0.051	291.79–291.85	0.005–0.070
15	293.62–293.68	0.020–0.057	293.91–293.96	0.008–0.046	293.85–293.93	0.010–0.064
16	297.70–297.77	0.035–0.064	297.97–298.03	0.010–0.059	297.92–298.00	0.010–0.059
17	301.76–301.83	0.029–0.071	302.03–302.09	0.017–0.051	302.01–302.08	0.019–0.062
18	305.85–305.90	0.025–0.078	306.11–306.18	0.010–0.055	306.08–306.14	0.021–0.053
19	309.94–309.98	0.029–0.061	310.16–310.22	0.016–0.051	310.15–310.21	0.008–0.067
20	314.00–314.06	0.021–0.054	314.25–314.29	0.013–0.045	314.21–314.28	0.010–0.059
21	318.10–318.13	0.021–0.057	318.31–318.37	0.010–0.043	318.26–318.37	0.008–0.054
22	322.22–322.28	0.015–0.055	322.44–322.50	0.005–0.053	322.42–322.49	0.010–0.062
23	326.22–326.28	0.025–0.064	326.43–326.51	0.013–0.057	326.42–326.49	0.015–0.076
24	330.27–330.34	0.022–0.067	330.51–330.56	0.010–0.041	330.49–330.57	0.013–0.073
25	334.34–334.41	0.018–0.062	334.56–334.63	0.008–0.056	334.56–334.63	0.010–0.059
26	338.44–338.49	0.022–0.055	338.63–338.69	0.005–0.050	338.63–338.72	0.010–0.055
27	342.48–342.56	0.024–0.068	342.71–342.76	0.006–0.052	342.72–342.79	0.014–0.059
D5S818						
7	370.14–370.21	0.037–0.079	370.66–370.71	0.015–0.068	370.60–370.70	0.006–0.077
8	374.15–374.21	0.029–0.059	374.68–374.75	0.010–0.064	374.65–374.73	0.013–0.079
9	378.14–378.20	0.019–0.063	378.71–378.76	0.010–0.074	378.67–378.75	0.018–0.076
10	382.14–382.20	0.016–0.068	382.71–382.79	0.010–0.065	382.69–382.77	0.013–0.076

Table 7 Precision results of multiple runs of the VeriFiler Plus Allelic Ladder *(continued)*

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
11	386.13–386.20	0.024–0.072	386.74–386.80	0.008–0.057	386.71–386.78	0.024–0.090
12	390.12–390.19	0.037–0.072	390.75–390.82	0.013–0.061	390.73–390.80	0.021–0.082
13	394.10–394.18	0.038–0.102	394.76–394.83	0.015–0.067	394.72–394.82	0.032–0.087
14	398.10–398.16	0.032–0.071	398.77–398.82	0.010–0.068	398.74–398.82	0.031–0.091
15	402.08–402.14	0.026–0.073	402.77–402.84	0.012–0.058	402.73–402.81	0.017–0.095
16	406.06–406.12	0.032–0.110	406.78–406.85	0.013–0.075	406.73–406.81	0.017–0.085
17	410.04–410.10	0.044–0.114	410.78–410.85	0.008–0.079	410.72–410.82	0.021–0.100
18	414.00–414.08	0.043–0.103	414.77–414.85	0.017–0.057	414.73–414.80	0.037–0.106
D2S441						
8	83.24–83.27	0.008–0.034	83.25–83.30	0.005–0.050	83.19–83.25	0.005–0.036
9	87.34–87.37	0.012–0.031	87.37–87.42	0.006–0.038	87.31–87.40	0.008–0.036
10	91.40–91.42	0.010–0.034	91.45–91.50	0.005–0.041	91.40–91.45	0.008–0.035
11	95.52–95.56	0.014–0.046	95.60–95.65	0.006–0.046	95.56–95.61	0.013–0.038
11	98.49–98.53	0.015–0.032	98.57–98.63	0.008–0.039	98.54–98.58	0.008–0.048
12	99.59–99.64	0.015–0.037	99.69–99.73	0.008–0.041	99.65–99.70	0.008–0.035
13	103.52–103.55	0.018–0.036	103.62–103.68	0.013–0.036	103.58–103.64	0.013–0.045
14	107.59–107.63	0.015–0.049	107.71–107.76	0.014–0.038	107.67–107.71	0.008–0.046
15	111.64–111.69	0.015–0.045	111.78–111.82	0.014–0.045	111.75–111.80	0.006–0.039
16	115.73–115.78	0.010–0.033	115.85–115.90	0.010–0.034	115.81–115.89	0.010–0.044
17	119.88–119.93	0.014–0.033	120.02–120.07	0.013–0.045	120.01–120.05	0.013–0.039
D19S433						
5	141.04–141.09	0.016–0.043	141.42–141.46	0.006–0.050	141.37–141.44	0.021–0.059
6	145.02–145.07	0.019–0.059	145.41–145.45	0.008–0.038	145.39–145.45	0.013–0.071
7	148.99–149.05	0.015–0.048	149.39–149.44	0.014–0.051	149.38–149.44	0.010–0.063
8	152.95–153.02	0.014–0.056	153.38–153.42	0.010–0.046	153.33–153.42	0.018–0.069
9	156.91–156.98	0.026–0.055	157.35–157.41	0.008–0.042	157.35–157.40	0.014–0.073
10	160.86–160.94	0.029–0.051	161.33–161.38	0.010–0.041	161.32–161.38	0.013–0.065

Table 7 Precision results of multiple runs of the VeriFiler Plus Allelic Ladder *(continued)*

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
11	164.82–164.89	0.017–0.048	165.30–165.36	0.010–0.052	165.28–165.36	0.017–0.070
12	168.78–168.85	0.029–0.054	169.26–169.32	0.008–0.052	169.24–169.33	0.024–0.084
12	170.89–170.97	0.032–0.059	171.38–171.44	0.006–0.046	171.37–171.45	0.005–0.076
13	172.74–172.81	0.018–0.060	173.24–173.29	0.005–0.048	173.22–173.30	0.027–0.080
13	174.72–174.80	0.025–0.062	175.22–175.28	0.010–0.045	175.21–175.30	0.027–0.079
14	176.68–176.76	0.030–0.065	177.20–177.26	0.008–0.054	177.16–177.27	0.028–0.082
14	178.65–178.74	0.026–0.069	179.17–179.25	0.005–0.037	179.16–179.27	0.018–0.088
15	180.62–180.70	0.029–0.070	181.14–181.21	0.005–0.039	181.11–181.23	0.024–0.098
15	182.63–182.69	0.028–0.064	183.15–183.22	0.006–0.046	183.15–183.22	0.028–0.088
16	184.56–184.66	0.034–0.060	185.10–185.17	0.006–0.046	185.07–185.19	0.031–0.077
16	186.57–186.66	0.028–0.063	187.11–187.17	0.005–0.043	187.13–187.20	0.029–0.083
17	188.49–188.59	0.028–0.070	189.06–189.13	0.005–0.053	189.05–189.15	0.032–0.087
17	190.53–190.62	0.031–0.064	191.09–191.15	0.005–0.062	191.07–191.16	0.030–0.096
18	194.56–194.64	0.032–0.060	195.12–195.19	0.008–0.032	195.12–195.20	0.037–0.077
19	198.49–198.58	0.031–0.062	199.08–199.14	0.010–0.051	199.08–199.17	0.028–0.105
FGA						
13	207.89–207.92	0.012–0.039	207.88–207.93	0.008–0.052	207.83–207.89	0.010–0.042
14	211.94–211.97	0.013–0.030	211.93–211.97	0.010–0.051	211.88–211.94	0.013–0.055
15	215.99–216.02	0.011–0.035	215.96–216.02	0.008–0.051	215.93–215.97	0.005–0.049
16	220.05–220.09	0.016–0.032	220.01–220.07	0.008–0.047	219.97–220.04	0.010–0.046
17	224.11–224.15	0.015–0.039	224.05–224.11	0.010–0.043	224.01–224.08	0.010–0.040
18	228.16–228.20	0.013–0.031	228.11–228.17	0.006–0.053	228.07–228.12	0.005–0.049
19	232.22–232.27	0.013–0.033	232.15–232.20	0.010–0.042	232.12–232.17	0.010–0.048
20	236.27–236.33	0.010–0.041	236.20–236.25	0.010–0.046	236.17–236.22	0.005–0.043
21	240.33–240.38	0.008–0.052	240.25–240.30	0.010–0.045	240.21–240.27	0.006–0.039
22	244.39–244.44	0.012–0.034	244.30–244.35	0.000–0.045	244.25–244.33	0.012–0.041
23	248.46–248.50	0.012–0.044	248.34–248.40	0.013–0.038	248.31–248.36	0.008–0.050

Table 7 Precision results of multiple runs of the VeriFiler Plus Allelic Ladder *(continued)*

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
24	252.51–252.56	0.014–0.040	252.39–252.45	0.010–0.046	252.38–252.43	0.010–0.044
25	256.58–256.62	0.016–0.033	256.46–256.51	0.014–0.044	256.41–256.49	0.010–0.042
26	260.63–260.68	0.014–0.046	260.48–260.54	0.005–0.052	260.46–260.52	0.010–0.050
26	262.66–262.71	0.014–0.063	262.53–262.59	0.010–0.050	262.49–262.55	0.005–0.041
27	264.66–264.70	0.012–0.040	264.52–264.56	0.010–0.041	264.48–264.53	0.010–0.048
28	268.72–268.76	0.010–0.046	268.58–268.61	0.010–0.048	268.53–268.59	0.008–0.045
29	272.78–272.83	0.017–0.063	272.63–272.69	0.013–0.038	272.61–272.66	0.005–0.049
30	276.86–276.92	0.011–0.048	276.71–276.78	0.008–0.045	276.68–276.73	0.006–0.045
30	278.69–278.75	0.012–0.044	278.55–278.60	0.010–0.053	278.51–278.58	0.006–0.048
31	282.76–282.81	0.012–0.034	282.59–282.67	0.008–0.045	282.55–282.63	0.008–0.048
32	286.83–286.88	0.018–0.038	286.68–286.73	0.010–0.042	286.62–286.69	0.013–0.051
33	290.89–290.94	0.014–0.036	290.71–290.77	0.013–0.041	290.67–290.75	0.008–0.049
42	327.62–327.67	0.014–0.050	327.39–327.43	0.010–0.053	327.38–327.46	0.008–0.057
43	331.67–331.73	0.006–0.061	331.44–331.49	0.010–0.049	331.42–331.51	0.015–0.063
44	335.72–335.79	0.015–0.046	335.50–335.57	0.010–0.046	335.53–335.59	0.013–0.062
45	339.80–339.86	0.019–0.048	339.57–339.63	0.000–0.050	339.59–339.65	0.010–0.068
46	343.77–343.84	0.013–0.054	343.52–343.59	0.005–0.057	343.54–343.61	0.005–0.077
47	347.84–347.90	0.013–0.047	347.57–347.64	0.013–0.041	347.59–347.67	0.013–0.067
48	351.92–351.98	0.012–0.043	351.66–351.72	0.010–0.048	351.67–351.75	0.006–0.076
49	355.98–356.04	0.014–0.044	355.71–355.77	0.000–0.045	355.74–355.80	0.012–0.060
50	360.03–360.07	0.018–0.054	359.73–359.80	0.013–0.050	359.75–359.82	0.017–0.079
51	364.08–364.14	0.018–0.078	363.80–363.85	0.014–0.056	363.82–363.88	0.017–0.073
D10S1248						
8	371.77–371.82	0.023–0.063	372.17–372.23	0.010–0.057	372.12–372.21	0.010–0.071
9	375.73–375.80	0.023–0.061	376.16–376.23	0.010–0.059	376.13–376.20	0.017–0.069
10	379.73–379.79	0.031–0.058	380.16–380.23	0.010–0.047	380.14–380.19	0.016–0.056
11	383.68–383.76	0.024–0.060	384.12–384.19	0.008–0.054	384.09–384.16	0.010–0.068

Table 7 Precision results of multiple runs of the VeriFiler Plus Allelic Ladder *(continued)*

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
12	387.70–387.76	0.020–0.056	388.15–388.22	0.012–0.054	388.13–388.18	0.021–0.058
13	391.69–391.75	0.019–0.163	392.14–392.20	0.013–0.062	392.11–392.17	0.010–0.072
14	395.65–395.72	0.028–0.088	396.10–396.17	0.013–0.054	396.07–396.15	0.008–0.076
15	399.64–399.72	0.028–0.128	400.11–400.17	0.013–0.054	400.07–400.14	0.017–0.074
16	403.61–403.67	0.024–0.062	404.10–404.19	0.013–0.058	404.07–404.14	0.017–0.077
17	407.63–407.68	0.025–0.081	408.13–408.18	0.005–0.057	408.08–408.14	0.017–0.085
18	411.56–411.60	0.019–0.115	412.04–412.12	0.008–0.052	412.00–412.09	0.014–0.067
19	415.49–415.57	0.021–0.102	416.02–416.10	0.015–0.056	416.00–416.05	0.017–0.080
D22S1045						
8	88.43–88.47	0.012–0.037	88.46–88.53	0.005–0.039	88.40–88.45	0.005–0.052
9	91.42–91.46	0.013–0.037	91.46–91.54	0.005–0.042	91.41–91.46	0.010–0.042
10	94.42–94.46	0.010–0.030	94.50–94.54	0.005–0.042	94.43–94.47	0.012–0.039
11	97.43–97.46	0.013–0.038	97.49–97.56	0.008–0.045	97.43–97.49	0.005–0.054
12	100.42–100.45	0.010–0.042	100.51–100.56	0.006–0.043	100.45–100.50	0.005–0.036
13	103.41–103.46	0.012–0.038	103.50–103.57	0.008–0.040	103.44–103.49	0.013–0.041
14	106.40–106.44	0.008–0.039	106.51–106.55	0.015–0.045	106.43–106.48	0.014–0.041
15	109.39–109.44	0.013–0.044	109.51–109.57	0.013–0.039	109.44–109.50	0.010–0.043
16	112.38–112.42	0.017–0.038	112.50–112.54	0.010–0.039	112.43–112.49	0.000–0.037
17	115.37–115.40	0.008–0.028	115.49–115.56	0.010–0.044	115.41–115.49	0.013–0.040
18	118.35–118.41	0.012–0.032	118.50–118.55	0.013–0.053	118.41–118.48	0.008–0.041
19	121.34–121.40	0.010–0.040	121.48–121.53	0.005–0.042	121.41–121.46	0.006–0.042
D1S1656						
9	146.83–146.86	0.011–0.039	146.87–146.93	0.010–0.043	146.86–146.91	0.005–0.041
10	150.72–150.76	0.015–0.034	150.77–150.83	0.010–0.039	150.78–150.81	0.017–0.043
11	154.72–154.77	0.010–0.038	154.78–154.83	0.008–0.044	154.77–154.84	0.014–0.050
12	158.81–158.85	0.014–0.031	158.86–158.92	0.010–0.042	158.88–158.91	0.013–0.048
13	162.74–162.76	0.017–0.034	162.80–162.85	0.014–0.037	162.80–162.85	0.010–0.043

Table 7 Precision results of multiple runs of the VeriFiler Plus Allelic Ladder *(continued)*

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
14	166.81–166.85	0.012–0.038	166.89–166.93	0.013–0.048	166.87–166.94	0.010–0.051
14	169.77–169.80	0.010–0.034	169.85–169.89	0.008–0.043	169.85–169.89	0.010–0.047
15	170.79–170.83	0.010–0.047	170.87–170.92	0.006–0.036	170.86–170.93	0.000–0.051
15	173.75–173.79	0.014–0.035	173.84–173.90	0.005–0.042	173.83–173.89	0.006–0.043
16	174.80–174.85	0.014–0.035	174.88–174.93	0.000–0.036	174.88–174.94	0.008–0.053
16	177.75–177.80	0.016–0.040	177.83–177.89	0.008–0.044	177.84–177.89	0.010–0.048
17	178.88–178.92	0.012–0.064	178.95–179.00	0.008–0.040	178.95–179.00	0.013–0.053
17	181.75–181.79	0.009–0.043	181.84–181.89	0.005–0.039	181.82–181.89	0.014–0.044
18	182.78–182.82	0.010–0.034	182.87–182.92	0.005–0.050	182.85–182.92	0.005–0.051
18	185.75–185.78	0.012–0.042	185.84–185.89	0.005–0.041	185.85–185.90	0.010–0.040
19	189.77–189.80	0.017–0.036	189.88–189.93	0.006–0.043	189.87–189.93	0.006–0.052
20	193.82–193.86	0.014–0.035	193.93–193.98	0.005–0.033	193.92–193.98	0.008–0.059
D13S317						
5	201.31–201.35	0.020–0.036	201.46–201.52	0.010–0.047	201.45–201.50	0.013–0.053
6	205.33–205.37	0.015–0.031	205.47–205.52	0.015–0.038	205.46–205.51	0.005–0.045
7	209.35–209.39	0.011–0.039	209.49–209.54	0.010–0.050	209.48–209.53	0.017–0.056
8	213.37–213.42	0.015–0.033	213.49–213.55	0.005–0.039	213.50–213.55	0.014–0.049
9	217.42–217.46	0.014–0.036	217.53–217.58	0.005–0.039	217.50–217.56	0.005–0.048
10	221.43–221.48	0.019–0.036	221.55–221.60	0.010–0.054	221.53–221.60	0.006–0.045
11	225.48–225.50	0.018–0.038	225.58–225.63	0.010–0.043	225.57–225.62	0.017–0.048
12	229.49–229.54	0.014–0.039	229.60–229.66	0.008–0.049	229.59–229.64	0.010–0.043
13	233.54–233.58	0.012–0.044	233.62–233.68	0.008–0.043	233.63–233.66	0.015–0.051
14	237.55–237.61	0.012–0.034	237.66–237.72	0.005–0.044	237.64–237.69	0.006–0.054
15	241.59–241.64	0.005–0.041	241.69–241.74	0.006–0.048	241.67–241.72	0.010–0.053
16	245.64–245.70	0.015–0.043	245.72–245.78	0.008–0.042	245.71–245.76	0.015–0.066
D7S820						
6	259.89–259.94	0.010–0.048	260.03–260.07	0.008–0.049	259.99–260.05	0.008–0.046

Table 7 Precision results of multiple runs of the VeriFiler Plus Allelic Ladder *(continued)*

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
7	263.90–263.96	0.008–0.045	264.06–264.11	0.013–0.042	264.00–264.07	0.014–0.049
8	267.92–267.97	0.018–0.044	268.08–268.13	0.010–0.048	268.05–268.09	0.010–0.056
9	271.95–271.99	0.009–0.045	272.10–272.15	0.017–0.045	272.07–272.12	0.010–0.050
10	275.96–276.01	0.017–0.042	276.13–276.19	0.010–0.047	276.10–276.16	0.010–0.061
11	279.97–280.02	0.012–0.057	280.16–280.22	0.010–0.047	280.10–280.19	0.014–0.052
12	284.00–284.04	0.017–0.042	284.20–284.25	0.012–0.039	284.14–284.21	0.012–0.056
13	288.02–288.08	0.012–0.040	288.21–288.28	0.014–0.042	288.18–288.24	0.008–0.061
14	292.05–292.09	0.013–0.045	292.24–292.30	0.013–0.048	292.21–292.27	0.010–0.062
15	296.07–296.11	0.017–0.040	296.26–296.33	0.010–0.052	296.24–296.28	0.010–0.053
Penta E						
5	310.56–310.66	0.075–0.110	311.23–311.28	0.010–0.059	311.20–311.26	0.044–0.096
6	315.69–315.78	0.068–0.113	316.34–316.39	0.014–0.055	316.30–316.38	0.014–0.097
7	320.72–320.82	0.073–0.121	321.41–321.47	0.005–0.051	321.39–321.46	0.028–0.099
8	325.78–325.88	0.060–0.108	326.44–326.50	0.010–0.057	326.42–326.48	0.029–0.096
9	330.82–330.91	0.060–0.109	331.48–331.54	0.008–0.052	331.46–331.54	0.025–0.090
10	335.88–335.97	0.071–0.103	336.53–336.59	0.010–0.058	336.53–336.60	0.040–0.097
11	340.87–341.01	0.064–0.117	341.58–341.66	0.005–0.056	341.59–341.65	0.040–0.094
12	345.96–346.05	0.078–0.118	346.63–346.69	0.013–0.070	346.64–346.71	0.036–0.081
13	351.02–351.12	0.065–0.120	351.69–351.75	0.005–0.055	351.70–351.78	0.017–0.091
14	356.06–356.18	0.075–0.123	356.74–356.80	0.018–0.066	356.74–356.83	0.030–0.090
15	361.13–361.23	0.093–0.137	361.80–361.86	0.013–0.055	361.78–361.87	0.033–0.090
16	366.21–366.29	0.073–0.122	366.83–366.91	0.013–0.067	366.82–366.91	0.019–0.082
17	371.24–371.35	0.069–0.117	371.90–371.98	0.013–0.070	371.87–371.96	0.021–0.080
18	376.29–376.36	0.066–0.116	376.96–377.02	0.019–0.068	376.93–377.00	0.030–0.083
19	381.31–381.41	0.070–0.104	382.00–382.07	0.014–0.059	381.98–382.03	0.012–0.083
20	386.35–386.43	0.069–0.108	387.04–387.09	0.010–0.070	387.01–387.10	0.031–0.109
21	391.38–391.46	0.068–0.106	392.07–392.15	0.008–0.056	392.05–392.14	0.005–0.087

Table 7 Precision results of multiple runs of the VeriFiler Plus Allelic Ladder *(continued)*

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
22	396.39–396.48	0.064–0.108	397.11–397.17	0.012–0.067	397.07–397.16	0.017–0.095
23	401.41–401.51	0.071–0.111	402.15–402.21	0.010–0.054	402.11–402.21	0.030–0.101
24	406.40–406.52	0.077–0.120	407.17–407.23	0.005–0.052	407.14–407.22	0.013–0.092
25	411.44–411.54	0.081–0.109	412.20–412.25	0.008–0.051	412.16–412.24	0.029–0.083
26	416.45–416.52	0.076–0.113	417.22–417.27	0.017–0.067	417.21–417.28	0.026–0.095
Penta D						
2	78.64–78.68	0.008–0.055	78.99–79.05	0.010–0.048	78.87–78.95	0.010–0.061
3	83.57–83.62	0.013–0.057	83.98–84.05	0.005–0.049	83.86–83.94	0.017–0.063
5	91.30–91.35	0.023–0.053	91.78–91.86	0.010–0.037	91.65–91.74	0.018–0.062
6	96.21–96.27	0.023–0.067	96.71–96.79	0.010–0.041	96.62–96.69	0.022–0.064
7	101.32–101.37	0.023–0.060	101.85–101.92	0.008–0.035	101.72–101.81	0.013–0.059
8	105.99–106.05	0.028–0.060	106.60–106.65	0.012–0.044	106.47–106.57	0.022–0.077
9	110.88–110.96	0.028–0.063	111.52–111.57	0.010–0.033	111.38–111.48	0.025–0.071
10	115.91–115.96	0.025–0.060	116.55–116.60	0.005–0.041	116.44–116.51	0.021–0.073
11	120.62–120.69	0.038–0.075	121.30–121.38	0.008–0.041	121.17–121.28	0.029–0.076
12	125.49–125.57	0.035–0.073	126.21–126.27	0.017–0.042	126.07–126.19	0.030–0.077
13	130.45–130.53	0.031–0.078	131.21–131.28	0.010–0.038	131.06–131.18	0.031–0.081
14	135.19–135.27	0.033–0.077	135.95–136.04	0.008–0.049	135.82–135.94	0.029–0.094
15	140.05–140.13	0.038–0.073	140.85–140.93	0.010–0.043	140.73–140.85	0.039–0.092
16	145.02–145.10	0.046–0.077	145.85–145.90	0.008–0.042	145.70–145.81	0.029–0.098
17	149.75–149.84	0.048–0.081	150.61–150.67	0.010–0.061	150.44–150.59	0.043–0.102
TH01						
4	169.86–169.92	0.029–0.060	170.35–170.40	0.005–0.045	170.30–170.38	0.017–0.069
5	173.87–173.94	0.018–0.055	174.34–174.42	0.000–0.047	174.30–174.39	0.013–0.068
6	177.86–177.93	0.023–0.060	178.36–178.41	0.008–0.048	178.31–178.38	0.017–0.076
7	181.86–181.94	0.030–0.053	182.36–182.40	0.005–0.036	182.32–182.40	0.021–0.075
8	185.86–185.94	0.022–0.060	186.35–186.43	0.005–0.043	186.33–186.40	0.016–0.078

Table 7 Precision results of multiple runs of the VeriFiler Plus Allelic Ladder *(continued)*

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
9	189.87–189.95	0.035–0.059	190.39–190.43	0.008–0.051	190.34–190.41	0.024–0.076
9	192.94–193.01	0.028–0.069	193.43–193.49	0.010–0.050	193.41–193.48	0.019–0.080
10	193.88–193.95	0.031–0.062	194.37–194.44	0.005–0.051	194.35–194.42	0.015–0.074
11	197.87–197.96	0.027–0.060	198.40–198.47	0.006–0.046	198.37–198.44	0.021–0.078
12	201.89–201.97	0.029–0.070	202.39–202.48	0.013–0.050	202.37–202.44	0.034–0.071
13	208.95–209.03	0.036–0.065	209.45–209.52	0.010–0.048	209.43–209.52	0.025–0.081
D12S391						
14	218.79–218.84	0.014–0.034	218.81–218.88	0.006–0.034	218.81–218.87	0.010–0.047
15	222.81–222.84	0.016–0.041	222.81–222.87	0.010–0.046	222.82–222.87	0.012–0.042
16	226.76–226.80	0.010–0.033	226.76–226.83	0.012–0.050	226.77–226.82	0.015–0.056
17	230.84–230.89	0.013–0.036	230.84–230.90	0.008–0.054	230.85–230.90	0.008–0.064
18	234.88–234.92	0.015–0.036	234.87–234.92	0.005–0.058	234.87–234.91	0.010–0.043
19	238.94–238.99	0.015–0.037	238.94–238.99	0.010–0.047	238.92–238.98	0.005–0.048
19	241.95–242.00	0.010–0.076	241.93–241.98	0.005–0.043	241.93–241.97	0.010–0.055
20	242.89–242.95	0.012–0.043	242.91–242.95	0.010–0.052	242.89–242.95	0.008–0.044
21	247.01–247.06	0.010–0.043	246.99–247.04	0.010–0.041	246.97–247.04	0.005–0.046
22	251.12–251.17	0.012–0.038	251.09–251.15	0.014–0.041	251.08–251.13	0.013–0.050
23	255.12–255.18	0.011–0.033	255.10–255.15	0.010–0.040	255.09–255.15	0.013–0.042
24	259.13–259.19	0.018–0.034	259.11–259.16	0.010–0.042	259.09–259.15	0.010–0.033
25	263.16–263.22	0.017–0.035	263.13–263.19	0.013–0.053	263.12–263.18	0.008–0.046
26	267.23–267.27	0.015–0.048	267.19–267.23	0.006–0.038	267.16–267.22	0.005–0.043
27	271.22–271.27	0.012–0.041	271.18–271.24	0.013–0.046	271.16–271.23	0.010–0.045
D2S1338						
11	280.86–280.95	0.042–0.072	281.45–281.48	0.008–0.058	281.40–281.48	0.040–0.085
12	284.85–284.94	0.031–0.069	285.45–285.50	0.017–0.043	285.41–285.48	0.041–0.093
13	288.87–288.94	0.031–0.075	289.42–289.51	0.013–0.057	289.41–289.48	0.033–0.089
14	292.86–292.96	0.024–0.065	293.43–293.48	0.010–0.062	293.40–293.50	0.021–0.088

Table 7 Precision results of multiple runs of the VeriFiler Plus Allelic Ladder *(continued)*

Marker/allele	3130*xl*		3500		3500xL	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
15	296.82–296.91	0.025–0.061	297.35–297.39	0.006–0.048	297.31–297.39	0.021–0.089
16	300.87–300.94	0.034–0.067	301.39–301.45	0.015–0.053	301.37–301.44	0.029–0.086
17	304.87–304.94	0.033–0.063	305.40–305.46	0.010–0.054	305.39–305.46	0.021–0.088
18	308.91–308.98	0.029–0.070	309.41–309.48	0.014–0.053	309.41–309.47	0.029–0.082
19	312.91–312.98	0.034–0.058	313.44–313.50	0.013–0.053	313.41–313.48	0.039–0.091
20	316.92–317.00	0.020–0.058	317.45–317.51	0.013–0.060	317.43–317.49	0.021–0.088
21	320.97–321.05	0.021–0.063	321.48–321.54	0.005–0.051	321.47–321.54	0.027–0.084
22	324.97–325.06	0.033–0.070	325.48–325.53	0.010–0.053	325.46–325.53	0.015–0.070
23	328.98–329.06	0.024–0.060	329.49–329.55	0.013–0.073	329.48–329.58	0.015–0.088
24	333.00–333.08	0.024–0.061	333.53–333.58	0.010–0.054	333.51–333.61	0.015–0.075
25	337.01–337.10	0.028–0.063	337.53–337.58	0.010–0.066	337.54–337.63	0.015–0.080
26	341.06–341.13	0.026–0.063	341.54–341.60	0.005–0.059	341.56–341.64	0.015–0.078
27	345.14–345.22	0.028–0.067	345.61–345.68	0.008–0.039	345.61–345.69	0.006–0.079
28	349.38–349.46	0.021–0.062	349.77–349.81	0.006–0.055	349.80–349.86	0.010–0.074
TPOX						
5	364.37–364.50	0.073–0.107	365.42–365.42	0.013–0.013	365.34–365.44	0.043–0.119
6	368.42–368.52	0.061–0.110	369.45–369.45	0.010–0.010	369.37–369.50	0.040–0.112
7	372.46–372.59	0.054–0.102	373.51–373.51	0.008–0.008	373.45–373.54	0.040–0.133
8	376.44–376.57	0.053–0.087	377.49–377.49	0.010–0.010	377.44–377.54	0.044–0.110
9	380.42–380.54	0.043–0.090	381.52–381.52	0.014–0.014	381.43–381.55	0.050–0.113
10	384.44–384.56	0.037–0.093	385.53–385.53	0.005–0.005	385.47–385.58	0.046–0.142
11	388.43–388.55	0.054–0.092	389.57–389.57	0.010–0.010	389.51–389.58	0.055–0.128
12	392.41–392.53	0.046–0.098	393.59–393.59	0.015–0.015	393.50–393.60	0.065–0.121
13	396.42–396.53	0.042–0.095	397.59–397.59	0.010–0.010	397.52–397.63	0.066–0.124
14	400.41–400.53	0.050–0.102	401.61–401.61	0.013–0.013	401.50–401.66	0.061–0.127
15	404.39–404.51	0.057–0.098	405.63–405.63	0.008–0.008	405.51–405.64	0.047–0.141

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 that are used in the kit. All data were generated on the 3500xL Genetic Analyzer. Some conclusions from these measurements and observations are:

- For each locus, the stutter percentage generally increases with allele length.
- Each allele within a locus displays a relatively consistent average stutter percentage.
- Peaks in the stutter position that are above the stutter filter percentage specified in the software are not filtered.
- The measurement of stutter percentage for allele peaks that are off-scale may be unusually high due to artificial truncation of the main allele peak.
- Stutter can be elevated when minus stutter and plus stutter overlap. This is typically observed when a given allele flanks another allele that is 2 repeat units away (as seen with the FGA locus in control 007 DNA).
- The magnitude and/or variability of stutter may increase with low DNA input amounts.

Figure 17 through Figure 21 show the stutter observed in the population study that are one repeat unit away from the alleles recorded. All data were generated on the 3500xL Genetic Analyzer.

The stutter filter settings that are derived from this data are listed in “Stutter percentage filter settings provided with GeneMapper™ ID-X Software” on page 88.

Plus-stutter was regularly observed and was more significant in trinucleotide repeat-containing loci (see “Plus-stutter peaks at the D22S1045 locus” on page 87).

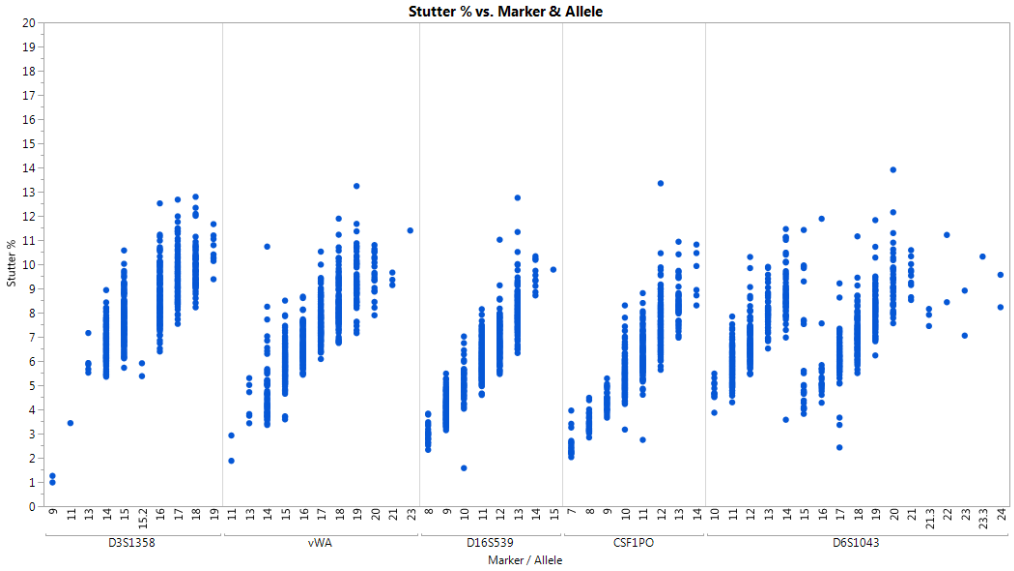


Figure 17 Stutter percentages for the VeriFiler™ Plus kit FAM™ dye (blue) channel loci: D3S1358, vWA, D16S539, CSF1PO, and D6S1043.

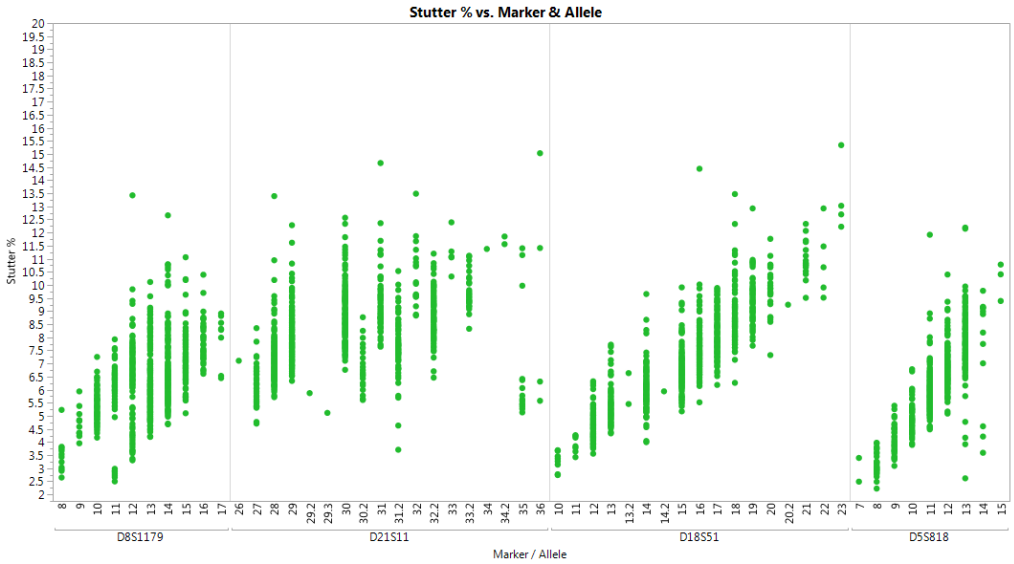


Figure 18 Stutter percentages for the VeriFiler™ Plus kit VIC™ dye (green) channel loci: D8S1179, D21S11, D18S51, and D5S818.

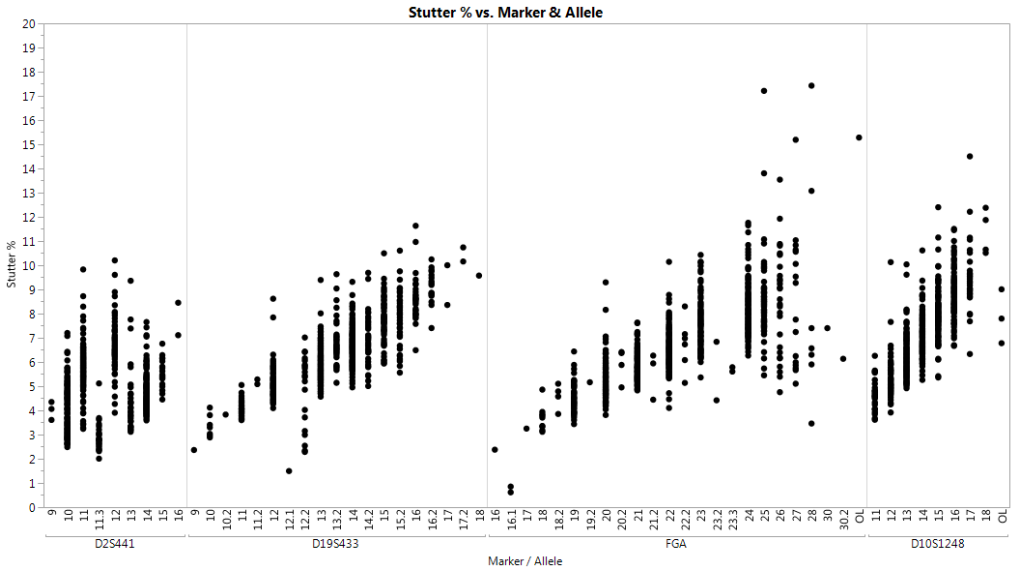


Figure 19 Stutter percentages for the VeriFiler™ Plus kit TED™ dye (yellow) loci: D2S441, D19S433, FGA, and D10S1248.

Note: In the electropherogram plot, yellow is displayed as black.

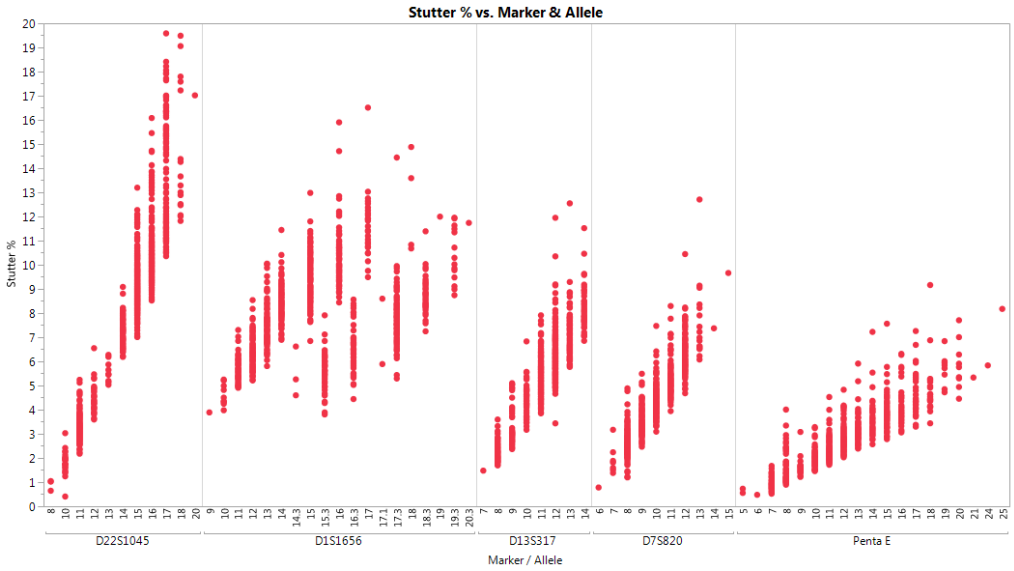


Figure 20 Stutter percentages for the VeriFiler™ Plus kit TAZ™ dye (red) loci: D22S1045, D1S1656, D13S317, D7S820, and Penta E.

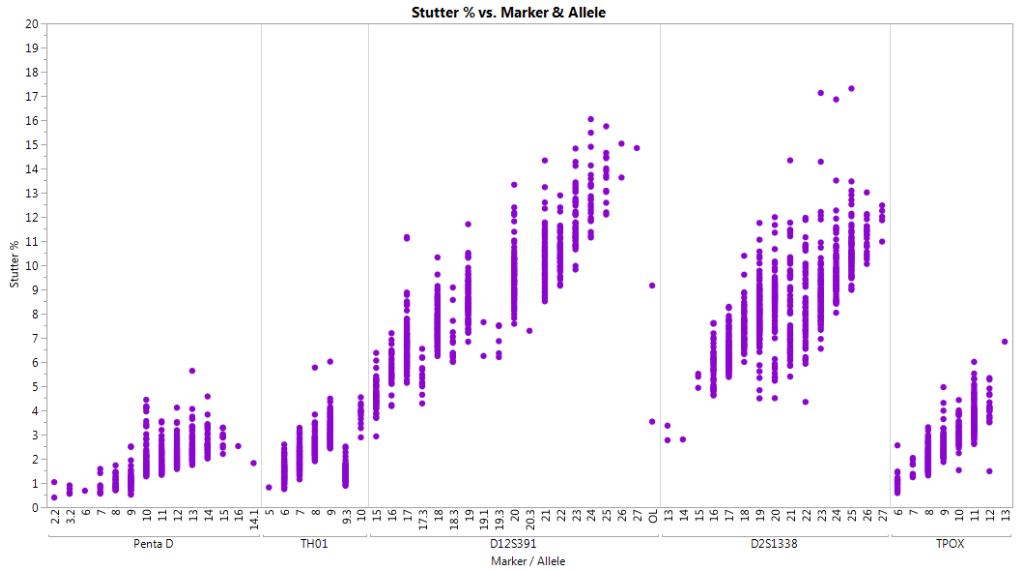


Figure 21 Stutter percentages for the VeriFiler™ Plus kit SID™ dye (purple) loci: Penta D, TH01, D12S391, D2S1338, and TPOX.

Plus-stutter peaks at the D22S1045 locus

The D22S1045 STR locus in the VeriFiler™ Plus kit is a trinucleotide repeat locus, and shows an elevated level of plus stutter (Figure 22). Other loci, such as FGA, may also exhibit elevated plus stutter.

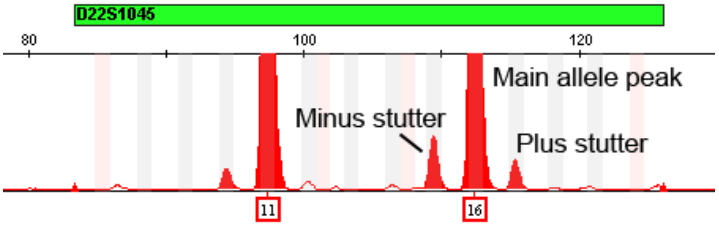


Figure 22 VeriFiler™ Plus kit electropherogram showing minus and plus stutter associated with the D22S1045 STR locus. Data produced on a 3500xL Genetic Analyzer.

STR loci such as D1S1656 (Figure 23) contain 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 VeriFiler™ Plus kit data contains a minus 2–nt stutter filter for D1S1656, as well as filters for commonly observed plus stutter, to prevent these peaks from being called in normal profiles.

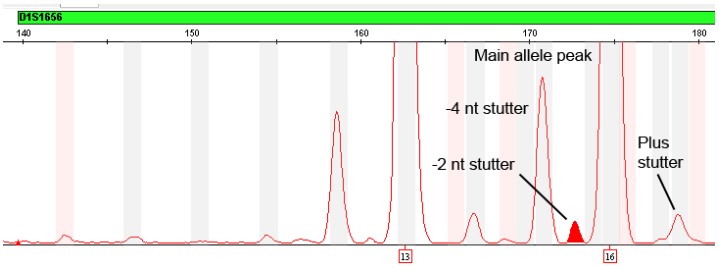


Figure 23 Example of non-standard stutter peaks in the D1S1656 locus. In addition to the main allele peaks and standard (–4 nt) stutter peaks, –2 nt minus stutter peaks and +4 nt plus stutter peaks can also be observed. Data produced on a 3500xL Genetic Analyzer.

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

The settings in Table 8 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.

Analysis showed that observed stutter data points were not normally distributed. As such, at each locus a best-fit, non-parametric statistical model was applied to the data and a threshold filter level that minimized the occurrence of non-filtered stutter peaks while allowing maximum peak-detection sensitivity was determined.

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

Table 8 Minus and plus stutter percentage filter settings provided with the GeneMapper™ *ID-X* Software

Note: Penta D and Penta E do not require plus stutter filters because their stutter levels are so low.

Locus [1]	Minus Stutter (%)	Plus Stutter (%)
D3S1358	12.61	3.74
vWA	11.86	4.46
D16S539	11.19	4.05
CSF1PO	11.00	5.42
D6S1043	12.05	4.58
D8S1179	11.54	4.85
D21S11	13.83	5.04
D18S51	13.73	5.96
D5S818	10.90	4.64
D2S441	9.71	3.71
D19S433	11.00	5.91
FGA	14.01	7.96
D10S1248	12.48	3.13
D22S1045	19.04	8.63
D1S1656	15.20	6.40

Table 8 Minus and plus stutter percentage filter settings provided with the GeneMapper ID-X Software *(continued)*

Locus [1]	Minus Stutter (%)	Plus Stutter (%)
D13S317	11.00	6.25
D7S820	9.83	4.27
Penta E	7.99	—
Penta D	4.59	—
TH01	5.55	3.95
D12S391	15.61	6.10
D2S1338	16.69	8.00
TPOX	5.82	3.49

[1] These percentages are used as stutter filters in VeriFiler_Plus_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 nucleotide addition observations

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

The VeriFiler™ Plus 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 final incubation at 60°C for 5 minutes.

This final extension step gives the DNA polymerase additional time to complete +A addition to all double-stranded PCR products.Figure 24 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.

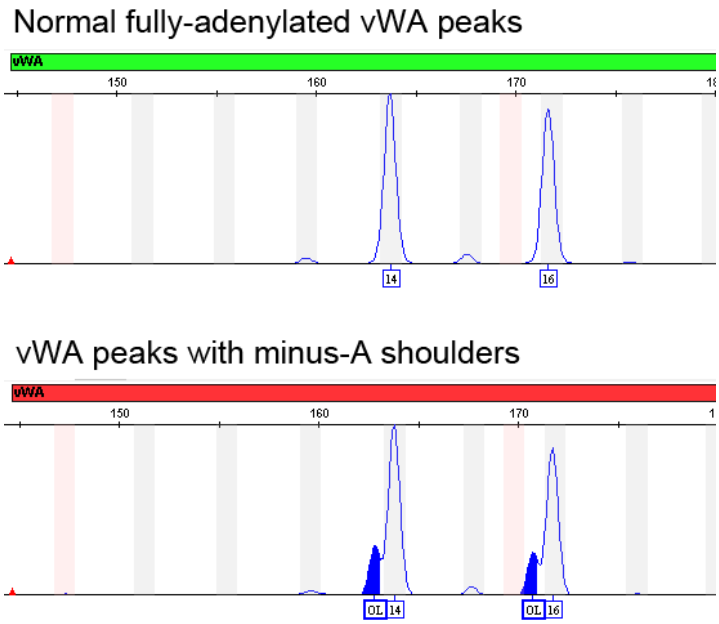


Figure 24 The top panel shows normal vWA peaks that are fully adenylated, with no visible shouldering and correct genotype calls. The bottom panel shows vWA peaks in which adenylation was incomplete, resulting in minus-A shoulders that were called as off-ladder (OL) peaks by the GeneMapper™ *ID-X* Software.

If the amount of input DNA is greater than recommended concentration, "shouldering" of allele peaks can be observed. Amplification of excess input DNA can also result in off-scale data and lowered IQCL peak heights. In this situation, the IQCL may also exhibit some shouldering due to incomplete +A nucleotide addition. Other adverse conditions may also result in incomplete adenylation, for example: PCR inhibition, expired PCR reagents, or not completing the final hold step during thermal cycling.

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

Because of improvements in PCR primer manufacturing processes, the incidence of artifacts has been greatly reduced in the VeriFiler™ Plus kit. Internal population studies show that kit electropherograms are free of reproducible dye artifacts in the kit read region of 64–458 nt. Two exceptions are as follows:

- A low-level 113–117 nt dye artifact in the VIC™ dye channel that has been detected.
- A low-level ~66 nt dye artifact in the TED™ dye channel. This artifact was approximately 40–80 RFU in our studies. The peak height observed may vary depending on the sensitivity of individual CE instruments.

Figure 25 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.

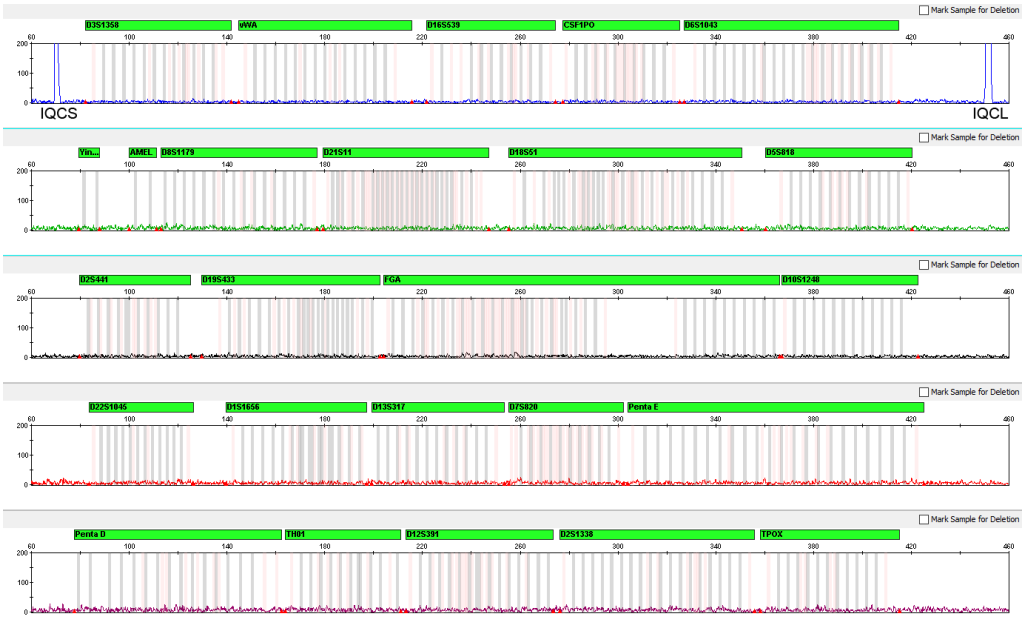


Figure 25 Examples of fluorescence background in data produced on a 3500xL Genetic Analyzer (Y-axis scale 0–200 RFU) (updated formulation)

Note the two FAM™ dye-labeled IQC peaks (IQCS and IQCL) that should normally be present in negative samples.

Some small PCR artifacts were occasionally observed in negative control reactions prepared with the VeriFiler™ Plus kit reagents that were subjected to long-term storage at elevated temperature as part of stability testing. The most prominent and consistent artifacts that were sometimes observed were:

- An artifact sizing at 120–130 bp in the FAM™ dye channel
- An artifact sizing at 110–120 bp in the VIC™ dye channel

For examples of these dye artifacts, see Figure 26. Such artifacts may form spontaneously upon long-term storage of PCR reagents, but their formation can be minimized by following the recommended storage conditions.

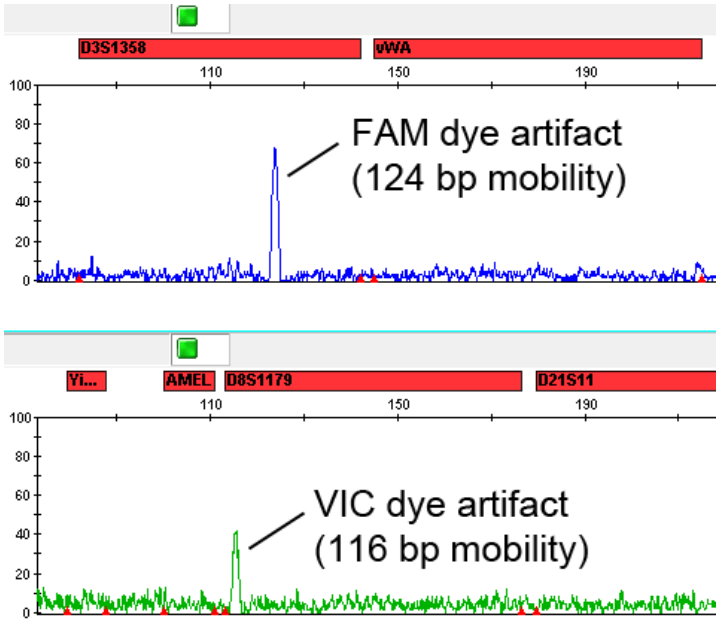


Figure 26 Examples of FAM™ dye and VIC™ dye artifacts in negative (NTC) reactions with VeriFiler™ Plus kit reagents that were subjected to long-term storage at elevated temperature

In this example, both artifact peaks are below 100 RFU.

Characterization of loci

SWGDAM guideline 3.1

"The basic characteristics of a genetic marker should be determined and documented." (SWGDAM, December 2016)

Loci in this kit

This section describes basic characteristics of the 23 autosomal STR loci, Y indel locus, and sex-determining marker (Amelogenin), that are amplified with the VeriFiler™ Plus 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 102-nt and 108-nt products from the X and Y chromosomes, respectively. The primers for the Y indel flank a region in the q arm of the Y chromosome (Yq11.221). Depending on the haplotype of the sample, the amplification generates either a 81-nt or a 87-nt product. (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.) Most of the STR loci present in the VeriFiler™ Plus kit contain tetranucleotide repeat units. However, the kit also contains one locus with

trinucleotide repeats (D22S1045) and two loci with pentanucleotide repeats (Penta D and Penta E). 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 VeriFiler™ Plus 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 loci present in the VeriFiler™ Plus kit 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

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

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

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

Table 9 Four sets of STR loci in the VeriFiler™ Plus kit that are located on the same chromosomes

Locus	Chromosome location		Distance apart (Mb)
	Map	Mb units	
D5S818	5q23.2	123.139	26.297
CSF	5q33.1	149.436	
vWA	12p13.31	5.963	6.378
D12S391	12p13.2	12.341	
D21S11	21q21.1	19.476	24.404
Penta D	21q22.3	43.88	
TPOX	2p25.3	1.472	66.741
D2S441	2p14	68.213	
D2S441	2p14	68.213	150.492
D2S1338	2q35	218.705	

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 2016)

Nonhuman studies

The VeriFiler™ Plus kit provides the required specificity for detecting human alleles. Species specificity testing was performed to confirm that there is minimal cross-reactivity with nonhuman DNA that may be present in forensic casework samples, and to document the rarely observed examples of cross-species detection.

The following species were tested (in the specified amounts) using standard PCR and capillary electrophoresis conditions for the VeriFiler™ Plus kit kit:

- Primates: chimpanzee, orangutan, and macaque (0.5 ng each)
- Non-primates: mouse, dog, sheep, pig, rabbit, cat, horse, rat, and cow (5.0 ng each)
- Microorganisms: *Streptococcus salivarius*, *Neisseria gonorrhea*, *Bacillus subtilis*, *Candida albicans*, *Lactobacillus delbrueckii*, *Escherichia coli*, and *Staphylococcus aureus* (pooled genomic DNAs, with approximately 100,000 copies of DNA from each species, per reaction)

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

Figure 27 shows example electropherogram results from the species specificity tests. Primate species (chimpanzee, macaque) produced more extensive cross-reactivity than non-primate species, and for a closely related species like chimpanzee, many peaks fell into allele bins. A few of the more distantly related mammals (bovine, dog, horse) produced peaks over the 175 RFU threshold, all of which genotyped as "OL" because they fell outside of allele bins. None of the remaining species that were tested gave peaks over the detection threshold.

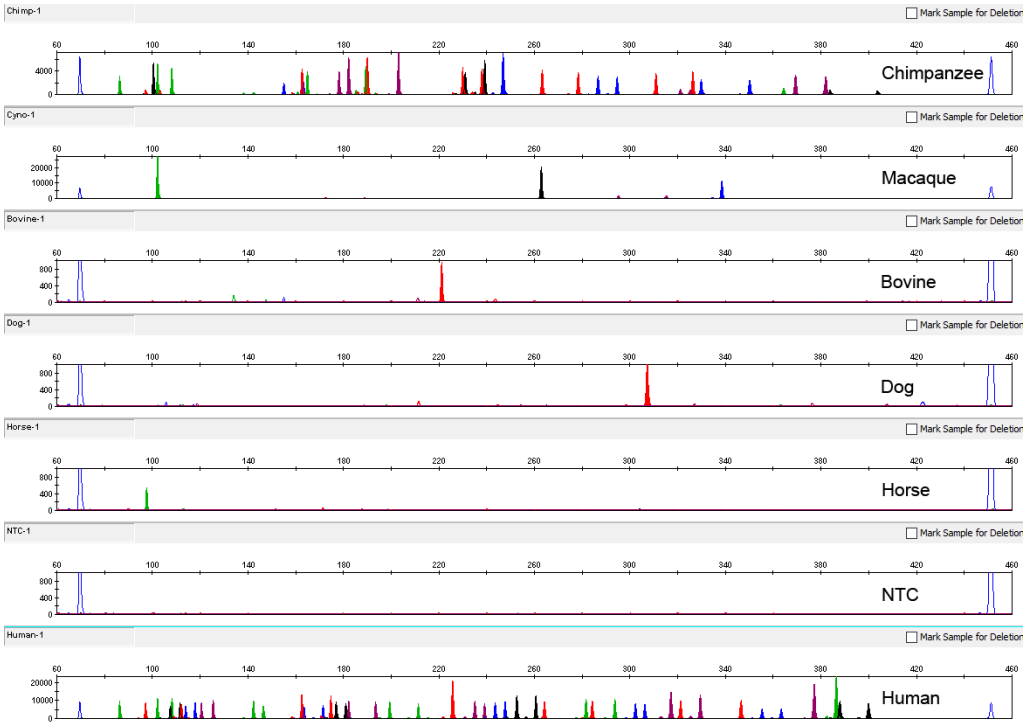


Figure 27 Representative electropherograms for some species tested in a species specificity study. Data produced on a 3500xL Genetic Analyzer (updated formulation)

Note: The individual panes are at different magnifications (zoom function) on the Y-axis.

Table 10 shows the most significant cross-reactive peaks that were observed among non-human, non-primate, genomic DNAs (that is, peaks over a 175 RFU Peak Amplitude Threshold on the 3500xL Genetic Analyzer), with the original and updated formulations of the VeriFiler™ Plus kit. Peaks were observed for dog, horse, hamster, bovine, and sheep. No peaks fell into human STR locus bins, and would therefore not be confused with human STR alleles. 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.

Table 10 Observed cross-reactive peaks for non-human, non-primate animals for the original and updated formulations of the VeriFiler™ Plus kit

Species	Dye channel	Size (bp)	VeriFiler™ Plus kit—Original formulation		VeriFiler™ Plus kit—Updated formulation	
			Average peak height (RFU)	Genotype call	Average peak height (RFU)	Genotype call
Dog	Blue	341	214	D6 OL	—	—
	Red	307	692	Penta E OL	716	Penta E OL
Horse	Green	98	630	Out of Marker Range (OMR)	828	OMR
Hamster	Green	130	243	D8 OL	—	—

Table 10 Observed cross-reactive peaks for non-human, non-primate animals for the original and updated formulations of the VeriFiler Plus kit *(continued)*

Species	Dye channel	Size (bp)	VeriFiler™ Plus kit—Original formulation		VeriFiler™ Plus kit—Updated formulation	
			Average peak height (RFU)	Genotype call	Average peak height (RFU)	Genotype call
Bovine	Red	221	399	D13 OL	489	D13 OL
Sheep	Purple	211	345	TH01 OL	301	TH01 OL

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 2016)

Sensitivity observation

The recommended amount of input DNA for the VeriFiler™ Plus kit is 0.5 ng for 29 cycles of amplification based on real-time PCR quantification, such as with the Quantifiler™ Trio DNA Quantification Kit or the Quantifiler™ HP DNA Quantification Kit. To determine the optimum input DNA, perform studies according to the quantification kit that you use.

If the sample contains degraded DNA, amplification of a higher amount of DNA may be beneficial. In Figure 28, DNA Control 007 was serially diluted from 1.0–0.016 ng. Full profiles (46 human alleles) were consistently obtained at 0.125 ng, but occasional partial profiles resulted at lower concentrations.

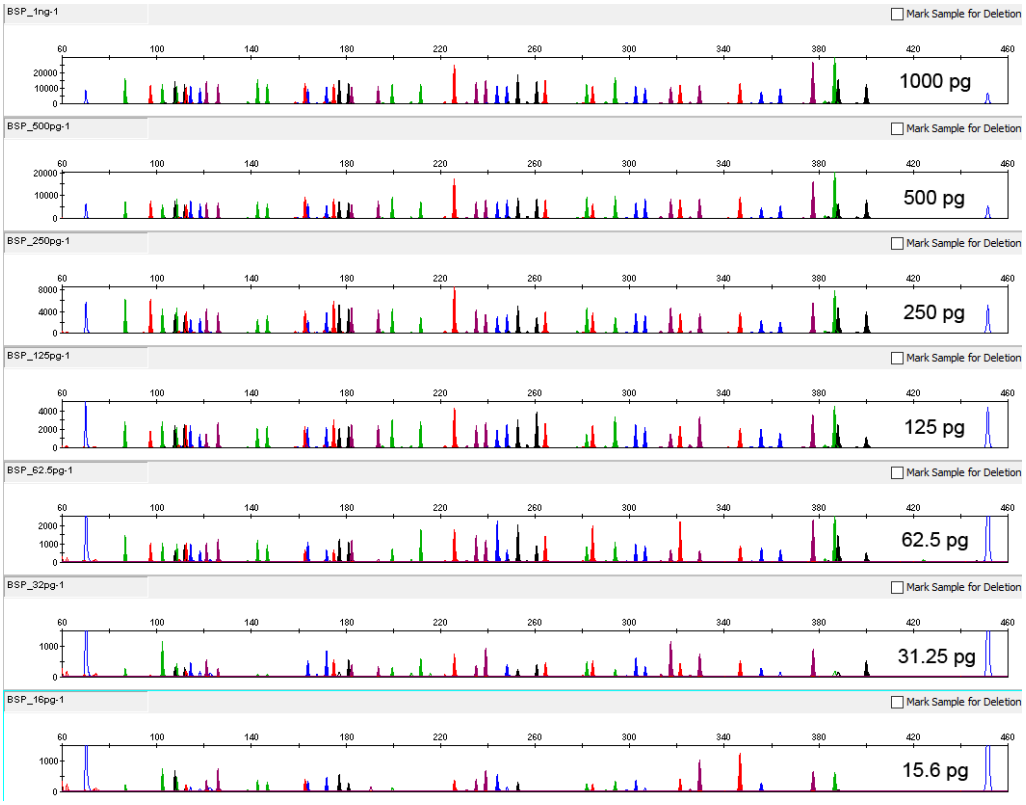


Figure 28 Electropherograms for amplifications using 1,000, 500, 250, 125, 63, 31, and 16 pg of DNA Control 007 (updated formulation)

Electrophoresis was performed on a 3500xL Genetic Analyzer. Note that because 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 2016)

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 DNA was sonicated and incubated with increasing doses of DNase I (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 0.5 ng of degraded DNA using the VeriFiler™ Plus 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.

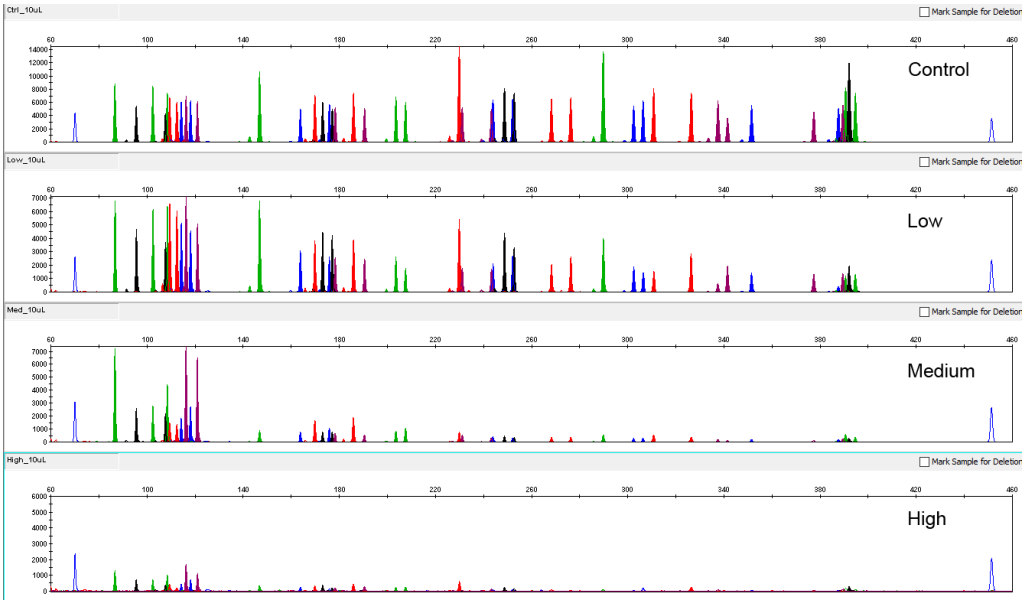


Figure 29 Amplification of a single donor DNA sample sonicated and incubated with increasing doses of DNase I (updated formulation)

The panels show the control (undegraded), low-, medium-, and high-degraded samples. Note that the Y-axis scale is magnified for more degraded samples, which generate lower peak heights.

Effect of inhibitors

Because of the substrates and environments from which they are collected, forensic DNA samples may be contaminated with substances that inhibit PCR amplification. The PCR chemistry for the VeriFiler™ Plus kit was optimized to be as robust as possible in the presence of such inhibitors. Model inhibitors tested during developmental validation were: hematin, a representative compound often extracted from blood stains (DeFranchis *et al*., 1988; Alkane *et al.*, 1994); humic acid, a simulant for soil-based inhibition; and tannic acid, a compound often co-extracted from leather sample substrates. 0.5 ng of DNA Control 007 was amplified in the presence of increasing concentrations of each model inhibitor. All samples were then amplified using the standard 29-cycle "touchdown" thermal cycling program.

Figure 30 and Figure 31 show representative electropherograms from the PCR inhibitor studies with humic acid, hematin, and tannic acid. Table 11 shows inhibitor final concentrations in 25-µL PCR reactions and average allele counts obtained in replicate reactions with the VeriFiler™ Plus kit.

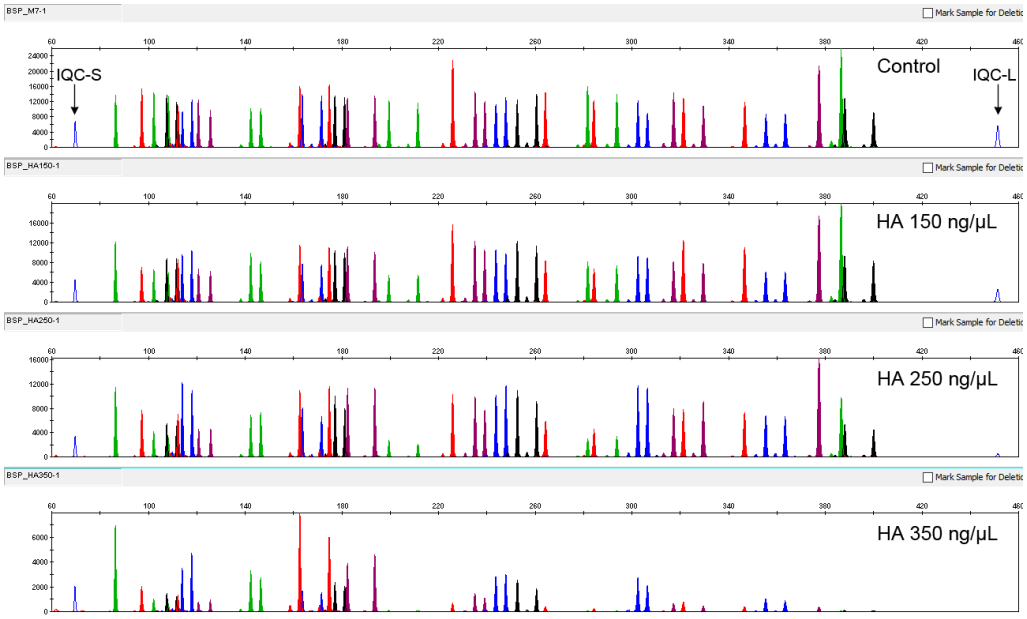


Figure 30 Electropherograms show the results of testing the VeriFiler™ Plus kit with the humic acid PCR inhibitor (updated formulation)

The top panel is the experimental control with 0.5 ng (total) of DNA Control 007 without humic acid. The remaining panels show 0.5 ng of DNA Control 007 with increasing levels of humic acid (150, 250, and 350 ng/µL). Note the behavior of the IQCS and IQCL peaks in response to increasing levels of inhibition, with the IQCL peak decreasing in peak height and eventually disappearing as the humic acid concentration increases.

Note: The individual panes are at different magnifications (zoom function) on the Y-axis.

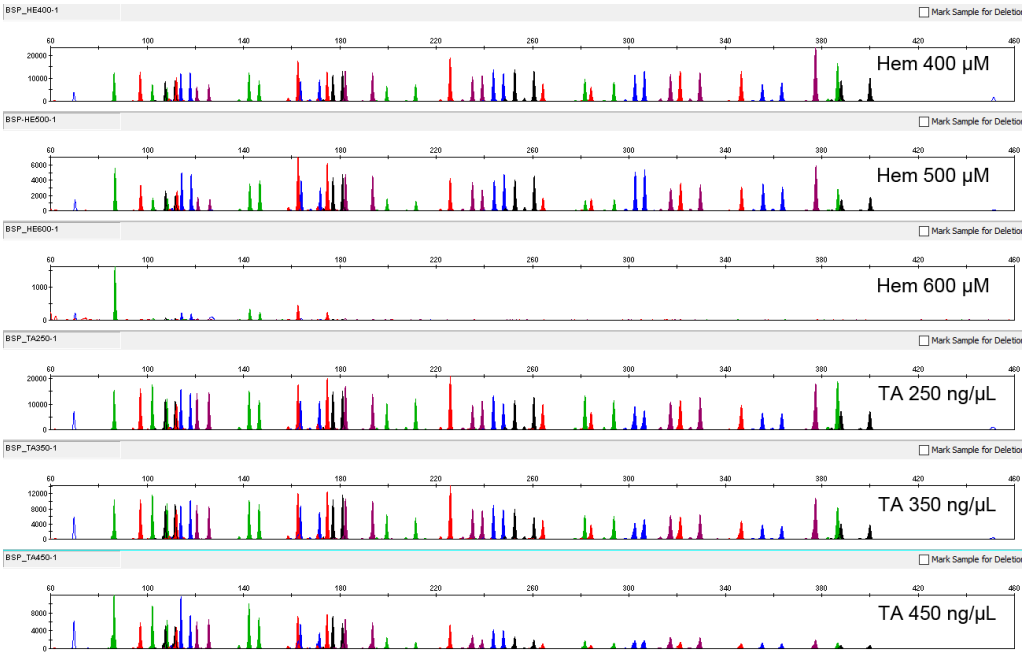


Figure 31 Example electropherograms show the results of testing hematin and tannic acid PCR inhibitors (updated formulation)

Each sample contained 0.5 ng (total) of human male DNA Control 007 with varying levels of hematin or tannic acid. Note that the IQCL peak was significantly reduced or absent while the IQCS peak still remained in the inhibitor-containing samples.

Note: The individual panes are at different magnifications (zoom function) on the Y-axis.

Table 11 VeriFiler™ Plus kit performance with PCR inhibitors (N = 4) (updated formulation)

Sample	Inhibitor concentration	Mean allele count
Control (no inhibitor)	0	46
Hematin low	400 µM	46
Hematin medium	500 µM	46
Hematin high	600 µM	8.75
Humic acid low	150 ng/µL	46
Humic acid medium	250 ng/µL	46
Humic acid high	350 ng/µL	42.5
Tannic acid low	250 ng/µL	46
Tannic acid medium	350 ng/µL	46
Tannic acid high	450 ng/µL	46

Mixture studies

SWGDAM guideline 3.8

"The ability to obtain reliable results from mixed source samples should be determined." (SWGDAM, December 2016)

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

Median, minimum, and maximum peak height ratios observed for alleles in the VeriFiler™ Plus kit loci in single-source human population database samples are shown in Figure 32. The population samples that are used are listed in “Population samples used in these studies” on page 108.

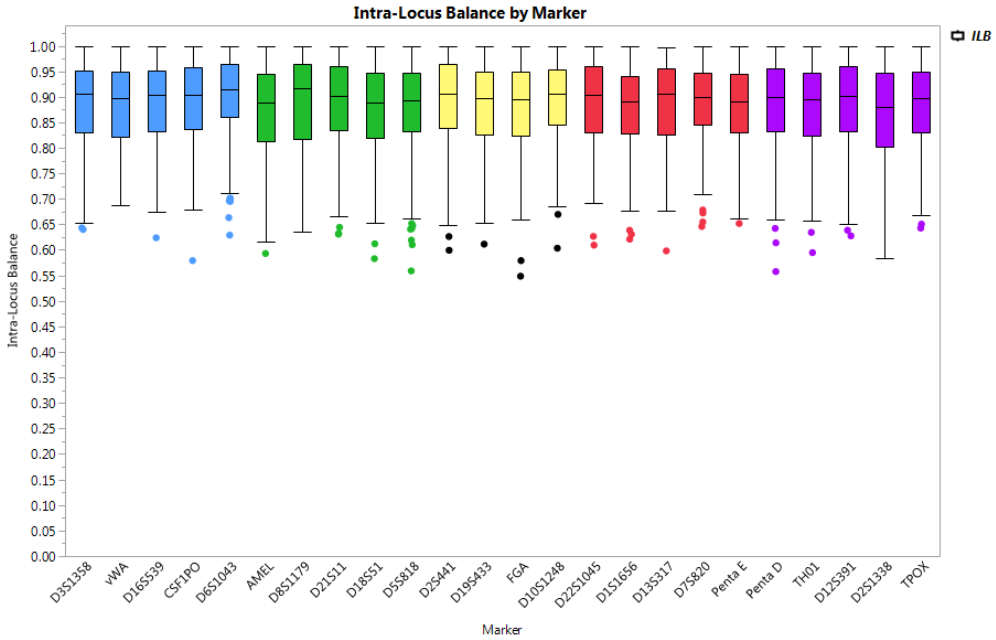


Figure 32 Heterozygote ratios for ~0.5 ng of input DNA. Intra-locus peak height ratio is calculated per individual profile by dividing the lower peak by the higher peak in each heterozygote allele pair, per marker. Box plot boxes and data points are colored according to the dye channel. Boxes show the 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. Black dots 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, 1:3, 1:7, 1:15, 1:30, 30:1, 15:1, 7:1, 3:1 and 1:0). The total amount of genomic input DNA mixed at each ratio was 0.5 ng. The samples were amplified in a ProFlex™ 96-well PCR System, then electrophoresed and detected using an 3500xL Genetic Analyzer.

The results of the mixed DNA samples are shown in Figure 33. The two human genomic DNAs, male 007 and female 9947a, 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 7:1 (0.438 ng of 007 and 0.063 ng of 9947a) and 1:7 (0.063 ng of 007 and 0.438 ng of 9947a), with 3/3 replicates of both samples giving full profiles of the respective minor contributor. 15:1, 30:1, 1:30, and 1:15 ratios resulted in partial profiles for the minor component (Table 13). The genotypes of each contributor DNA are shown in Table 12.

Table 13 shows mixture sample compositions and mean STR allele counts from VeriFiler™ Plus kit assays.

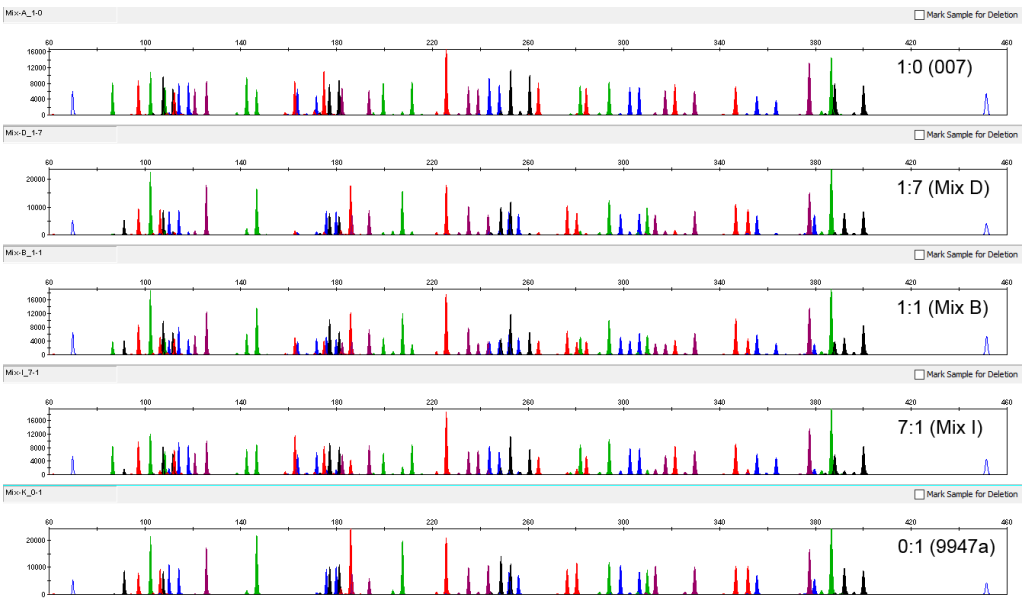


Figure 33 Amplification of DNA mixtures at various ratios (updated formulation)
Panels show electropherograms for 1:0 (male 007 DNA only), 1:7 mixture, 1:1 mixture, 7:1 mixture, and 0:1 (female 9957a DNA only).

Table 12 Genotypes of mixture sample contributors (updated formulation)

Asterisks denote alleles that are unique for each individual, and also apart from plus- or minus-stutter positions of other alleles present in the individuals.

Marker	Male 007 genotype		Female 9947a genotype	
	Allele 1	Allele 2	Allele 1	Allele 2
D3S1358	15	16*	14	15
vWA	14*	16	17*	18*
D16S539	9*	10	11*	12*
CSF1PO	11	12	10	12
TPOX	8	—	8	—
Yindel	2*	—	—	—
AMEL	X	Y*	X	—
D8S1179	12	13	13	—
D21S11	28*	31*	30	—
D18S51	12*	15	15	19*
D2S441	14	15*	10*	14
D19S433	14	15	14	15
TH01	7	9.3	8*	9.3

Table 12 Genotypes of mixture sample contributors (updated formulation) *(continued)*

Marker	Male 007 genotype		Female 9947a genotype	
	Allele 1	Allele 2	Allele 1	Allele 2
FGA	24	26*	23	24
D22S1045	11	16*	11	14*
D5S818	11	—	11	—
D13S317	11	—	11	—
D7S820	7*	12*	10*	11
D10S1248	12	15	13*	15
D1S1656	13*	16*	18.3*	—
D12S391	18	19	18	20*
D2S1338	20*	23	19	23
D6S1043	12	14*	12	18*
Penta E	7*	12	12	13*
Penta D	11	12	12	—

Table 13 Mixture sample compositions and mean STR allele counts from VeriFiler™ Plus kit assays (updated formulation)

Mixture sample	Ratio (A:B)	DNA amounts (ng/reaction)		Mean allele count[1]	
		Male 007 ("A")	Female 9947a ("B")	Male 007 ("A")	Female 9947a ("B")
A	1:0	0.500	0.000	18.00	0.00
B	1:1	0.250	0.250	18.00	14.00
C	1:3	0.125	0.375	18.00	14.00
D	1:7	0.063	0.438	18.00	14.00
E	1:15	0.031	0.469	15.33	14.00
F	1:30	0.016	0.484	12.67	14.00
G	30:1	0.484	0.016	18.00	7.33
H	15:1	0.469	0.031	18.00	10.67
I	7:1	0.438	0.063	18.00	14.00

Table 13 Mixture sample compositions and mean STR allele counts from VeriFiler Plus kit assays (updated formulation) *(continued)*

Mixture sample	Ratio (A:B)	DNA amounts (ng/reaction)		Mean allele count[1]	
		Male 007 ("A")	Female 9947a ("B")	Male 007 ("A")	Female 9947a ("B")
J	3:1	0.375	0.125	18.00	13.67
K	0:1	0.000	0.500	0.00	14.00

[1] Mixture sample compositions and STR allele counts from VeriFiler™ Plus kit assays. The average number of unique, distinct alleles (that is, alleles neither present in the other contributor genotype nor located in a stutter position) obtained in 3 replicate reactions per mixture sample. The full profiles of unique alleles in 007 and 9947a DNAs were 18 and 14, respectively.

Population data

SWGDAM guideline 3.7

"The distribution of genetic markers in populations should be determined in relevant population groups." (SWGDAM, December 2016)

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 VeriFiler™ Plus PCR Amplification Kit was designed and optimized primarily for the analysis of forensic casework samples. It was designed as a companion kit to the VeriFiler™ Express PCR Amplification Kit (Cat. No. A32014) and the Huaxia™ Platinum™ PCR Amplification Kit (Cat. No. A31323), and contains the same set of 25 loci.

Population samples used in these studies

The VeriFiler™ Plus kit has high genotypic concordance (>99.5%) to its databasing counterparts, the VeriFiler™ Express kit and Huaxia™ Platinum™ kit (all three kits contain the same 25 loci). An initial population study of individuals of different ethnic populations within the United States and China was conducted with 2,475 individuals. This initial study was the basis for the allele frequency tables and associated random match probability tables at the end of this section.

After the minor redesign of certain primers in the VeriFiler™ Plus kit multiplex to reduce the incidence of artifact peaks in samples with high levels of bacterial DNA, a new population study was performed to check:

- Concordance between the original and redesigned VeriFiler™ Plus kits
- Concordance with the GlobalFiler™ kit reference genotypes

As with the original population study, the study with the updated formulation included in-house testing of domestic United States individuals as well as population samples collected and tested by laboratories in China. See Table 14.

Table 14 Population samples (updated formulation)

Sample source (population)	Total samples	Sample type	No. of samples by ethnic origin
In-house testing of domestic United States individuals	1,308	• Extracted DNAs from 1,108 blood samples • 200 samples from blood cards that were tested in direct amplification mode	• African-American—285 • Asian—316 • Caucasian—322 • Hispanic—385
Chinese laboratories	987	Blood cards that were tested in direct amplification mode	Individuals of Chinese origin—987

Concordance studies

Concordance between the original VeriFiler™ Plus kit and the redesigned kit was 100% for 2,288 samples tested in-house and externally. When genotype results were compared between the VeriFiler™ Plus kit and the GlobalFiler™ kit, the following level of concordance was observed:

Sample source (population)	Sample type	Level of discordance
In-house testing of domestic United States individuals	Extracted DNAs from 1,108 blood samples	1,099/1,108 (discordances observed in D10S1248 and D3S1358)
	200 samples from blood cards that were tested in direct amplification mode	200/200 (0 discordances observed)
Chinese laboratories	Blood cards that were tested in direct amplification mode	986/987 (1 discordance observed in D5S818)

Among the in-house extracted samples, several instances of discordance were observed that were caused by a 1-bp insertion in the flanking region of D10S1248. Affected individuals genotyped as off-ladder (OL). The same individuals tested with the GlobalFiler™ kitdid not give the OL genotypes. All individuals that contained the 1-bp insertion were of African descent, and subsequent database searches confirmed that this indel is found almost exclusively in individuals of African ancestry.

D10S2148 in the VeriFiler™ Plus kit

The VeriFiler™ Express kit and Huaxia™ Platinum™ kit were designed for database workflows that involve pristine single-source samples. The VeriFiler™ Plus kit was designed for casework workflows that involve challenging samples. Although the three kits are complementary, the VeriFiler™ Plus kit was modified to optimize casework performance and discrimination power with degraded samples. In the VeriFiler™ Plus kit, more discriminating markers (such as the Penta D marker) were given priority for amplification and located in the smaller molecular weight regions, while the D10S1248 marker, because of its lower discrimination power, was positioned in the larger molecular weight region.

To move the D10S1248 marker from a smaller base pair size in the SID™ dye channel to the larger base pair size in the TED™ dye channel, the flanking region that borders the primer binding and STR sites was extended. By sequencing additional base pairs in the flanking region of the D10S1248 marker, the opportunity to observe rare, sample-specific mutations increases.

In the case of six African-American (out of 330) and one Hispanic (out of 393) population samples, an insertion in the flanking region for the D10S1248 marker caused a 1-bp difference when comparing results from the VeriFiler™ Plus kit to the VeriFiler™ Express kit, Huaxia™ Platinum™ kit, and GlobalFiler™ kit (Figure 34). This was seen with alleles 13, 14, and 15 reported as 13.1, 14.1, and 15.1. The discrepancy has not been observed in any other population samples tested (1,335

total combined Caucasian and Asian samples). Subsequent sequencing of the sample showed that it is consistent with African lineage. This D10S1248 insertion is observed in the original and updated formulations of the VeriFiler™ Plus kit.

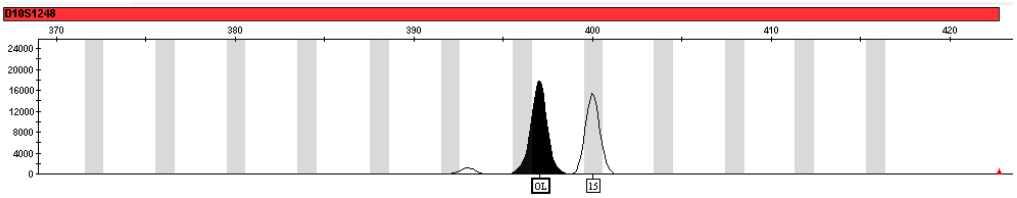


Figure 34 D10S1248 marker from an African American population sample amplified with the VeriFiler™ Plus kit

The off-ladder (OL) peak sizes at the 14.1 base pair position because of a single base pair insertion in the flanking region between the primer and STR region. When amplified with the VeriFiler™ Express kit, Huaxia™ Platinum™ kit, and GlobalFiler™ kit, the genotype at this marker would be 14, 15.

Supporting data

Additional studies were performed at the National Institute of Standards and Technology (NIST, C.R. Steffan). Those studies confirmed the above conclusions, showing the 1-bp difference in nine out of 355 African-American samples.

For expanded frequency analysis of this insertion, we examined the sequencing data for the specific insertion at the D10S1248 marker from the following organizations:

- Broad Institute (**https://gnomad.broadinstitute.org/variant/10-131092326-T-TA?dataset=gnomad_r2_1**)
- 1,000 Genomes Project (**https://www.internationalgenome.org/about**)
- NCBI (**https://www.ncbi.nlm.nih.gov/snp/rs567190580#frequency_tab**)

Out of 5,044 total African-related samples, there were 80 observations. Out of 771 total Hispanic-related samples, the insertion was not observed.

Next steps and considerations

The potential to observe discrepant genotypes among STR PCR kits that use different primer sequences is a known phenomenon in the forensic community. Depending on workflow, interpretation protocols, and database search parameters, each laboratory may take a different approach to handling discrepancies across STR PCR kits.

If your laboratory has an established protocol for handling these discrepancies, we recommend that you follow that protocol for this occurrence. If your laboratory does not have an established protocol, we recommend that you consider different analysis and database search approaches, some of which include:

- Evaluate previously generated VeriFiler™ Plus kit files that contain 0.1 microvariant calls at the D10S1248 marker.
- Confirm the genotype through amplification with a secondary STR PCR kit, such as the GlobalFiler™ kit.
- Exclude the D10S1248 marker from upload to a database if the 0.1 microvariant is observed.
- Perform database searches that include one mismatch when a database is composed of profiles from different STR PCR kits.

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

Figure 35 through Figure 39 show the autosomal STR allele frequencies at VeriFiler™ Plus kit loci by population group.

Note: These tables show observed allele frequencies among different population groups. To ensure a conservative approach when performing statistical calculations such as profile frequency estimates, minimum allele frequencies should be used for very rare alleles that may be insufficiently sampled in a population. For autosomal markers, minimum allele frequencies are typically calculated as 5/2N, where N is the number of individuals sampled in a given population (Butler, 2010).

Table 15 shows the Random Match Probability (RMP) values of the VeriFiler™ Plus kit loci individually and combined.

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
Sample Size	2463	2462	2455	2463	2461	2462	2460	2463	2462	2462	2447	2463	2458	2458	2463	2463	2463	2461	2462	2460	2454	2438	2463
4							0.0002																
5							0.0002												0.0002	0.0467	0.0002		
6	0.0002						0.0002									0.0002			0.0039	0.0004	0.1057	0.0002	
7	0.0037			0.0008	0.0002		0.0002							0.0173	0.0008	0.0032			0.0104	0.0124	0.2675	0.0016	
8	0.0018	0.0022		0.2542	0.0128			0.0006						0.0028	0.0035	0.1592	0.0022		0.0471	0.0053	0.0607	0.5455	
9	0.0477			0.1326	0.2485		0.0002					0.0006		0.0663	0.0012	0.0666	0.0022		0.3148	0.0116	0.4817	0.1081	
9.1												0.0199				0.0035							
9.2																0.0008							
9.3																					0.0591		
10	0.2326	0.0010		0.1435	0.1117	0.0012	0.0002	0.0006		0.0002		0.2485		0.1937	0.0298	0.1661	0.1139		0.1265	0.0488	0.0247	0.0277	0.0004
10.1												0.0014				0.0004							
10.2							0.0004																
10.3																						0.0002	
11	0.2420	0.0028		0.2426	0.2802	0.0043	0.0028	0.0607		0.2279		0.3388	0.0002	0.3216	0.1407	0.3244	0.0751		0.1877	0.1163	0.0002	0.2859	0.0002
11.1																0.0002							
11.2							0.0004																
11.3												0.0406											
12	0.3871	0.0701		0.1657	0.2263	0.0426	0.0431	0.0585		0.0032		0.1665	0.0012	0.2496	0.1360	0.2328	0.1242		0.1446	0.1195	0.0002	0.0287	0.0002
12.1	0.0002						0.0002																
12.2							0.0041																
12.3												0.0012			0.0006								
13	0.0696	0.3400		0.0491	0.1052	0.2074	0.2677	0.1076		0.0051	0.0004	0.0264	0.0024	0.1391	0.1240	0.0384	0.2288	0.0002	0.1188	0.0543		0.0014	0.0008
13.2							0.0402																
13.3												0.0004											
14	0.0132	0.2400	0.0002	0.0110	0.0144	0.1881	0.2500	0.0818		0.0268		0.1447	0.0391	0.0077	0.1236	0.0041	0.1908	0.0004	0.0329	0.0876		0.0004	0.2245
14.2							0.1037																
14.3														0.0002									
15	0.0018	0.2226	0.0155	0.0004	0.0006	0.1673	0.0858	0.2722		0.2685		0.0104	0.3519	0.0014	0.0148	0.0002	0.1724		0.0097	0.1035			0.0335

Figure 35 Combined China populations (minimum allele frequency = 0.0010)

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
15.2							0.1370																0.0002
15.3								0.0047															
16		0.0981	0.0094	0.0002		0.1355	0.0189	0.2259		0.2547	0.0119	0.0006	0.3255		0.0032		0.0771	0.0006	0.0032	0.0785		0.0002	0.1908
16.1								0.0002															
16.2							0.0380													0.0002			
16.3								0.0085															
17		0.0213	0.1061			0.0810	0.0018	0.0855		0.1879	0.0719		0.2040		0.0376		0.0118	0.0008	0.0002	0.0772			0.2540
17.2							0.0039											0.0002					
17.3			0.0010					0.0583							0.0010								
17.4																				0.0002			
18		0.0018	0.2399			0.0437	0.0002	0.0108		0.0242	0.1030		0.0710		0.1726		0.0012	0.0254		0.0791			0.1841
18.1															0.0002								
18.2			0.0002				0.0006								0.0008								
18.3			0.0006					0.0191															
18.4																				0.0012			
19			0.1980			0.0469		0.0010		0.0016	0.1841		0.0045	0.0002	0.1439		0.0002	0.0453		0.0547			0.0962
19.2																		0.0002					
19.3			0.0004					0.0037							0.0002								
19.4																				0.0012			
20			0.1664			0.0303		0.0002			0.1165		0.0002		0.0540			0.0498		0.0437			0.0132
20.2																		0.0006					
20.3			0.0002					0.0002							0.0006								
21			0.1018			0.0217					0.0345				0.0071			0.1121		0.0209			0.0018
21.1						0.0002																	
21.2																		0.0035					
21.3															0.0024								
22			0.0843			0.0158					0.0499				0.0010			0.1772		0.0211			
22.2																		0.0049					
22.3			0.0002												0.0002								
23			0.0446			0.0077					0.2058							0.2117		0.0089			

Combined China populations *(continued)*

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
23.2																		0.0102					
23.3											0.0002												
24			0.0187			0.0041			0.0002		0.1385							0.1784		0.0047			
24.2																		0.0069					
25			0.0094			0.0014					0.0666							0.1065		0.0016			
25.2																		0.0047					
26			0.0029			0.0006					0.0110							0.0427		0.0004			
26.2																		0.0010					
27			0.0002			0.0002			0.0028		0.0043							0.0116					
27.2																		0.0006					
28									0.0520		0.0012							0.0037					
28.2									0.0120														
29									0.2626		0.0002							0.0010					
29.2									0.0020														
30									0.2571														
30.1									0.0002														
30.2									0.0185														
30.3									0.0035														
31									0.0952														
31.2									0.0796														
31.3									0.0002														
32									0.0268														
32.2									0.1320														
33									0.0037														
33.2									0.0455														
34									0.0016														
34.2									0.0037														
35.2									0.0006														
39									0.0002														

Combined China populations *(continued)*

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
Sample Size	304	304	304	304	304	304	304	304	304	304	304	304	304	304	304	304	304	304	304	304	304	304	304
2.2																			0.1151				
3.2																			0.0263				
5																			0.0576	0.0789	0.0049		
6																0.0033			0.0115		0.1628	0.0822	
6.1																					0.0016		
7	0.0345													0.0033		0.0049			0.0230	0.1168	0.3618	0.0164	
7.3	0.0016																						
8	0.0905			0.0263	0.0329					0.0049		0.0016		0.0691		0.2039	0.0033		0.1003	0.1842	0.2023	0.3618	
9	0.0378	0.0016		0.0247	0.2155		0.0016	0.0016					0.0033	0.0214		0.1234	0.0033		0.1497	0.0493	0.1645	0.1891	
9.3																					0.0872		
10	0.2582			0.0280	0.1036	0.0033	0.0115	0.0132		0.0444		0.0855		0.0707	0.0148	0.3059	0.0362		0.1201	0.0395	0.0148	0.1020	
10.2							0.0016																
11	0.2253	0.0378		0.2878	0.2993	0.0049	0.0970	0.0510		0.1546		0.3536		0.2451	0.1053	0.2007	0.0609		0.1595	0.0658		0.2138	0.0033
11.2							0.0033																
11.3												0.0345											
12	0.2928	0.1414		0.4391	0.2007	0.0691	0.1086	0.0855		0.0658		0.1974		0.3438	0.2155	0.1332	0.1151		0.1266	0.1217		0.0329	
12.2							0.0395												0.0016				
13	0.0477	0.2204		0.1431	0.1332	0.0378	0.2681	0.1151		0.0016	0.0016	0.0378	0.0066	0.2270	0.0839	0.0230	0.1891		0.0789	0.1036		0.0016	0.0099
13.2						0.0033	0.0559																
14	0.0115	0.2829		0.0493	0.0148	0.0526	0.1941	0.2484		0.0806		0.2697	0.0872	0.0164	0.0789	0.0016	0.3421		0.0247	0.0691			0.0740
14.2						0.0033	0.0559																
14.3								0.0099															
15		0.1941	0.0691	0.0016		0.1628	0.0674	0.1776		0.2270	0.0033	0.0197	0.2780	0.0033	0.0444		0.1826		0.0049	0.0757			0.2253
15.1			0.0016																				
15.2							0.0444						0.0033										
15.3								0.0164															
16		0.0954	0.0526			0.1842	0.0148	0.0938		0.2039	0.0526		0.3322		0.0378		0.0559			0.0477			0.2615
16.1			0.0016															0.0033					

Figure 36 United States—African-American population (minimum allele frequency = 0.0082)

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
16.2							0.0329																
16.3								0.0691															
17		0.0230	0.1678			0.1645		0.0280		0.1957	0.0921		0.2237		0.0905		0.0115			0.0280			0.1924
17.1			0.0016																				
17.2							0.0033																
17.3			0.0049					0.0609															
18		0.0033	0.2632			0.1447		0.0049		0.0214	0.0477		0.0609		0.1365			0.0099		0.0164			0.1398
18.2																		0.0082					
18.3			0.0082					0.0164															
19			0.1365			0.0938		0.0016			0.1563		0.0049		0.1118			0.0658		0.0016			0.0658
19.1			0.0049																				
19.2																		0.0033					
19.3			0.0033					0.0049															
20			0.1184			0.0461					0.1036				0.0625			0.0674		0.0016			0.0214
20.2						0.0016												0.0033					
20.3								0.0016															
21			0.0658			0.0197					0.1168				0.0049			0.1234					0.0066
21.2																		0.0016					
21.3			0.0016												0.0016								
22			0.0461			0.0049					0.1283				0.0016			0.1760					
22.2																		0.0016					
23			0.0362			0.0033					0.0987				0.0066			0.1612					
23.3																		0.0033					
24			0.0066								0.0938				0.0033			0.1908					
25			0.0099								0.0707							0.0938					
26											0.0280							0.0428					
27									0.0576		0.0066							0.0296					
28									0.2549									0.0115					
29									0.1579														
29.3									0.0016														

United States—African-American population *(continued)*

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
30									0.2138									0.0016					
30.2									0.0132									0.0016					
31									0.0872														
31.2									0.0428														
32									0.0115														
32.2									0.0724														
33									0.0082														
33.2									0.0345														
34									0.0016														
35									0.0345														
36									0.0082														

United States—African-American population *(continued)*

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
Sample Size	376	376	376	376	376	376	376	376	376	376	376	376	376	376	376	376	376	376	376	376	376	376	376
5																				0.0426			
6																			0.0053	0.0013	0.1117		
7	0.0053			0.0013										0.0146	0.0040	0.0027			0.0080	0.0133	0.2739		
8	0.0013	0.0013		0.2301	0.0066			0.0013						0.0013	0.0027	0.1556	0.0027		0.0372	0.0027	0.0505	0.5133	
9	0.0545			0.1423	0.2779							0.0013		0.0572	0.0013	0.0505	0.0013		0.2979	0.0053	0.4973	0.1197	
9.1												0.0253				0.0027							
9.3																					0.0465		
10	0.2181	0.0013		0.1303	0.1184							0.2646		0.1915	0.0319	0.1569	0.0851		0.1423	0.0638	0.0186	0.0146	
10.1												0.0013											
11	0.2367			0.2766	0.2926	0.0040	0.0040	0.0598		0.2380		0.3511		0.3364	0.1316	0.3590	0.0718		0.1742	0.1104	0.0013	0.3245	
11.3												0.0372											
12	0.4122	0.0691		0.1689	0.1862	0.0346	0.0439	0.0465		0.0066		0.1423	0.0027	0.2380	0.1476	0.2327	0.1210		0.1569	0.1144		0.0253	
12.1	0.0013																						
12.2							0.0053																
12.3												0.0027											
13	0.0545	0.3471		0.0426	0.0997	0.2035	0.2620	0.0997		0.0040		0.0239		0.1516	0.1184	0.0372	0.2194	0.0013	0.1330	0.0585			
13.2							0.0572																
14	0.0093	0.2633		0.0080	0.0186	0.2074	0.2726	0.0811		0.0279		0.1370	0.0412	0.0080	0.1303	0.0027	0.1915		0.0306	0.1051		0.0027	0.2447
14.2							0.1170																
14.3														0.0013									
15	0.0066	0.2114	0.0160			0.1715	0.0864	0.3112		0.2660		0.0133	0.3684		0.0160		0.2008		0.0093	0.1077			0.0412
15.2							0.1024																
15.3								0.0040															
16		0.0864	0.0106			0.1316	0.0120	0.2101		0.2340	0.0080		0.3085		0.0066		0.0931		0.0053	0.0838			0.1662
16.2							0.0319																
16.3								0.0093															
17		0.0186	0.0944			0.0785		0.0851		0.1902	0.0638		0.1848		0.0279		0.0120			0.0745			0.2354
17.2							0.0040											0.0013					

Figure 37 United States—Asian-American population (minimum allele frequency = 0.0066)

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
17.3								0.0532															
18		0.0013	0.2367			0.0372		0.0106		0.0319	0.1144		0.0891		0.1955		0.0013	0.0306		0.0718			0.1822
18.2							0.0013								0.0013								
18.3								0.0199															
19			0.1862			0.0399		0.0027		0.0013	0.1582		0.0053		0.1383			0.0346		0.0505			0.1130
19.3			0.0013					0.0053															
20			0.1662			0.0359					0.0931				0.0386			0.0612		0.0346			0.0146
21			0.1011			0.0239					0.0399				0.0040			0.1130		0.0239			0.0027
21.1						0.0013																	
21.3															0.0027								
22			0.1051			0.0213					0.0492				0.0013			0.1862		0.0239			
23			0.0532			0.0013					0.2168							0.2420		0.0080			
23.2																		0.0080					
24			0.0133			0.0040					0.1622							0.1569		0.0027			
24.2																		0.0066					
25			0.0120			0.0040					0.0745							0.1064		0.0013			
26			0.0027								0.0160							0.0426					
27			0.0013						0.0013		0.0027							0.0066					
28									0.0532		0.0013							0.0027					
28.2									0.0080														
29									0.2620														
29.2									0.0013														
30									0.2553														
30.2									0.0160														
30.3									0.0066														
31									0.1144														
31.2									0.0798														
32									0.0279														
32.2									0.1157														
33									0.0027														

United States—Asian-American population *(continued)*

United States—Asian-American population *(continued)*

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
33.2									0.0532														
34.2									0.0013														
35.2									0.0013														

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
Sample Size	337	337	337	337	337	337	337	337	337	337	337	337	337	337	337	337	337	337	337	337	337	337	337
2.2																			0.0015				
5																				0.0682	0.0015		
6																			0.0015	0.0030	0.2255	0.0015	
7				0.0015												0.0119			0.0074	0.1528	0.1780		
8	0.0030			0.1098	0.0148									0.0074		0.1588	0.0193		0.0089	0.0119	0.1098	0.5059	
9	0.0267			0.0742	0.1291							0.0059		0.0534		0.1662	0.0134		0.1855	0.0148	0.1706	0.1276	
9.3																					0.3056		
10	0.2849			0.0682	0.0415	0.0119	0.0015	0.0030		0.0030		0.1988		0.0534	0.0074	0.2745	0.1083		0.1350	0.1068	0.0089	0.0490	
11	0.3056	0.0059		0.2834	0.3190	0.0089		0.0623		0.1380		0.3412	0.0030	0.3442	0.3086	0.2166	0.0653		0.1602	0.1246		0.2789	
11.3												0.0504											
12	0.3131	0.0341		0.3071	0.3056	0.1528	0.0653	0.1602		0.0045		0.0401		0.3724	0.2567	0.1454	0.1558		0.2181	0.1780		0.0371	
12.1							0.0015																
12.2							0.0015																
12.3												0.0015											
13	0.0638	0.2982		0.1113	0.1706	0.1187	0.2760	0.0697		0.0074	0.0015	0.0326	0.0015	0.1484	0.0742	0.0237	0.3249		0.2062	0.0831			0.0015
13.2							0.0163																
14	0.0030	0.2997		0.0445	0.0178	0.1751	0.3501	0.0623		0.0371	0.0015	0.2849	0.1528	0.0178	0.0490	0.0015	0.1914		0.0579	0.0519			0.0861
14.1																			0.0015				
14.2							0.0223																
14.3								0.0030															
15		0.1914	0.0445		0.0015	0.1528	0.1662	0.1528		0.3650	0.0015	0.0430	0.2774	0.0030	0.0134	0.0015	0.0905		0.0134	0.0475			0.1246
15.2							0.0356																
15.3								0.0890															
16		0.1320	0.0341			0.1098	0.0549	0.0979		0.3680	0.0430	0.0015	0.2329		0.0045		0.0282	0.0015	0.0030	0.0519			0.2166
16.2							0.0030																
16.3								0.0490															
17		0.0356	0.0994			0.1098	0.0030	0.0445		0.0712	0.1869		0.1988		0.0653		0.0030	0.0015		0.0415			0.2760
17.1								0.0030															

Figure 38 United States—Caucasian population (minimum allele frequency = 0.0074)

United States—Caucasian population *(continued)*

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
17.2							0.0015																
17.3			0.0208					0.1246															
18		0.0030	0.1632			0.0861	0.0015	0.0030		0.0059	0.0846		0.1187		0.0638			0.0119		0.0326			0.1840
18.3			0.0223					0.0579															
19			0.1202			0.0445					0.1454		0.0148		0.1039			0.0549		0.0119			0.0979
19.3			0.0059					0.0178															
20			0.0994			0.0134					0.1573				0.0341			0.1513		0.0148			0.0134
20.2																		0.0045					
20.3			0.0015																				
21			0.1365			0.0104					0.0282				0.0119			0.1810		0.0015			
21.2																		0.0030					
21.3															0.0045								
22			0.1068			0.0030					0.0178				0.0015			0.1899					
22.2																		0.0089					
23			0.0816			0.0030					0.0979							0.1469					
23.2																		0.0045					
23.3															0.0015								
24			0.0386								0.1009							0.1454		0.0015			
25			0.0208								0.1128							0.0697		0.0015			
26			0.0030						0.0030		0.0178							0.0178					
27			0.0015						0.0282		0.0030							0.0059					
28									0.1632									0.0015					
29									0.2389														
29.2									0.0015														
29.3									0.0015														
30									0.2389														
30.2									0.0267														
31									0.0682														
31.2									0.0890														
32									0.0237														

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
32.2									0.0920														
33.2									0.0208														
34.2									0.0045														

United States—Caucasian population *(continued)*

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
Sample Size	382	382	382	382	382	382	382	382	382	382	382	382	382	382	382	382	382	382	382	382	382	382	382
2.2																			0.0223				
3.2																			0.0026				
5																			0.0079	0.0524			
6					0.0013															0.0013	0.2788	0.0079	
7	0.0092		0.0026											0.0537		0.0118			0.0052	0.1021	0.3207	0.0013	
8	0.0052	0.0013		0.0916	0.0183									0.0131		0.1139	0.0065		0.0406	0.0380	0.0903	0.4830	
9	0.0236	0.0013		0.1597	0.1047	0.0013		0.0013				0.0013	0.0013	0.0484		0.0798	0.0026		0.1767	0.0118	0.1230	0.0733	
9.3			0.0013																		0.1715		
10	0.2605	0.0013		0.0942	0.1558	0.0065	0.0039	0.0026		0.0052		0.3089		0.0471	0.0105	0.2579	0.0982		0.1832	0.0694	0.0144	0.0563	
10.3																0.0013							
11	0.2814	0.0026		0.2277	0.3207	0.0118	0.0144	0.0366		0.0746		0.3220		0.3822	0.1649	0.2906	0.0510		0.1296	0.0668		0.2631	0.0013
11.1	0.0013																						
11.2							0.0026																
11.3												0.0432				0.0013							
12	0.3691	0.0445		0.2866	0.2435	0.1086	0.0812	0.0916		0.0105		0.0419	0.0013	0.2958	0.1715	0.1963	0.1257		0.1819	0.1688	0.0013	0.1086	0.0026
12.2							0.0144																
12.3												0.0039											
13	0.0419	0.2592	0.0013	0.0929	0.1401	0.1178	0.1872	0.0681		0.0092		0.0170	0.0039	0.1492	0.1047	0.0419	0.3220	0.0013	0.1688	0.1008		0.0013	0.0013
13.2							0.0707																
14	0.0052	0.3547	0.0013	0.0432	0.0131	0.1558	0.3010	0.1099		0.0223		0.2264	0.0877	0.0105	0.1427	0.0052	0.2356		0.0589	0.0707		0.0013	0.0654
14.2						0.0013	0.0445																
14.3								0.0026															
15	0.0026	0.2291	0.0366	0.0013	0.0026	0.1257	0.1309	0.1636		0.4254		0.0327	0.3442		0.0445		0.1178		0.0183	0.0812			0.0969
15.2						0.0013	0.0733																
15.3								0.0275															
16		0.0812	0.0497			0.1257	0.0406	0.1571		0.3599	0.0366	0.0026	0.2723		0.0065		0.0340		0.0013	0.0602			0.3076
16.1								0.0026															
16.2							0.0249																

Figure 39 United States—Hispanic population (minimum allele frequency = 0.0065)

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
16.3								0.0537															
17		0.0236	0.0733	0.0026		0.1636	0.0065	0.0707		0.0798	0.1780		0.1780		0.0537		0.0065			0.0576			0.2709
17.1			0.0026																				
17.2							0.0039																
17.3			0.0118					0.1558															
18			0.2003			0.0851		0.0065		0.0118	0.0681		0.1021		0.0995			0.0065		0.0340			0.1819
18.3			0.0209					0.0432															
19			0.1885			0.0380					0.1846		0.0092		0.0720			0.0694	0.0026	0.0183			0.0654
19.3			0.0105					0.0065							0.0039								
20			0.1688			0.0183				0.0013	0.1361				0.0432			0.0903		0.0301			0.0065
20.2																		0.0026					
20.3															0.0118								
21			0.0916			0.0236					0.0366				0.0105			0.1401		0.0209			
21.3															0.0432								
22			0.0668			0.0065					0.0628							0.1387		0.0105			
22.2																		0.0065					
22.3															0.0131								
23			0.0366			0.0052					0.1401							0.1243		0.0052		0.0039	
23.2																		0.0039					
23.3															0.0039								
24		0.0013	0.0183			0.0026					0.0838							0.1649					
24.2									0.0026														
25			0.0118			0.0013					0.0550							0.1401					
26			0.0026						0.0039		0.0157							0.0681					
27			0.0026						0.0144		0.0013							0.0301					
28									0.1139		0.0013							0.0079					
28.2									0.0013														
29									0.2107									0.0039					
30									0.2723									0.0013					
30.2									0.0144														

United States—Hispanic population *(continued)*

Allele	CSF1PO	D10S1248	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D6S1043	D7S820	D8S1179	FGA	PentaD	PentaE	TH01	TPOX	vWA
31									0.0497														
31.2									0.1139														
32									0.0157														
32.2									0.1257														
33									0.0013														
33.2									0.0550														
35									0.0026														
36									0.0013														
38									0.0013														

United States—Hispanic population *(continued)*

Table 15 Random Match Probability (RMP) values for VeriFiler™ Plus kit markers

Allele	Combined China	African-American	Caucasian	Asian-American	Hispanic
CSF1PO	0.1168	0.0792	0.1289	0.1221	0.1289
D10S1248	0.0938	0.0672	0.0931	0.1007	0.0931
D12S391	0.0427	0.0362	0.0198	0.0408	0.0198
D13S317	0.0643	0.1363	0.0735	0.0675	0.0735
D16S539	0.0799	0.0727	0.0981	0.0836	0.0981
D18S51	0.0343	0.0300	0.0289	0.0366	0.0289
D19S433	0.0526	0.0361	0.0895	0.0549	0.0895
D1S1656	0.0440	0.0315	0.0195	0.0490	0.0195
D21S11	0.0509	0.0419	0.0466	0.0508	0.0466
D22S1045	0.0883	0.0504	0.1367	0.0844	0.1367
D2S1338	0.0318	0.0198	0.0279	0.0321	0.0279
D2S441	0.0858	0.0996	0.0976	0.0897	0.0976

Table 15 Random Match Probability (RMP) values for VeriFiler Plus kit markers *(continued)*

Allele	Combined China	African-American	Caucasian	Asian-American	Hispanic
D3S1358	0.1262	0.1033	0.0757	0.1222	0.0757
D5S818	0.0870	0.0952	0.1290	0.0907	0.1290
D6S1043	0.0287	0.0248	0.0584	0.0313	0.0584
D7S820	0.0799	0.0747	0.0678	0.0905	0.0678
D8S1179	0.0449	0.0700	0.0604	0.0462	0.0604
FGA	0.0345	0.0301	0.0370	0.0393	0.0370
Penta D	0.0594	0.0237	0.0530	0.0577	0.0530
Penta E	0.0115	0.0188	0.0199	0.0121	0.0199
TH01	0.1490	0.0885	0.0811	0.1641	0.0811
TPOX	0.2129	0.0860	0.1785	0.2141	0.1785
vWA	0.0674	0.0602	0.0624	0.0641	0.0624
Cumulative	**1.34×10^{-28}**	**2.13×10^{-30}**	**1.24×10^{-28}**	**3.09×10^{-28}**	**1.24×10^{-28}**

Probability of paternity exclusion observation

If your laboratory requires the probability of paternity exclusion calculation, contact your Thermo Fisher Scientific HID representative.

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, including the IQC markers	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. (Do not use a compression pad with the 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.

Observation	Possible cause	Recommended action
Positive signal from DNA Control 007 but partial or no signal from DNA test samples when IQC peaks are present and balanced	The quantity of test DNA sample is below the assay sensitivity.	Quantify DNA and (when possible) add 500 pg of DNA. For low concentration samples, add up to 17.5 µL of the DNA sample to the reaction mix.
	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 consider using the Precision ID GlobalFiler™ NGS STR Panel.
	The test sample was diluted in the wrong buffer (for example, a TE buffer with an incorrect EDTA concentration).	Redilute DNA using nuclease-free water or low-TE buffer (with 0.1 mM EDTA).
Positive signal from DNA Control 007 but partial or no signal from DNA test samples when IQC peaks are present and unbalanced	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 was diluted in the wrong buffer (for example, a TE buffer with an incorrect EDTA concentration).	Redilute DNA using nuclease-free water or low-TE buffer (with 0.1 mM EDTA).
Positive signal from DNA Control 007 and elevated signal from DNA test samples when IQC peaks are present and unbalanced	The quantity of the test sample DNA is in excess of recommended input amount, which can cause loss of balance in IQC peaks.	Quantify DNA, then use 500 pg.
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.	Ensure that you apply the provided locus-specific stutter filters.
	Stutter filters apply to sample input amounts equal or greater than 250 pg. Samples well below the recommended input amount may exhibit stutters values exceeding the filters due to the stochastic effects of the PCR.	Increase the sample input above 250 pg, if possible. (Note, the optimum sample input is 500 pg.)
	The test sample contained mixed DNA.	If a mixed profile is not expected, check that laboratory protocols relating to cleanliness are followed.

Observation	Possible cause	Recommended action
More than two alleles present at a locus *(continued)*	Incomplete 3´ A base addition (n–1 nt position) occured.	Include the final extension step of 60°C for 5 minutes in the PCR.
		Remove amplified plate from storage (thaw if necessary) and place on thermal cycler at 60°C for 15 minutes.
		Check the quantity of the original sample DNA to ensure input is less than 750 pg per reaction. Adjust input as necessary during re-amplification.
		If the total amount of DNA in the reaction exceeds 1 ng, adjust the final extension time to 15 minutes to minimize incomplete 3′ A base addition.
	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.
		Check that you are using the recommended injection conditions on the instrument.
	Poor spectral separation occurred.	Perform a spectral calibration.
		Confirm that Filter Set J6-T modules are installed and used for analysis.
	Too much DNA was present in the reaction.	Use the recommended amount of template DNA: 500 pg for 29 PCR cycles.
	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 DNA quantity was too low, leading to stochastic effects.	Load more DNA sample or concentrate the DNA.
STR profiles contain many off-scale alleles	DNA quantification was not performed or not accurate.	Ensure that DNA quantification is accurate.

B Materials required but not supplied

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

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
Hi-Di™ Formamide, 25-mL	4311320
Invitrogen™ Nuclease-Free Water (not DEPC-Treated)	AM9937
(Optional) Invitrogen™ TE Buffer (low-TE buffer; 10 mM Tris-HCl pH 8.0 and 0.1 mM EDTA)	12090015 or see "(Optional) Prepare low-TE buffer" on page 22.

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

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 Series Data Collection Software 4, v4.0.1 software patch	A40059 or A40376[1]
3500 Series Data Collection Software 3.1 Upgrade (RUO)	A26287[1]
3500 Series Data Collection Software 3.1 (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-37 Matrix Standard Kit (Dye Set J6-T)	A31234
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

(continued)

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 required materials

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	4478404
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3130/3130*xl* Genetic Analyzer 16-Capillary Array, 36 cm	4315931
POP-4™ Polymer for the 3130/3130*xl* Genetic Analyzer	4352755
Running Buffer, 10×	402824
DS-37 Matrix Standard Kit (Dye Set J6-T)	A31234
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

C

PCR work areas

Work area setup and lab design

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

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

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

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

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

PCR setup work area materials

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

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

- 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 “Instruments and software compatibility” on page 17.

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.

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	
VeriFiler™ Plus PCR Amplification Kit—PCR Amplification and CE Quick Reference	MAN0017495
VeriFiler™ Plus PCR Amplification Kit—PCR Setup Quick Reference	MAN0017494
Quantification kits	
Quantifiler™ HP and Quantifiler™ Trio DNA Quantification Kits User Guide	4485354
Thermal cyclers	
ProFlex™ PCR System User Guide	MAN0007697
Veriti™ Thermal Cycler User Guide	4375799
GeneAmp™ PCR System 9700 Base Module User Manual	4303481
Direct Amplification of Reference Samples Using the VeriFiler™ Plus PCR Amplification Kit	—
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	—
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 3.1 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*xl* 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

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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

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Document title	Pub. No.
GeneMapper™ ID-X Software v1.5 Installation Guide	100031706
GeneMapper™ ID-X Software v1.5 Administrator Guide	100031703
GeneMapper™ ID-X Software v1.5 Reference Guide	100031707
GeneMapper™ *ID-X* Software v1.6	
GeneMapper™ ID-X Software v1.6 New Features and Software Verification User Bulletin	100073905
Precision ID Ancestry Panel and Precision ID Identity Panel	
Precision ID Panels with the Ion S5™ System Application Guide	MAN0015831

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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