applied biosystems

Yfiler™ Plus PCR Amplification Kit

USER GUIDE

Catalog Numbers 4484678 and 4482730

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Revision D

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Revision history: Pub. No. 4485610

Revision	Date	Description
D	10 January 2019	• Throughout the user guide, update DYS387S1 to DYF387S1. • In Table 2 on page 13, update the number of primer set tubes to 2 tubes for Cat. No. 4484678. • In "Perform PCR" on page 28, update the cycle number recommendation to "CYCLE (Direct Amplification 26–29) (Extracted DNA 30)". • Update the following stutter filter values to reflect rounding used in the analysis files: – In Table 3 on page 75, 2 nt stutter filters for DYS19 and DYS481; minus stutter filter for DYS391. – In Table 4 on page 76, plus stutter filters for DYS635, DYS392, DYS570.
C	27 December 2016	Add the following information to Chapter 5: • Example electropherograms of DYS437 artifacts • Table of % minus and % plus stutter which includes minimum, maximum, and mean stutter values Non-technical changes: reorganized content
B	October 2014	Add Chapter 5, Experiments and Results.
A	July 2014	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™ Yfiler™ Plus PCR Amplification Kit is a 6-dye, short tandem repeat (STR) multiplex assay optimized to allow amplification from multiple male-specific sample types such as male-male, male-female mixtures. The kit amplifies 27 Y-STR loci, see Table 1.

The Yfiler™ Plus PCR Amplification Kit uses the same improved process for synthesis and purification of the amplification primers that are previously developed for other next-generation Thermo Fisher Scientific STR chemistries. The improved amplification primers deliver clean electrophoretic backgrounds that help interpretation.

Single-source sample types supported

The Yfiler™ Plus PCR Amplification Kit is optimized to allow direct amplification from the following types of single-source samples without the need for sample purification:

- Blood and buccal samples on treated paper substrates.
- Blood samples collected on untreated paper substrates and treated with Prep-n-Go™ Buffer.
- Buccal samples collected on swab substrates and treated with Prep-n-Go™ Buffer.

About the primers

Non-nucleotide linkers are used in primer synthesis for the following loci: DYS389I/II, DYS635, DYS627, DYS19, YGATAH4, DYS448, DYS391, DYS390, DYS438, DYS391, DYS390, DYS438, DYS392, DYS518, DYS437, and DYS449.

For these primers, non-nucleotide linkers are placed between the primers and the fluorescent dye during oligonucleotide synthesis (Butler 2005, Grossman *et al*., 1994). Non-nucleotide linkers enable reproducible positioning of the alleles to facilitate interlocus spacing. The combination of a 6-dye fluorescent system and the use of non-nucleotide linkers allows simultaneous amplification and efficient separation of the 27 Y-STR loci during automated DNA fragment analysis.

Dyes used in the kit

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

Loci amplified by the kit

Table 1 Yfiler™ Plus PCR Amplification Kit loci and alleles

Locus designation	Alleles included in Allelic Ladder	Dye label	DNA Control 007
DYS576	10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25	6-FAM™	19
DYS389I	9, 10, 11, 12, 13, 14, 15, 16, 17		13
DYS635	15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30		24
DYS389II	24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35		29
DYS627	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27		21
DYS460	7, 8, 9, 10, 11, 12, 13, 14	VIC™	11
DYS458	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24		17
DYS19	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19		15
YGATAH4	8, 9, 10, 11, 12, 13, 14, 15		13
DYS448	14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24		19
DYS391	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16		11
DYS456	10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24	NED™	15
DYS390	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29		24
DYS438	6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16		12
DYS392	4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20		13
DYS518	32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49		37
DYS570	10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26	TAZ™	17
DYS437	10, 11, 12, 13, 14, 15, 16, 17, 18		15
DYS385 a/b	6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28		11,14
DYS449	22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40		30
DYS393	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18	SID™	13
DYS439	6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17		12
DYS481	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32		22
DYF387S1	30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44		35,37
DYS533	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17		13

Standards and controls that are required

For the Yfiler™ Plus PCR Amplification 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 Yfiler™ Plus Allelic Ladder. DNA Control 007 is present in the kit. See "DNA Control 007 profile" on page 12.
- **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.
- **Yfiler™ 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 "Allelic ladder profile" on page 11.

Allelic ladder profile

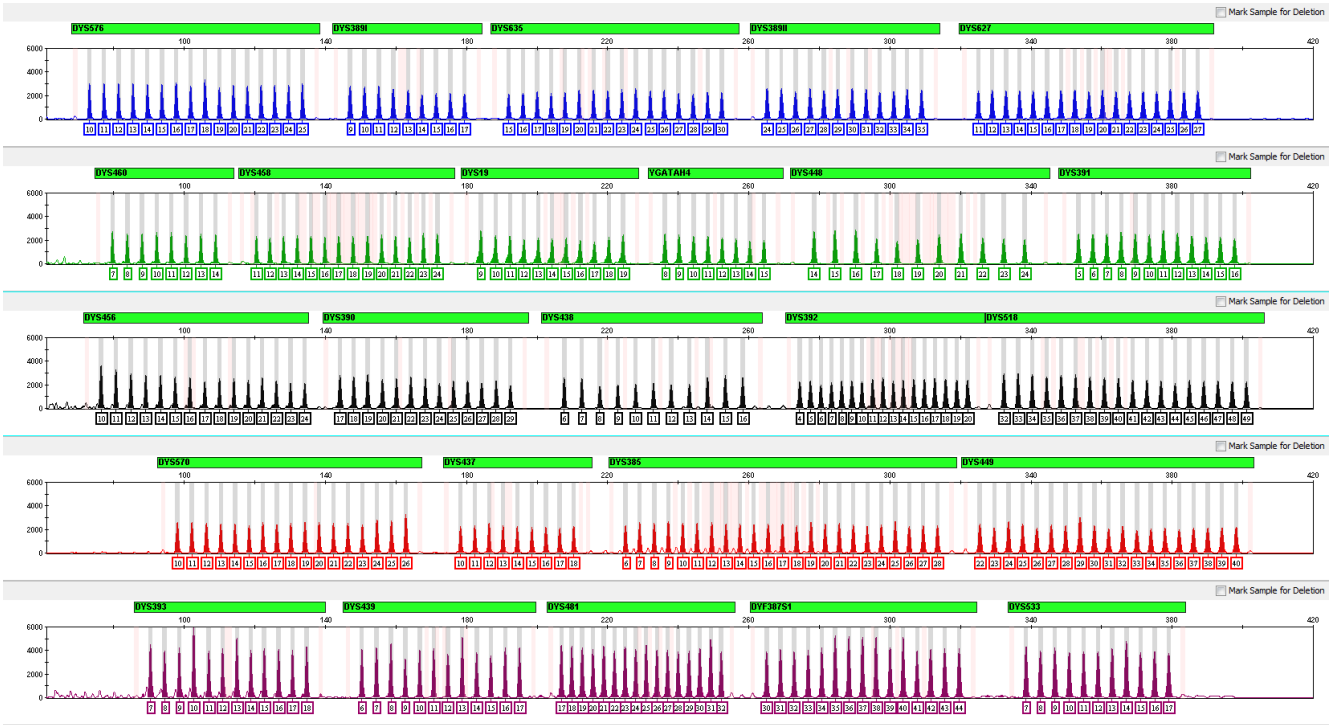


Figure 1 GeneMapper™ *ID-X* Software plot of the Yfiler™ Plus Allelic Ladder (Y-axis scale 0 to 6,000 RFU).

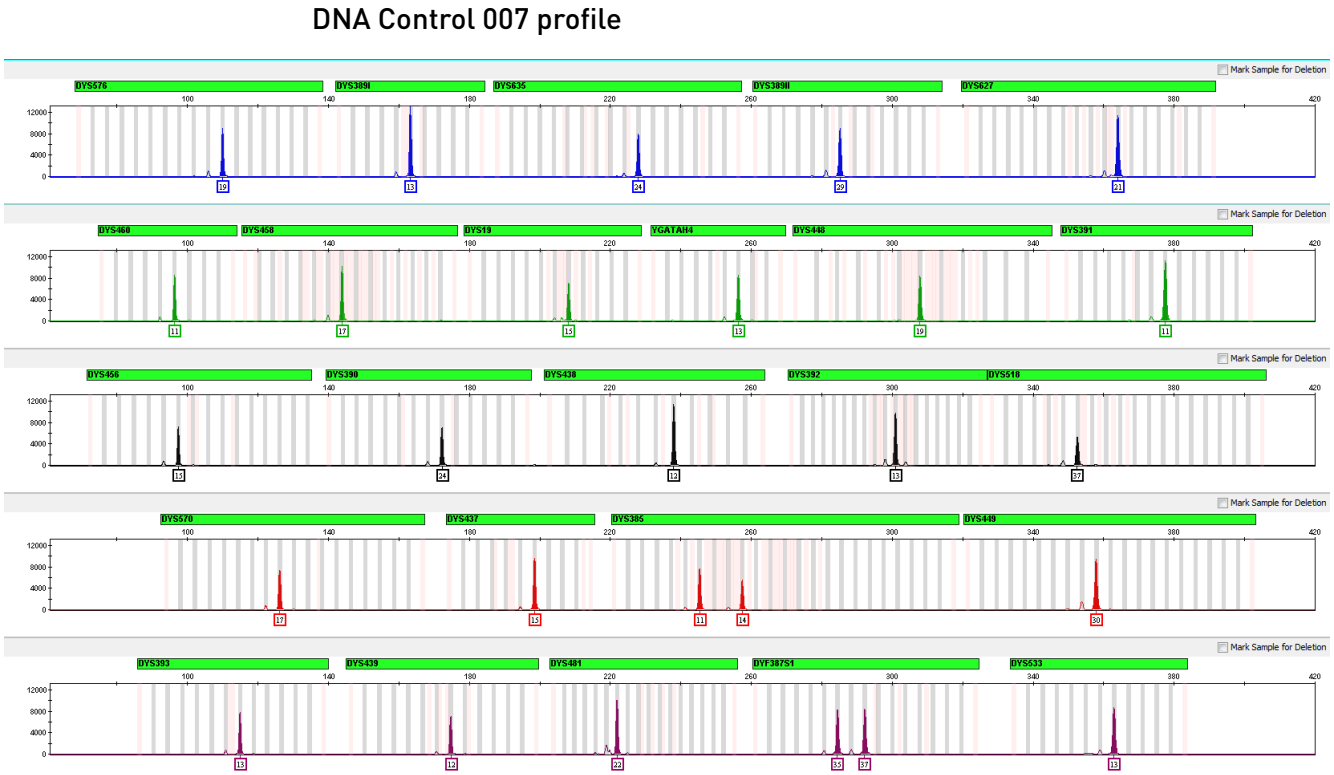


Figure 2 DNA Control 007 (1 ng) amplified with the Yfiler™ Plus PCR Amplification Kit and analyzed on an Applied Biosystems™ 3500xL Genetic Analyzer (Y-axis scale 0 to 12,000 RFU).

Contents and storage

The Yfiler™ Plus PCR Amplification Kit contains sufficient quantities of the following reagents to perform: 100 (Cat. No. 4484678) or 500 (Cat. No. 4482730) amplifications at 25 µL/amplification.

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

IMPORTANT! The fluorescent dyes that are 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 2 Yfiler™ Plus PCR Amplification Kit

Contents	100 reactions (Cat. No. 4484678)	500 reactions (Cat. No. 4482730)	Storage
Yfiler™ Plus Master Mix Contains $MgCl_2$, dATP, dGTP, dCTP, and dTTP, bovine serum albumin, enzyme, and 0.05% sodium azide in buffer and salt.	2 × 0.5 mL	4 × 1.25 mL	–25°C to –15°C on receipt. 2°C to 8°C after first use.
Yfiler™ Plus Primer Set Contains locus-specific 6-FAM™, VIC™, NED™, TAZ™, and SID™ dye-labeled and unlabeled primers in buffer. The primers amplify the Y-STR loci DYS19, DYS385 a/b, DYF387S1 a/b, DYS389 I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS449, DYS456, DYS458, DYS460, DYS481, DYS518, DYS533, DYS570, DYS576, DYS627, DYS635 (Y GATA C4), and Y GATA H4.	2 × 0.25 mL	2 × 1.25 mL	–25°C to –15°C on receipt. 2°C to 8°C after first use. Store protected from light.

Contents	100 reactions (Cat. No. 4484678)	500 reactions (Cat. No. 4482730)	Storage
Yfiler™ Plus Allelic Ladder Contains the following amplified alleles: • 6-FAM™ dye (blue): DYS389I 9–17; DYS389II 24–35; DYS576 10–25; DYS627 11–27; DYS635 15–30. • VIC™ dye (green): DYS19 10–19; DYS391 5–16; DYS448 14–24; DYS458 11–24; DYS460 7–14; Y GATA H4 8–15. • NED™ dye (yellow): DYS390 17–29; DYS392 4–20; DYS438 6–16; DYS456 10–24; DYS518 32–49. • TAZ™ dye (red): DYS385 a/b 32–49; DYS437 10–18; DYS449 22–40; DYS570 10–26. • SID™ dye (purple): DYF387S1 a/b 30–44; DYS393 7–18; DYS439 6–17; DYS481 17–32; DYS533 7–17.	2 × 0.025 mL	2 × 0.05 mL	–25°C to –15°C on receipt. 2°C to 8°C after first use. Store protected from light. **IMPORTANT!** The allelic ladder contains PCR products. Do not amplify. To avoid contamination, store the allelic ladder separate from the other kit components and unamplified DNA.
DNA Control 007 Contains 2 ng/µL of human male genomic DNA in 0.05% sodium azide and buffer.	1 × 0.05 mL	2 × 0.05 mL	–25°C to –15°C on receipt. 2°C to 8°C after first use.

Required materials not supplied

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

Instruments and software compatibility

Instrument type	Validated models
Thermal cyclers	• ProFlex™ 96-well PCR System (Cat. No. 4484075) • 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) **IMPORTANT!** The Yfiler™ Plus kit is NOT validated for use with: • ProFlex™ 2 × Flat PCR System (Cat. No. 4484078) • ProFlex™ 2 × 384-well PCR System (Cat. No. 4484077) • Veriti™ 96-Well *Fast* Thermal Cycler (Cat. No. 4375305) • GeneAmp™ PCR System 9700 with the aluminium 96-well block (Cat. No. 4314879)
Genetic analyzers[1]	• 3500/3500xL Genetic Analyzer with any of the following: – 3500 Data Collection Software v1 (Windows™ Vista operating system) and HID Updater 3500 Data Collection Software v2 (Cat. No. 4480670) – 3500 Data Collection v2 Software (Windows™ 7 operating system) and HID Updater 3500 Data Collection Software v2 (Cat. No. 4480670) – 3500 Data Collection v3 Software (Windows™ 7 operating system) • 3130/3130*xl* Genetic Analyzer with: – Data Collection Software v4 (Windows™ 7 operating system) – 3130/3730 Data Collection v4 6-Dye Module v1
Analysis software	GeneMapper™ *ID-X* Software v1.4 or later Windows™ XP or Windows™ 7 operating system

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

Workflow

Process casework (extracted DNA) samples with the Yfiler™ Plus PCR Amplification Kit

"Extracted DNA: Prepare the amplification kit reactions" on page 19

Process database (direct amplification) samples with the Yfiler™ Plus PCR Amplification Kit

"Direct amplification: Treated and untreated paper substrates" on page 22 or

"Direct amplification: Swab substrates" on page 24

▼

"Perform PCR" on page 28

For compatible thermal cyclers, see "Instruments and software compatibility" on page 15

▼

Perform electrophoresis

"Set up the 3500/3500xL instruments for electrophoresis (before first use of the kit)" on page 30 or

"Set up the 3130/3130xl instruments for electrophoresis (before first use of the kit)" on page 35 or

▼

"Prepare samples for electrophoresis (3500 Series and 3130 Series instruments)" on page 34

▼

Analyze data

"Set up the GeneMapper™ ID-X Software for analysis (before first use of the kit)" on page 41

▼

"Create an analysis method" on page 46

▼

"Create a size standard definition file if needed" on page 54

▼

"Analyze and edit sample files with GeneMapper™ ID-X Software" on page 56

▼

"Examine or edit a project" on page 57

2 Perform PCR

Extracted DNA: DNA quantification

Importance of quantification before STR analysis

DNA quantification can be used to determine:

- If the sample contains sufficient human DNA and/or human male DNA to proceed with short tandem repeat (STR) analysis.
- The amount of sample to use in STR analysis applications.
- For the Quantifiler™ Trio DNA Quantification Kit, the relative quantities of human male and female DNA in a sample that can help in the selection of the appropriate STR chemistry.
- The DNA quality, regarding inhibition level (all kits) or the inhibition level and the DNA degradation level (Quantifiler™ HP DNA Quantification Kit and Quantifiler™ Trio DNA Quantification Kit only). This metric is useful for determining the likelihood of recovery of STR loci with larger amplicon sizes.
- Highly degraded samples that cannot be recovered by STR analysis with capillary electrophoresis can be analyzed with the Precision ID NGS System and Panels. Optimized for degraded samples, the Precision ID Identity Panel provides discrimination of individuals similar to STR genotype match probabilities. Also, the Precision ID Ancestry Panel infers biogeographical ancestry for investigative leads.
- If PCR inhibitors are present in a sample that may require additional purification before proceeding to STR analysis.

Effect of DNA quantity on results

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

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

Off-scale data is a problem because:

  - Quantification (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause the corresponding stutter peak to appear higher in relative intensity, thus increasing the calculated percent stutter.
  - Multicomponent analysis of off-scale data is not accurate. This inaccuracy results in poor spectral separation ("pull-up").
- Incomplete +A nucleotide addition.

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

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

Methods of quantifying DNA

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

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

Kit and user guide	Detects	How it works
Quantifiler™ HP DNA Quantification Kit (Cat. No. 4482911) For more information, see *Quantifiler™ HP and Quantifiler™ Trio DNA Quantification Kits User Guide* (Pub. No. 4485354)	• Total human DNA (two targets—one small amplicon and one larger amplicon) • Degraded DNA	• Uses 5′ nuclease assays with multiple-copy target loci, for improved detection sensitivity:[1] – The human-specific target loci are multiple copy, and dispersed on various autosomal chromosomes. – The primary quantification targets have relatively short amplicons (75 to 80 bases), to improve the detection of degraded DNA samples. • Uses features that maximize consistency of quantification: – Genomic targets have conserved primer- and probe-binding sites. – Minimal copy number variation between different individuals and population groups. • Contains a Large Autosomal target with a longer amplicon (>200 bases) to help determine if a DNA sample is degraded.
Quantifiler™ Trio DNA Quantification Kit (Cat. No. 4482910) For more information, see *Quantifiler™ HP and Quantifiler™ Trio DNA Quantification Kits User Guide* (Pub. No. 4485354)	• Total human DNA (two targets—one small amplicon and one larger amplicon) • Human male DNA • Degraded DNA	

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

Extracted DNA: Before you begin

Thaw reagents (before first use of the kit)

Thaw the Master Mix and Primer Set.

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

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

Extracted DNA: Prepare the amplification kit reactions

Prepare low-TE buffer

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

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

   Note: Adjust the volumes accordingly for specific needs.

2. Aliquot, then autoclave the solutions.
3. Store the aliquots at room temperature.

Prepare the amplification kit reactions: extracted DNA

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

1. Vortex the Master Mix and Primer Set for 3 seconds. Before opening the tubes or bottles, remove droplets from the caps by centrifuging the tubes briefly or tapping the bottles on the bench.
2. Pipette the required volumes of components into an appropriately sized clear (non-colored) polypropylene tube:

Reaction component	Volume per reaction
Master Mix	10.0 µL
Primer Set	5.0 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 15 µL of reaction mixture into each reaction well of a MicroAmp™ Optical 96-Well Reaction Plate or each MicroAmp™ tube.
5. Prepare samples as shown in the following table, then add them to the appropriate well or tube (final reaction volume is 25 µL).

Sample	30-cycle protocol
Negative control	Add 10 µL of low-TE buffer.
Test sample	Add test DNA sample to a total amount of 1.0 ng.
Positive control	Add 007 control DNA to a total amount of 1.0 ng. **Note:** 007 control DNA contains 2 ng/µL of human male genomic DNA.

6. Seal the MicroAmp™ Optical 96-Well Reaction Plate with MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical Adhesive Film, or cap the tubes.
7. Centrifuge the tubes or plate at 3,000 rpm for about 20 seconds in a tabletop centrifuge (with plate holders, if using 96-well plates).
8. Amplify the samples as described in Chapter 2, "Perform PCR".

IMPORTANT! This kit is not validated for use with the GeneAmp™ PCR System 9700 with the aluminum 96-well block. Use of this thermal cycling platform may adversely affect performance of this kit.

Direct amplification: Optimize PCR cycle number

Before using the Yfiler™ Plus PCR Amplification Kit for the first time, perform a single initial sensitivity experiment. The experiment determines the appropriate cycle number to use during internal validation studies and operational use of the kit. It also accounts for instrument-to-instrument and sample-to-sample variations. If you are processing multiple sample type and substrate combinations (for example, buccal samples on treated paper and buccal samples on swabs), perform separate sensitivity experiments for each sample type and substrate to be used for testing.

Procedural guidelines: Optimize PCR cycle number

- Select 26 samples so that you can complete electrophoresis using a single 96-well plate. This minimizes the impact of run-to-run variation on the results. Examples of PCR and electrophoresis plate layouts are provided on page 117.
- To maximize result quality, prepare and amplify Plate 1, then repeat for Plates 2 and 3. Do not prepare all 3 plates before amplification.
- To minimize the effect of instrument-to-instrument variation, use the same thermal cycler to amplify all 3 plates.

Select samples and prepare plates

1. Select 26 of each sample+substrate type. Ensure that the selected samples represent a "typical" range of samples analyzed in your laboratory.
2. Prepare the samples and the reactions as described in the appropriate protocols later in this chapter. To complete amplification of three replicate plates, prepare sufficient PCR reagents.
3. Create the first of 3 identical PCR plates, see Appendix D, “Plate layouts“.
4. Amplify each plate using a different cycle number to determine the optimum conditions for use in your laboratory.

   Suggested cycle numbers for different sample type and substrate combinations are listed in the following table.

Sample type	Substrate
	Treated or untreated paper
Blood	27, 28, 29 cycles
Buccal	27, 28, 29 cycles

Note: To minimize the effect of instrument-to-instrument variation, use the same thermal cycler to amplify all three plates. To maximize result quality, prepare and amplify Plate 1 then repeat for Plates 2 and 3. Do not prepare all three plates simultaneously.

If the average peak heights fall outside of the range of values that are listed below, test higher or lower cycle numbers to attain optimal peak heights.

Determine optimum PCR conditions

1. Run the PCR products on the appropriate CE platform using the recommended protocol that is described in Chapter 3, “Perform electrophoresis“.
2. Based on the results of the sensitivity study, select the appropriate PCR cycle number for future experiments.

Our studies indicate the optimum PCR cycle number generates profiles with the following heterozygote peak heights, with no instances of allelic dropout and minimal occurrence of off-scale allele peaks:

Instrument	Heterozygous peak height
3500 Series	5,000–12,000 RFU
31xx	2,500–4,000 RFU

When amplifying single-source, unpurified samples, greater sample-to-sample variation in peak height can be observed than with purified samples. Careful optimization of the cycle number helps to minimize this variation.

Direct amplification: Before you begin

Thaw reagents (before first use of the kit)

Thaw the Master Mix and Primer Set.

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

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

Direct amplification: Treated and untreated paper substrates

Sample preparation guidelines: treated or untreated paper substrate

- Do not add water to the wells on the reaction plate before adding the punches. If you observe static issues with the paper discs, you can prepare and dispense the 25-µL reaction mix into the wells of the reaction plate before adding the punches.
- To ensure optimum peak intensity, make the punch as close as possible to the center of the sample. Increasing the size of the punch can cause inhibition during PCR amplification.
- For manual punching: Place the tip of a 1.2 mm Harris Micro-Punch on the card, hold the barrel of the Harris Micro-Punch (do not touch the plunger), gently press and twist 1/4-turn, then eject the punch in to the appropriate well on the reaction plate.
- For automated punching: See the User Guide of your automated or semiautomated disc punch instrument for proper guidance.
- For blood on untreated paper samples, add 2 µL of Prep-n-Go™ Buffer (for use with untreated paper substrates) on top of the 1.2-mm sample punch.

Prepare low-TE buffer

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

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

   Note: Adjust the volumes accordingly for specific needs.
2. Aliquot, then autoclave the solutions.
3. Store the aliquots at room temperature.

Prepare the reactions: treated or untreated paper substrates

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.

If this is the first time you are using the kit, follow the instructions in "Thaw reagents (before first use of the kit)" on page 19 before proceeding.

1. Add samples to the reaction plate:

To these wells ...	Add...	
Negative control	1.2 mm blank disc	
Test samples	1.2 mm sample disc	
Positive control **IMPORTANT!** Do not add a blank disc to the positive control well.	For 26 cycles	3 µL of Control DNA 007
	For 27 cycles	2 µL of Control DNA 007
	For 28 cycles	1 µL of Control DNA 007
	For 29 cycles	1 µL of Control DNA 007

Note: If the peak heights are too high or too low for your optimized cycle number, the suggested volumes of positive control can be adjusted.

2. Vortex the Master Mix and Primer Set for 3 seconds. Before opening the tubes or bottles, remove droplets from the caps by centrifuging the tubes briefly or tapping the bottles on the bench.
3. Pipet the required volumes of components into an appropriately sized polypropylene tube.

Reaction component	Volume per reaction
Master Mix	10.0 µL
Primer Set	5.0 µL
Low TE buffer	10.0 µL

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

IMPORTANT! This kit is optimized for a 25-µL PCR volume to overcome the PCR inhibition that is expected when amplifying unpurified samples. Using a lower PCR reaction volume may reduce the ability of the kit chemistry to generate full STR profiles.

4. Vortex the reaction mix for 3 seconds, then centrifuge briefly.
5. Dispense 25 µL of the reaction mix into each reaction well of a MicroAmp™ Optical 96-Well Reaction Plate.
6. Seal the plate with MicroAmp™ Clear Adhesive Film (Cat. No. 4306311) or MicroAmp™ Optical Adhesive Film (Cat. No. 4311971).

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

IMPORTANT! If you are using the GeneAmp™ PCR System 9700 with silver or gold-plated silver block 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. Other validated thermal cyclers do not require a compression pad.

7. Centrifuge the plate at 3,000 rpm for about 20 seconds in a tabletop centrifuge with plate holders.
8. Amplify the samples as described in Chapter 2, "Perform PCR".

IMPORTANT! This kit is not validated for use with the GeneAmp™ PCR System 9700 with the aluminum 96-well block. Use of this thermal cycling platform may adversely affect performance of this kit.

Direct amplification: Swab substrates

Sample preparation guidelines: swab substrate

- Detach each buccal swab head from the swab shaft before lysis.
- If you are using the heated lysis protocol, perform lysis in either of the following formats:
  - 1.5-mL tubes with a heat block (VWR™ Scientific Select dry heat block or similar)
  - PrepFiler™ 96-Well Processing Plates (Cat. No. 4392904)
  - Robbins Scientific™ Model 400 Hybridization Incubator or similar
  - Agilent™ Benchtop Rack for 200 µL Tubes/V Bottom Plates (metal) or similar (Cat. No. 410094)

    IMPORTANT! Do not use a plastic plate adaptor.
- For optimum performance, lyse the entire swab. If you need to preserve the sample, use half of the lysate prepared from the entire swab.

Prepare the sample lysate: room temperature

This protocol may improve the performance for challenging or aged samples.

1. Add 400 µL Prep-n-Go™ Buffer (Cat. No. 4471406) to 1.5-mL tubes or the appropriate wells of a PrepFiler™ 96-Well Processing Plate (Cat. No. 4392904).
2. Into each tube or well, put the entire head of each swab, then let stand for 20 minutes at room temperature (20°C to 25°C) to lyse the sample.
3. After 20 minutes, transfer the sample lysate out of the sample plate into tubes or plates for storage, then discard the deep-well plate containing the swab heads.

   Note: To minimize the risk of contamination, do not remove the swab heads from the sample lysate plate before transferring the lysate.
4. Go to "Prepare the reactions: swab substrate" on page 26 or "Store the sample lysate" on page 27.

Prepare the sample lysate: heat protocol

This protocol may improve the performance for challenging or aged samples.

1. Preheat the heat block to 90°C or the oven with metal plate adaptor to 99°C.
2. Add 400 µL Prep-n-Go™ Buffer (for buccal swabs, Cat. No. 4471406) to 1.5-mL tubes or the appropriate wells of a PrepFiler™ 96-Well Processing Plate (Cat. No. 4392904).
3. Into each tube or well, put the entire head of each swab. If you are using tubes, cap the tubes. Let the tubes or plate stand for 20 minutes in the preheated heat block or oven to lyse the sample.
4. After 20 minutes, remove the tubes or the deep-well plate from the heat block or oven.
5. Let the lysate stand at room temperature for at least 15 minutes to cool the lysate (for accurate pipetting).
6. Transfer the sample lysate out of the 1.5-mL tubes or sample plate into tubes or plates for storage. Discard the 1.5-mL tubes or deep-well plate containing the swab heads.

   Note: To minimize the risk of contamination, do not remove the swab heads from the sample lysate plate before transferring the lysate.
7. Go to "Prepare the reactions: swab substrate" on page 26 or "Store the sample lysate" on page 27.

Prepare the reactions: swab substrate

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.

If this is the first time you are using the kit, follow the instructions in "Thaw reagents (before first use of the kit)" on page 19 before proceeding.

1. Add Prep-n-Go™ Buffer (Cat. No. 4471406) to the control wells in the MicroAmp™ Optical 96-Well Reaction Plate:

To these wells ...	Add...	
Negative control	3 µL of Prep-n-Go™ Buffer	
Positive control	For 26 cycles	0 µL of Prep-n-Go™ Buffer
	For 27 cycles	1 µL of Prep-n-Go™ Buffer
	For 28 and 29 cycles	2 µL of Prep-n-Go™ Buffer

2. Vortex the Master Mix and Primer Set for 3 seconds. Before opening the tubes or bottles, remove droplets from the caps by centrifuging the tubes briefly or tapping the bottles on the bench.

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

Reaction component	Volume per reaction
Master Mix	10.0 µL
Primer Set	5.0 µL
Low TE buffer	10.0 µL

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

IMPORTANT! This kit is optimized for a 25-µL PCR volume to overcome the PCR inhibition that is expected when amplifying unpurified samples. Using a lower PCR reaction volume may reduce the ability of the kit chemistry to generate full STR profiles.

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

5. Dispense 25 µL of the reaction mix into each reaction well of a MicroAmp™ Optical 96-Well Reaction Plate.

   The final volume in each well is 28 µL (reaction mix plus Prep-n-Go™ Buffer or sample lysate or positive control).

6. Add samples to the reaction plate:

To these well(s) of a MicroAmp™ Optical 96-Well Reaction Plate...	Add...	
Test samples	3 µL of sample lysate	
Positive control	For 26 cycles	3 µL of Control DNA 007
	For 27 cycles	2 µL of Control DNA 007
	For 28 and 29 cycles	1 µL of Control DNA 007

Note: If peak heights are too high or too low for your optimized cycle number, the suggested volumes of positive control can be adjusted.

7. Seal the plate with MicroAmp™ Clear Adhesive Film (Cat. No. 4306311) or MicroAmp™ Optical Adhesive Film (Cat. No. 4311971).

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

IMPORTANT! If you are using the GeneAmp™ PCR System 9700 with silver or gold-plated silver block 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. Other validated thermal cyclers do not require a compression pad.

8. Vortex the reaction mix at medium speed for 3 seconds.

9. Centrifuge the plate at 3,000 rpm for about 20 seconds in a tabletop centrifuge with plate holders.

10. Amplify the samples as described in Chapter 2, "Perform PCR".

IMPORTANT! This kit is not validated for use with the GeneAmp™ PCR System 9700 with the aluminum 96-well block. Use of this thermal cycling platform may adversely affect performance of this kit.

Store the sample lysate

1. Cap the sample lysate storage tubes or seal the sample lysate storage plate with MicroAmp™ Clear Adhesive Film.

2. Store the sample lysate as needed:

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

Note: The effects of multiple freeze/thaw cycles on the lysate have not been fully evaluated. Therefore, multiple freeze/thaw cycles are not recommended.

Perform PCR

IMPORTANT! This kit is validated for use with the thermal cyclers listed in "Instruments and software compatibility" on page 15 .

1. Program the thermal cycling conditions.

   IMPORTANT!
   - If you are using the ProFlex™ 96-well PCR System, then run in the GeneAmp™ **PCR System 9600 Simulation Mode**.
     For instructions on how to configure the ProFlex™ 96-well PCR System to run GeneAmp™ PCR System 9600 Simulation Mode, see the *ProFlex™ PCR System User Guide* (Cat. No. MAN0007697).
   - If you are using the GeneAmp™ PCR System 9700 with a 96-well silver or gold-plated silver block, then select **9600 Emulation Mode**.
     For instructions on how to configure the GeneAmp™ PCR System 9700 to run 9600 Emulation Mode, see the *GeneAmp™ PCR System 9700 Base Module User Manual* (Cat. No. 4303481).
   - If you are using the Veriti™ 96-Well Thermal Cycler, then select **9600 Emulation Mode**.
     For instructions on how to configure the Veriti™ 96-Well Thermal Cycler to run 9600 Emulation Mode, see the *Veriti™ 96-Well Thermal Cycler AmpFℓSTR™ Kit Validation User Bulletin* (Cat. No. 4440754).

Initial incubation step	Optimum cycle number[1]		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE (Direct Amplification 26–29) (Extracted DNA 30)		HOLD	HOLD
95°C, 1 minute	94°C, 4 seconds	61.5°C, 1 minute	60°C, 22 minutes	4°C, up to 24 hours[2]

[1] See "Direct amplification: Optimize PCR cycle number" on page 21.
[2] The infinity (∞) setting allows an unlimited hold time.

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

   IMPORTANT! If you are using adhesive clear film instead of caps to seal the plate wells, be sure to 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 does 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.

3 Perform electrophoresis

Allelic ladder requirements for electrophoresis

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

Instrument	Number of allelic ladders to run	One injection equals	Number of samples per allelic ladder(s)
3500	1 per 3 injections	8 samples	23 samples + 1 allelic ladder
3500*xl*	1 per injection	24 samples	23 samples + 1 allelic ladder
3130	1 per 4 injections	4 samples	15 samples + 1 allelic ladder
3130*xl*	1 per injection	16 samples	15 samples + 1 allelic ladder

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

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

Materials required for electrophoresis

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

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

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

Electrophoresis software setup

The following table lists the data collection software and the run modules that you can use to analyze PCR products generated by this kit. For details on the procedures, see the documents listed in "Documentation and support" on page 123.

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

Genetic Analyzer	Operating System	Data Collection Software	Additional software	Plate templates, assays, run modules, and conditions (installed with the HID Updater)
3500 3500xL	Windows™ Vista	3500 Data Collection Software v2	HID Updater 3500 DC v2 (Cat. No. 4480670)	Plate templates: 6dye_36_POP4 (and _xl) Assays: GF+Norm_POP4 (and _xl) and GF_POP4 (and _xl), which contain instrument protocol HID36_POP4 (and_xl)_J6_NT3200 with the following conditions: • Run module: HID36_POP4 (and _xl) • Injection conditions: 1.2 kV/16 sec (24 sec for xl)[1] • Alternate injection conditions: 1.5 kV/16 sec (24 sec for xl)[2] • Run conditions: 13 kV/1550 sec • Dye Set J6
3500 3500xL	Windows™ 7	3500 Data Collection Software v2	HID Updater 3500 DC v2 (Cat. No. 4480670)	Same as 3500 Data Collection Software v2 listed above
3500 3500xL	Windows™ 7	3500 Data Collection Software v3	None	Same as 3500 Data Collection Software v2 listed above

[1] This kit was developed using an injection time of 16 seconds on the 3500 instrument. This is different than the default injection time of 15 seconds. The instrument protocol will need to be modified accordingly.

[2] This kit was developed using two injection voltage conditions for the 3500 instrument; 1.2 kV/16 sec and 1.5 kV/16 sec. You are encouraged to explore both options during validation to determine which protocol provides the best results on your instrumentation.

Obtain and run the HID Updater (v1 and v2 software only)

Perform this procedure if you are using 3500 Series Software v1 or v2.

You can run 6-dye samples on 3500 Data Collection Software. Before running on either system for the first time, run the HID Updater 3500 DC v2 (Cat. No. 4480670). The HID Updater installs plate templates, assays, and instrument protocols that can be used to run Yfiler™ Plus PCR Amplification Kit samples. For more information, refer to the release notes provided with the Updater.

Note: If you have a new instrument installed by a Thermo Fisher Scientific representative, the updater may have been run during installation.

1. Shut down the 3500/3500xL Data Collection Software.
2. Download the updater from **www.thermofisher.com/us/en/home/technical-resources/software-downloads/3500-Series-Genetic-Analyzers-for-Human-Identification.html**.
3. Open the Read me file and review the software release notes.
4. Click the **updater .exe** file.
5. Follow the on-screen prompts.
6. Restart the computer.

Create a Yfiler™ Plus assay

The Yfiler™ Plus assay has an instrument protocol and a QC protocol. The easiest way to create an assay is to start off with an existing one that can be modified independently. To modify an existing assay:

1. Access the Assays library by selecting the Library tab and then the Assays tab under Library Resources.
2. Select an existing assay (for example, GF_POP4), click **Duplicate** then give it a new name (for example, YFP_POP4).
3. Select the new assay, then click **Edit** to open it.
4. Open the instrument protocol by clicking **Edit**, then modify the injection and run conditions specific to your instrument class and as shown in "Electrophoresis software setup" on page 30. Save the modified instrument protocol by clicking **Save to Library**, then give the protocol a new name.
5. To edit the QC protocol, follow the instructions.

Modify 3500 QC protocol

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

Before using the QC protocol to acquire data, modify it to:

- Change the Baseline Window and Peak Window Settings default settings to the settings shown in the following figure.
- Change the size calling default setting to to Local Southern, if needed.

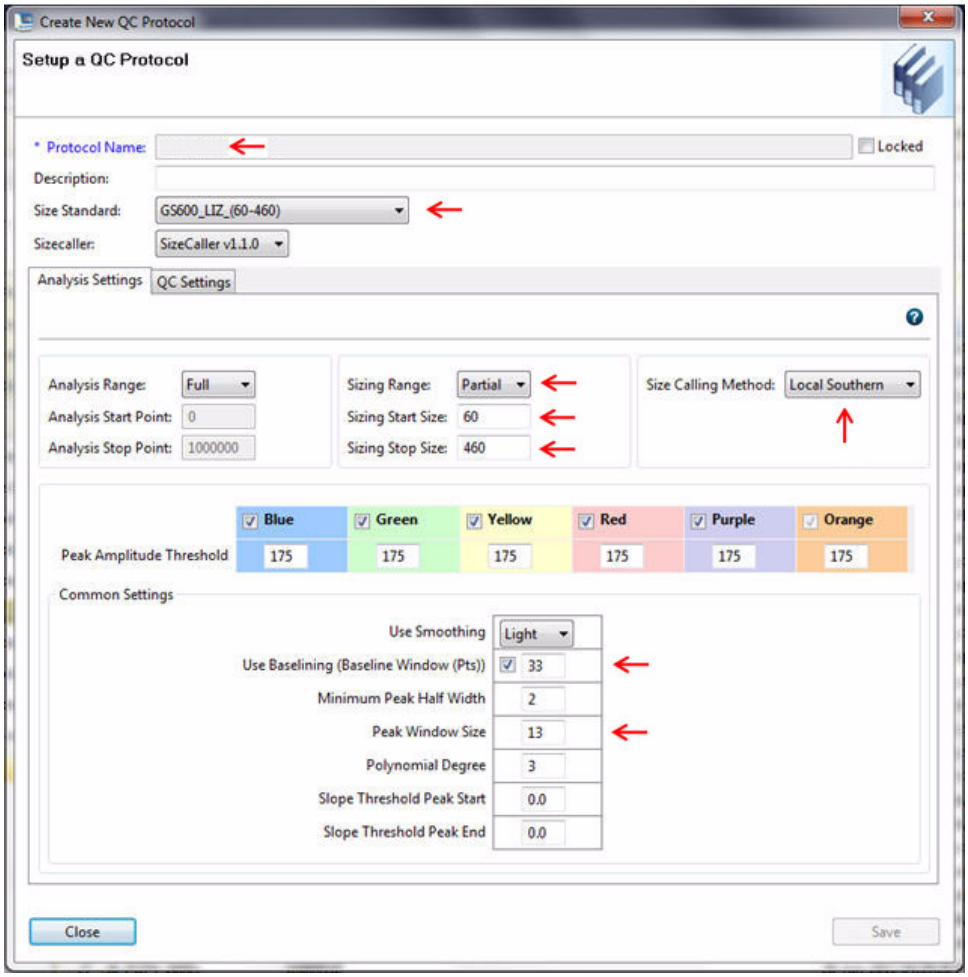
1. In the **Library** tab, open the **QC Protocol** window.

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 Method or 3rd Order Least Squares
After checking the "Use Baselining" box: Baseline Window Pts.	33
Peak Window Size	13

c. Click **Save**.

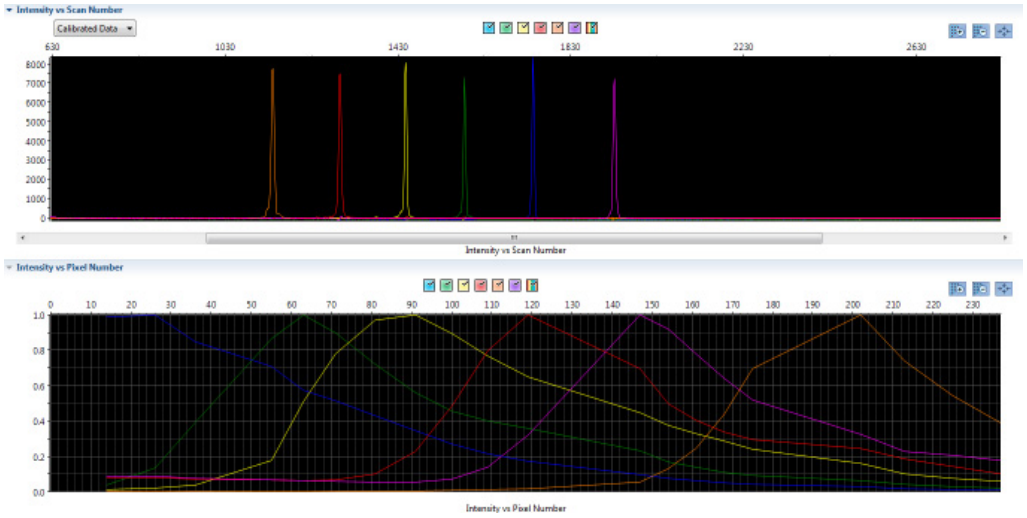


3. Add the QC protocol to the HID assay.

Perform spectral calibration

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

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



Prepare samples for electrophoresis (3500 Series and 3130 Series instruments)

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

Prepare the samples for electrophoresis immediately before loading.

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

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

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

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

2. Vortex the tube, then briefly centrifuge.

3. Into each well of a MicroAmp™ Optical 96-Well Reaction Plate, add:
   - 10 µL of the formamide/size standard mixture
   - 1 µL of PCR product or Allelic Ladder

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

4. Seal the reaction plate with appropriate septa, then briefly vortex and centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.
5. Heat the reaction plate in a thermal cycler at 95°C for 3 minutes.
6. Immediately place the plate on ice for 3 minutes.
7. Place the sample tray on the autosampler, then start the electrophoresis run.

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 123.

Note: We conducted validation studies for the Yfiler™ Plus PCR Amplification Kit using the 3130xl, 3500, or 3500xL configurations.

Genetic Analyzer	Operating System	Data Collection Software	Additional software	Run modules and conditions
3130	Windows™ 7	Data Collection Software v4[1]	3130/3730 DC v4 6-Dye Module v1	• HIDFragmentAnalysis36_POP4_1 Injection conditions: 3 kV/5 sec • Run conditions: 15 kV/1500 sec • Dye Set J6
3130 *xl*				• HIDFragmentAnalysis36_POP4_1 Injection conditions: 3 kV/10 sec • Alternate injection condition for the 3130*xl*: 3 kV/13 sec[2] • Run conditions: 15 kV/1500 sec • Dye Set J6

[1] Requires activation of 6-dye license.

[2] This kit was developed using two injection voltage conditions for the 3130*xl*; 3 kV/10 sec and 3 kV/13 sec. You are encouraged to explore both options during validation to determine which protocol provides the best results on your instrumentation.

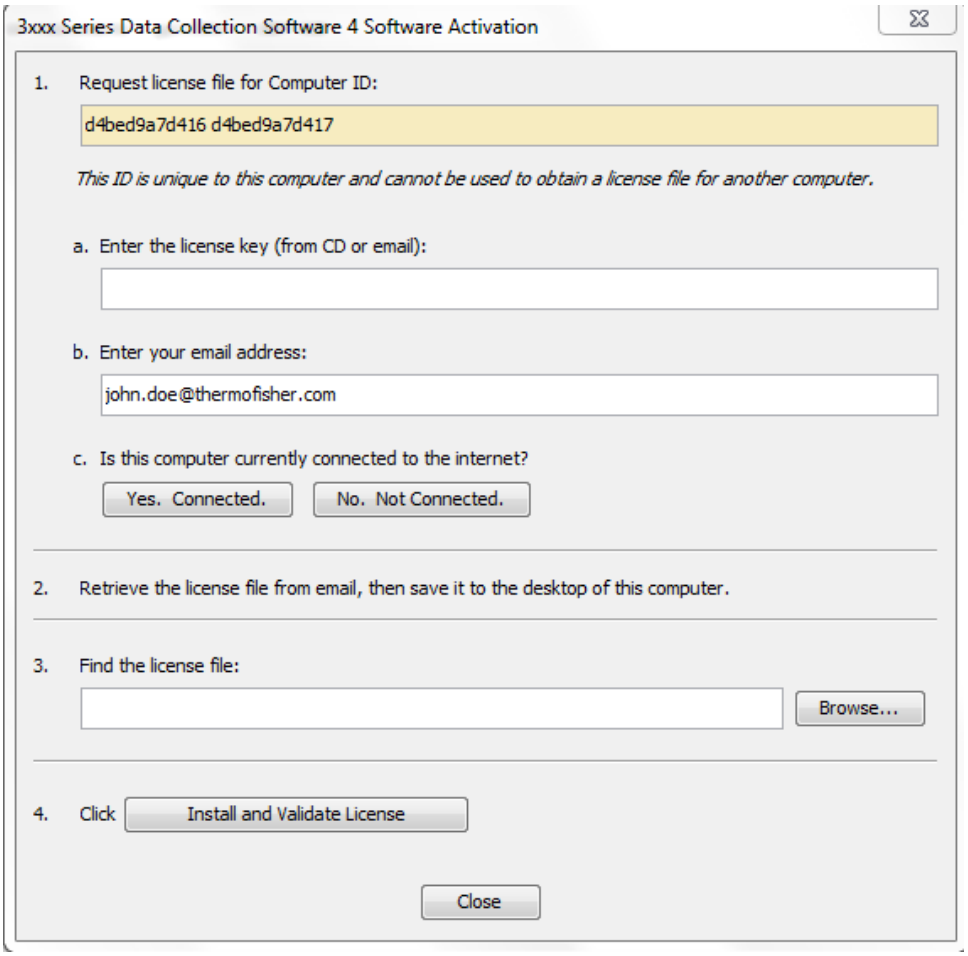
Obtain and activate 6-dye license

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.

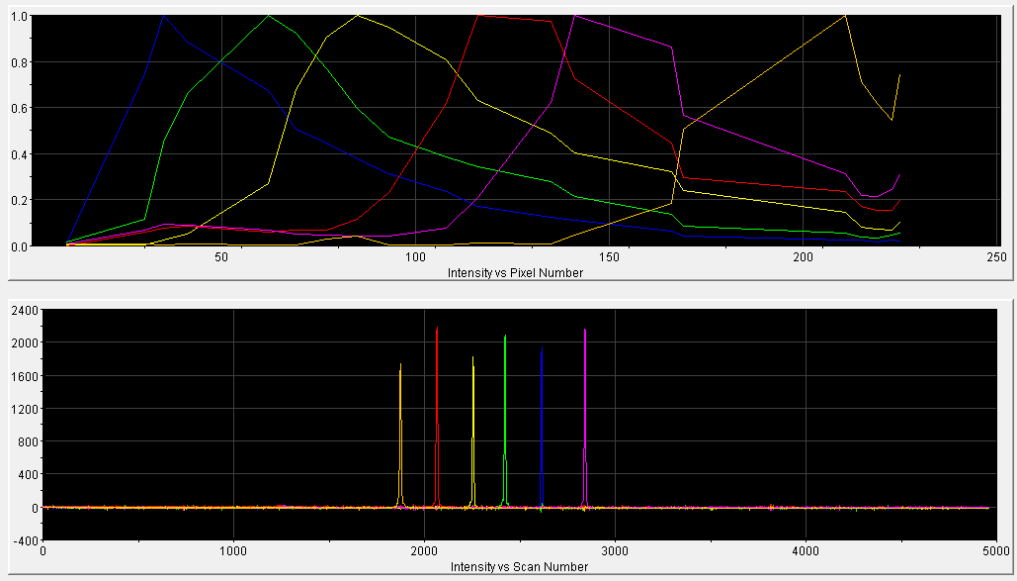
9. If the Software Activation dialog box has closed, select **Tools** ▸ **License Manager**.

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-36 Matrix Standard Kit (Dye set J6, 6-dye) (Cat. No. 4425042). Select the J6 dye set for the 3130 instrument, or the J6-RCT dye set for the 3730 instrument.

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



Prepare samples for electrophoresis (3500 Series and 3130 Series instruments)

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

Prepare the samples for electrophoresis immediately before loading.

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

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

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

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

2. Vortex the tube, then briefly centrifuge.

3. Into each well of a MicroAmp™ Optical 96-Well Reaction Plate, add:
   - 10 µL of the formamide/size standard mixture
   - 1 µL of PCR product or Allelic Ladder

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

4. Seal the reaction plate with appropriate septa, then briefly vortex and centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.

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

6. Immediately place the plate on ice for 3 minutes.

7. Place the sample tray on the autosampler, then start the electrophoresis run.

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

Overview of GeneMapper™ *ID-X* Software

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

GeneMapper™ *ID-X* Software v1.4 or later analyzes 4-dye, 5-dye, and 6-dye data and is required to correctly analyze data that is generated using the Yfiler™ Plus PCR Amplification 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.

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 41

▼

"(If needed) Download newer versions of panel, bin, and stutter files" on page 41

▼

"Import panels, bins, and marker stutter" on page 42

▼

"(Optional) Define custom table or plot settings" on page 45

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

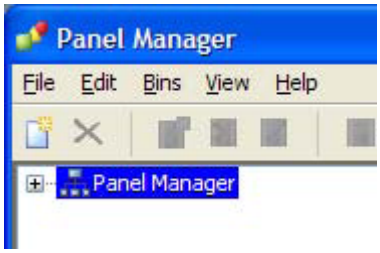
1. Go to **thermofisher.com/us/en/home/technical-resources/software-downloads/genemapper-id-x-software.html**.
2. If the file versions listed are newer than the versions on your computer, download the file **Yfiler Plus Analysis Files**.

   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:

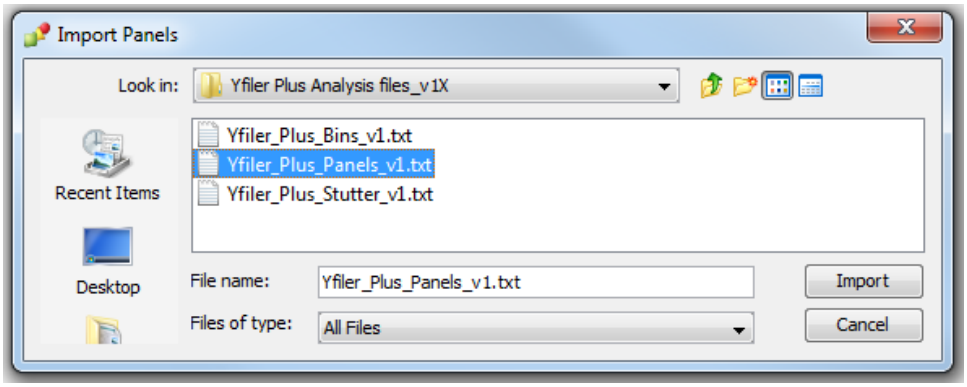


   a. Select **Panel Manager**, then select **File** ▸ **Import Panels** to open the **Import Panels** dialog box.

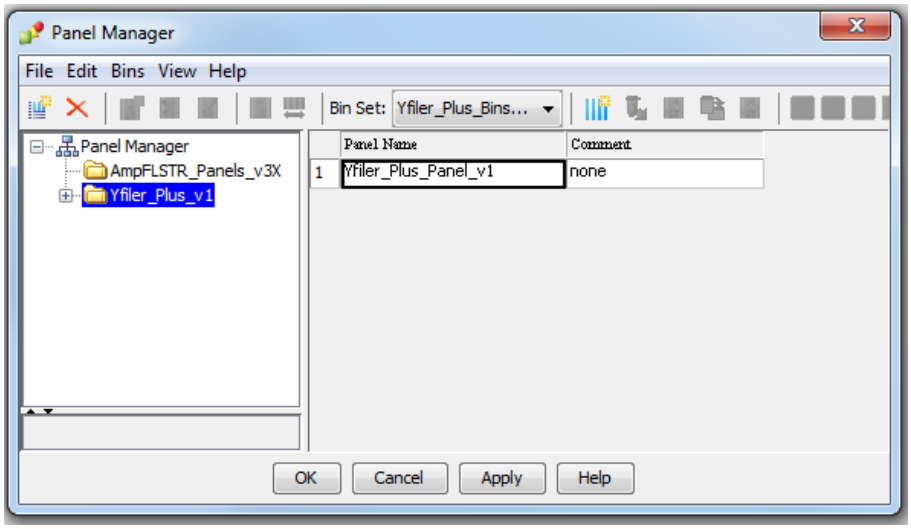
   b. Navigate to, then open the **Yfiler Plus Analysis Files** folder that you unzipped in the previous procedure.

4. Select **Yfiler_Plus_Panels.txt**, then click **Import**.

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



5. Import the bins file:
   a. Select the **Yfiler_Plus_Panels** folder in the navigation pane.

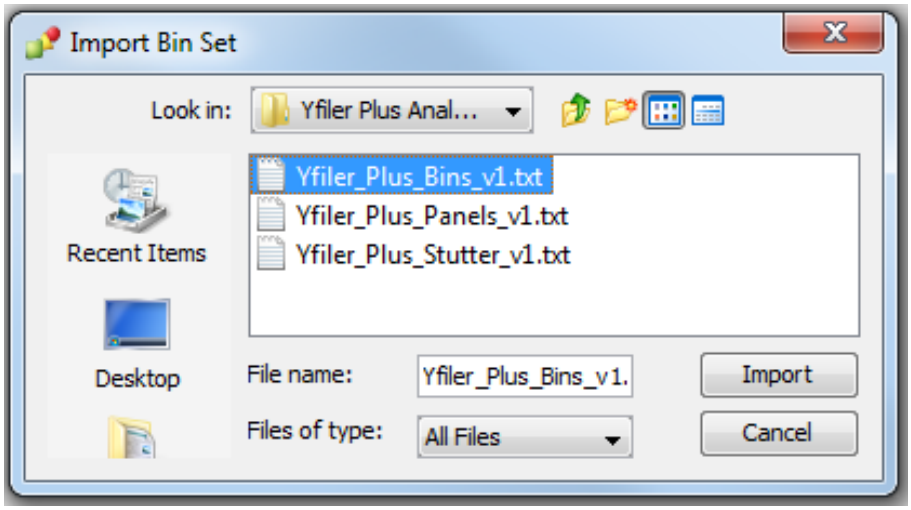


   b. Select **File ▸ Import Bin Set** to open the **Import Bin Set** dialog box.

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

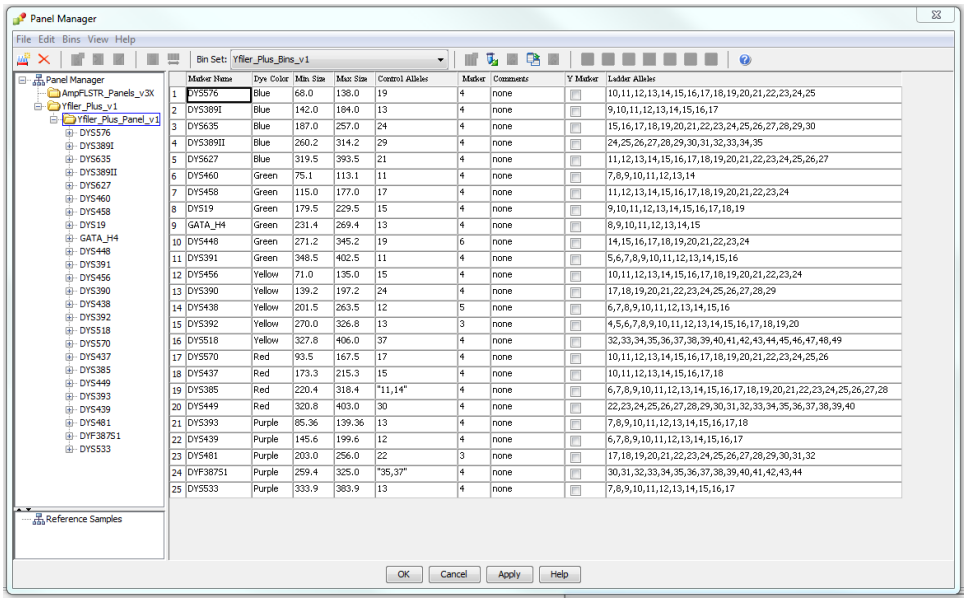
   d. Select **Yfiler_Plus_Bins.txt**, then click **Import**.

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



6. *(Optional)* View the imported panels and bins in the navigation pane: Double-click the **Yfiler_Plus_Panels** folder.

   The panel information is displayed in the right pane and the markers are displayed below it.



7. Import the stutter file:

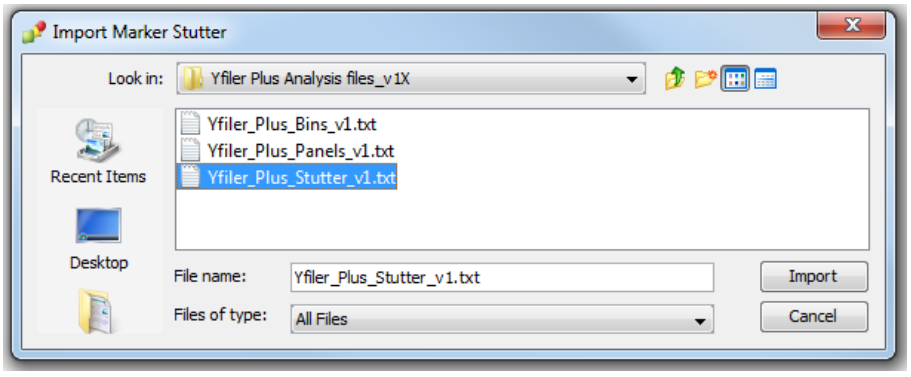
   a. Select the **Yfiler_Plus_Panels** folder in the navigation panel.

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

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

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

      Note: Importing this file associates the marker stutter ratio with the bin set in the **Yfiler_Plus_Panels** folder and overwrites any existing stutter ratios associated with the panels and bins in that folder.

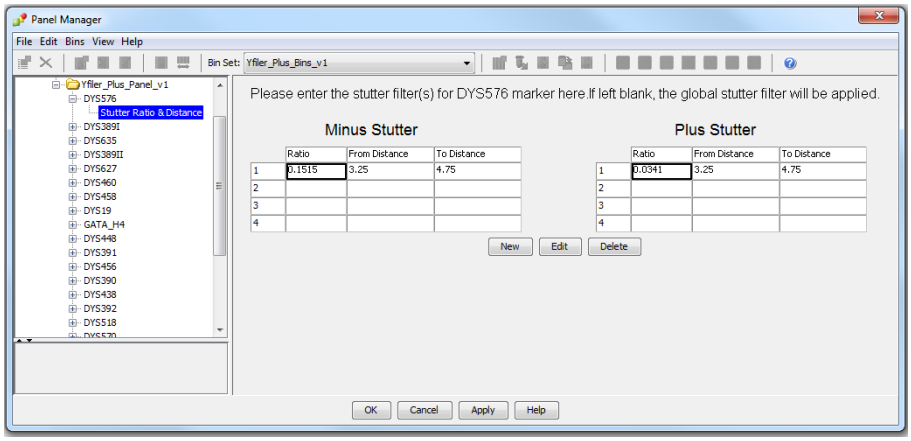


8. View the imported marker stutters in the navigation pane:

   a. Double-click the **Yfiler_Plus_Panels** folder to display the folder.

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

c. Double-click a marker to display the **Stutter Ratio & Distance** view for the marker in the right pane.



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

IMPORTANT! If you close the **Panel Manager** without clicking **Apply**, the panels, bin sets, and marker stutter are not imported into the GeneMapper™ *ID-X* Software database.

(Optional) Define custom table or plot settings

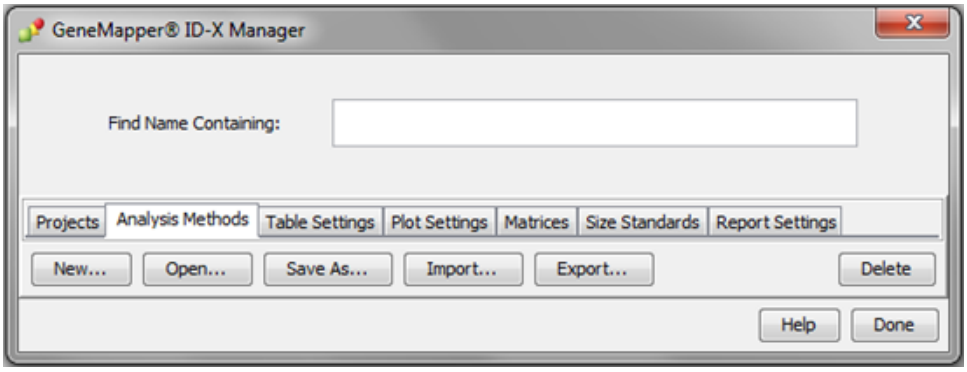
Default views for table and plot settings are provided with the software. For information on defining custom views, see *GeneMapper™ ID-X Software Getting Started Guide— Basic Features*.

Create an analysis method

Create an analysis method

IMPORTANT! Analysis methods are version-specific, so you must create an analysis method for each version of the software. For example, an analysis method that is created in GeneMapper™ *ID-X* Software version 1.2 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.

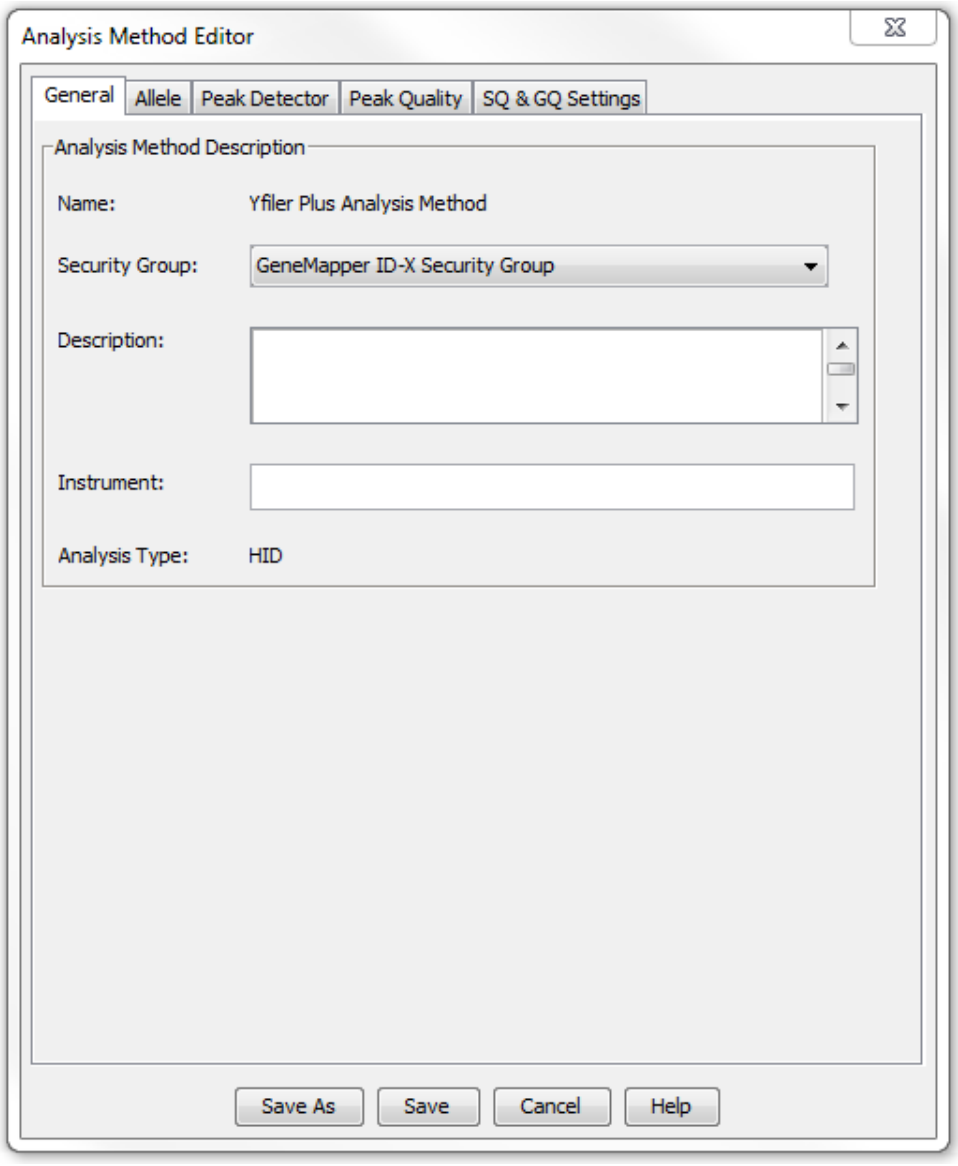
   Note: The **Analysis Method Editor** closes when you save your settings. To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.

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! To determine the appropriate settings to use, perform appropriate internal validation studies.

1. Select the **Yfiler_Plus_Bins** bin set.

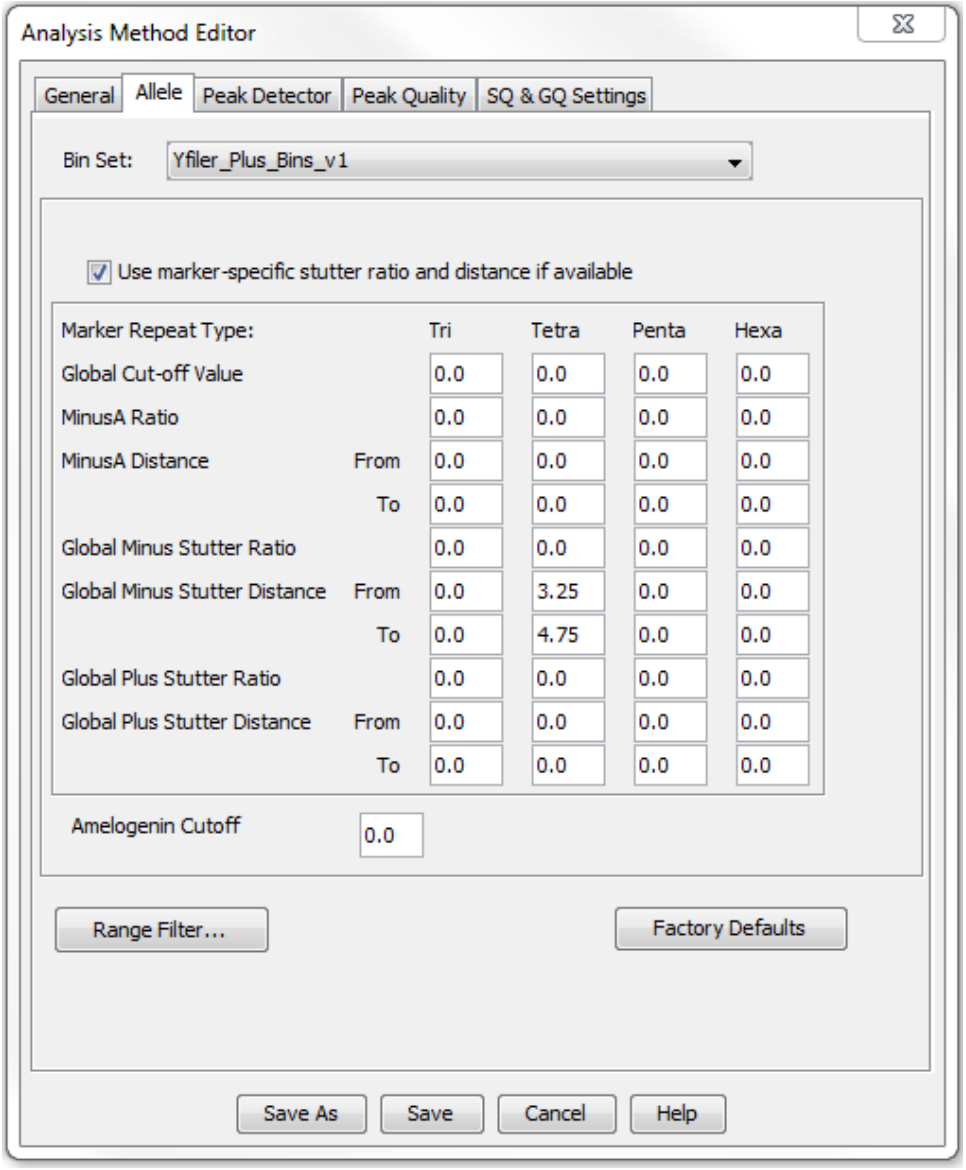


Figure 3 Settings used in developmental validation of the kit

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

3. If using GeneMapper™ *ID-X* Software v1.4 or later, enter values for the 4 **Marker Repeat Types**.

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.	—
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 these parameters to specify the minimum peak height, in order to limit the number of detected peaks. Although GeneMapper™ *ID-X* Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the genotype of these peaks.
Smoothing and Baseline	Enter the values shown in Figure 4, or adjust if needed dependent on the polymer you are using. 3730 DNA Analyzer with POP-7™ polymer only: With the **Smoothing** setting of **None**, the instances of spacing failures for the D2S441 and D1S1656 markers in some allelic ladder samples are significantly reduced. With the default **Smoothing** setting of **Light**, failures of base-pair spacing quality assessment are observed.	For more information, see the *GeneMapper™ ID-X Software v1.4 New Features and Installation Procedures User Bulletin* (Pub. No. 4477684 Rev. B), "Known issues: 3730 DNA Analyzer allelic ladder failures".

Field	Values to enter or select	Additional information
Size Calling Method	Select **3rd Order Least Squares** method or **Local Southern Method** or another method if validated by your laboratory. **IMPORTANT!** The Yfiler™ Plus PCR Amplification 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).	—
Normalization	*(Optional)* Select the **Normalization** checkbox. Ensure that your internal validation supports the selection of this feature.	A **Normalization** checkbox is available on this tab in GeneMapper™ *ID-X* Software for use in conjunction with data run on the 3500 Series Genetic Analyzers.

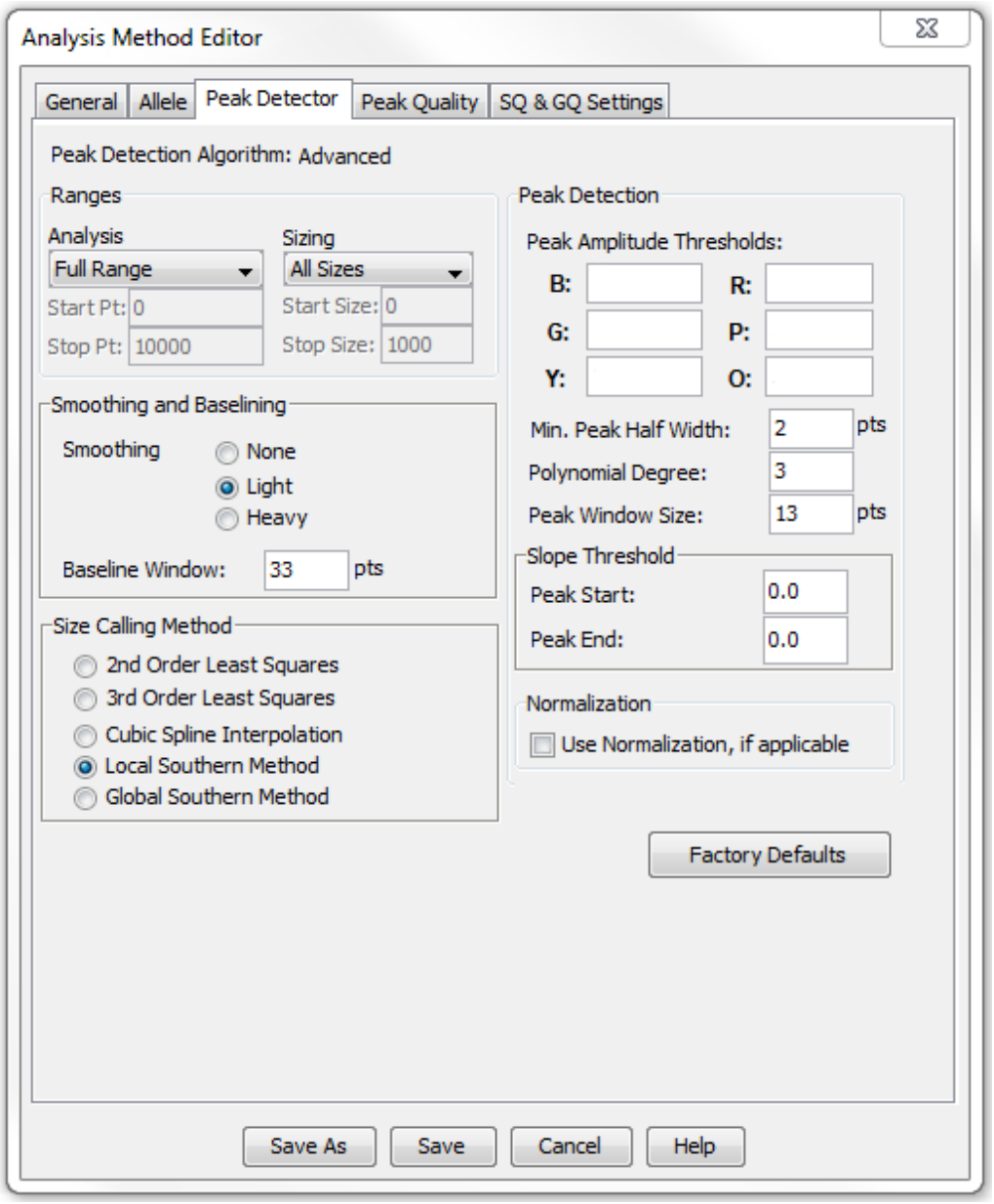
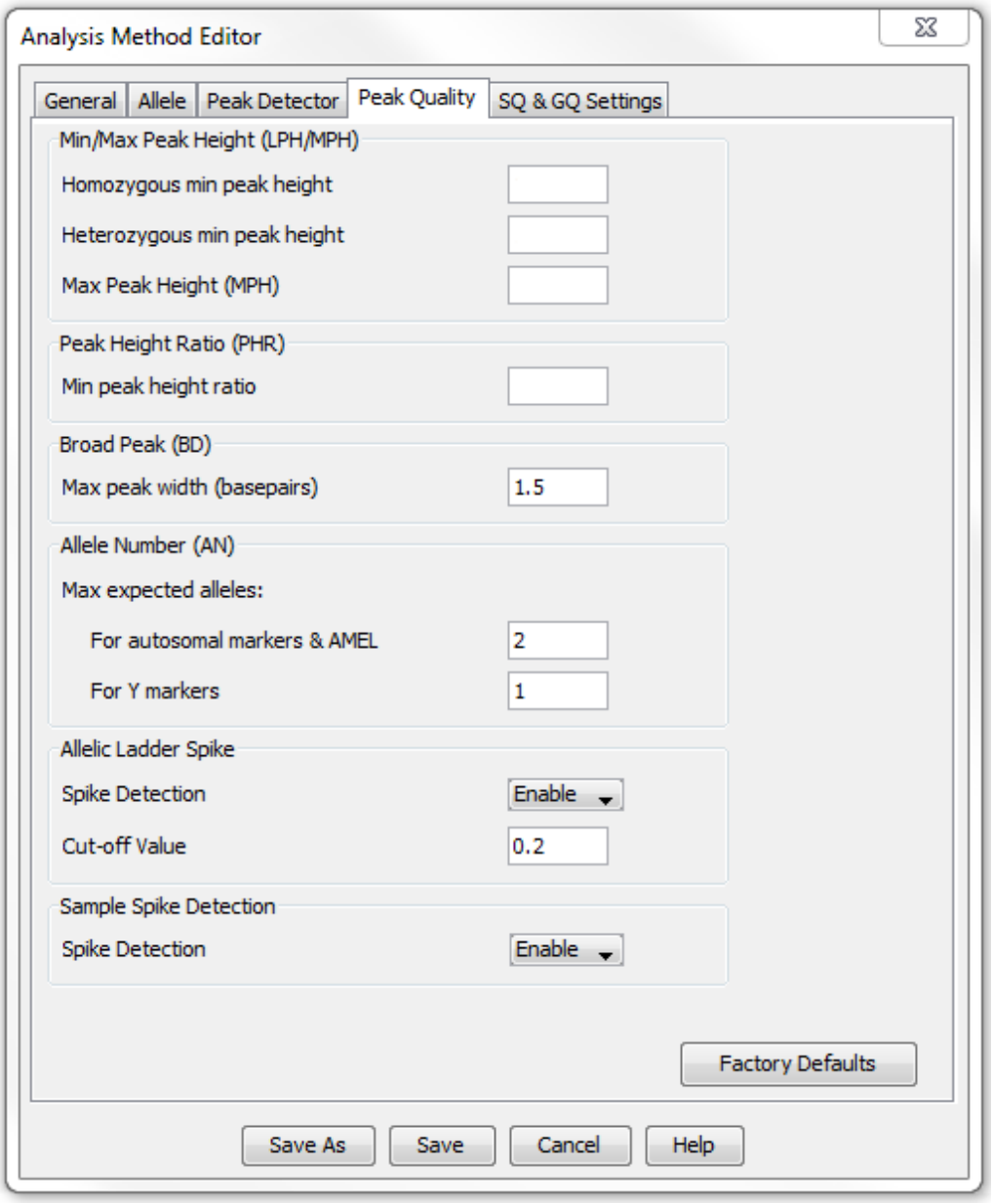


Figure 4 Settings used in the internal validation of the kit

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.

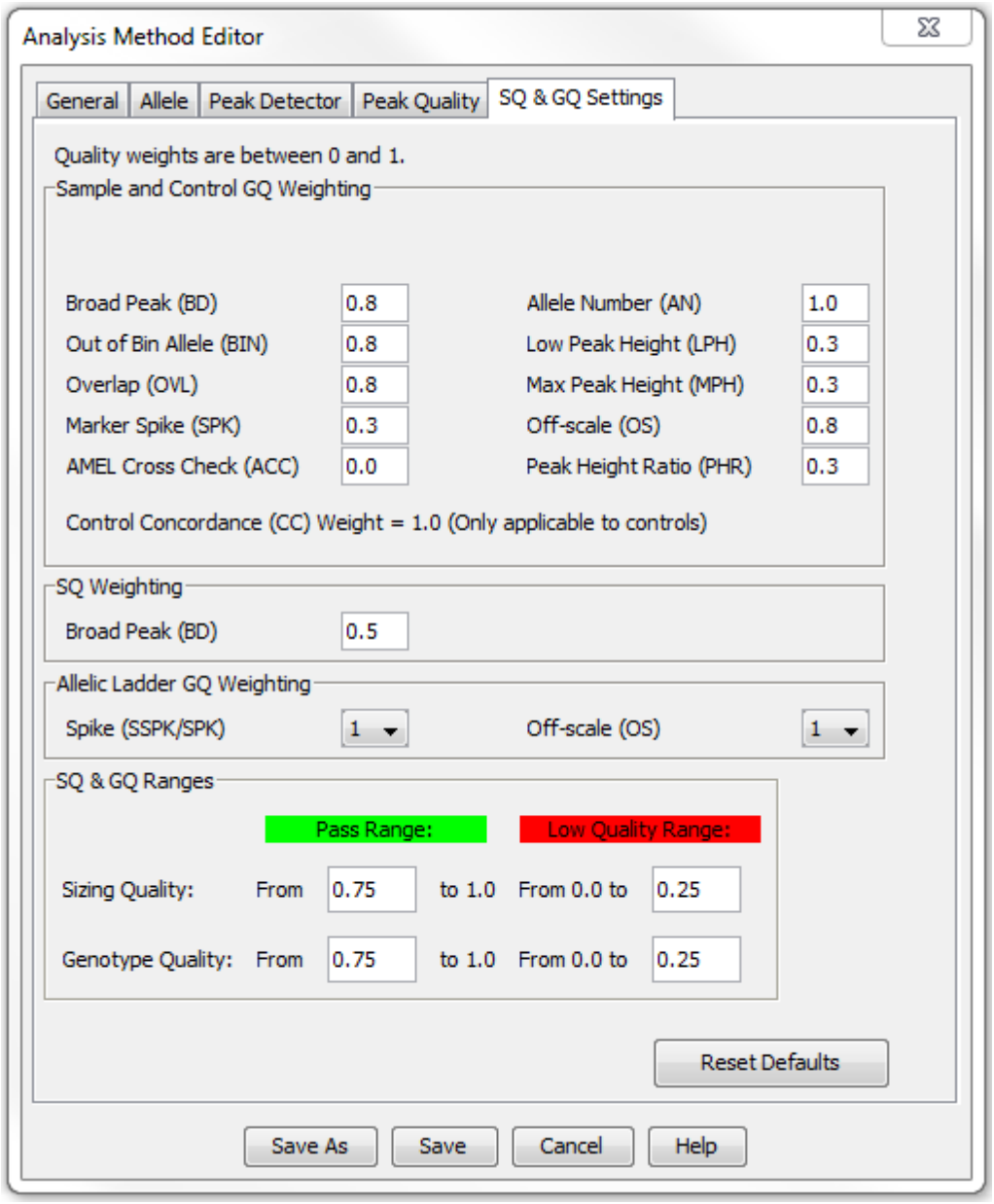
Enter the following values:



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. To determine the appropriate values to use, perform appropriate internal validation studies.

Enter the following values:



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.

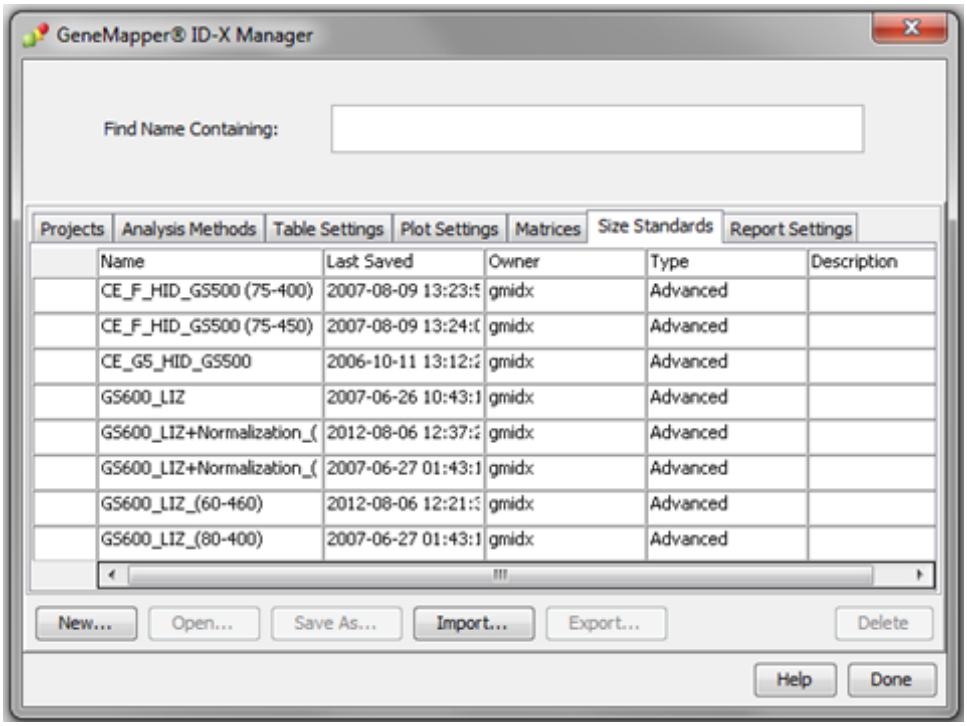
About the GS600_LIZ_ (60–460) size standard definition file

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 15 . If you need to create your own size standard definition, see "Create a size standard definition file" on page 54.

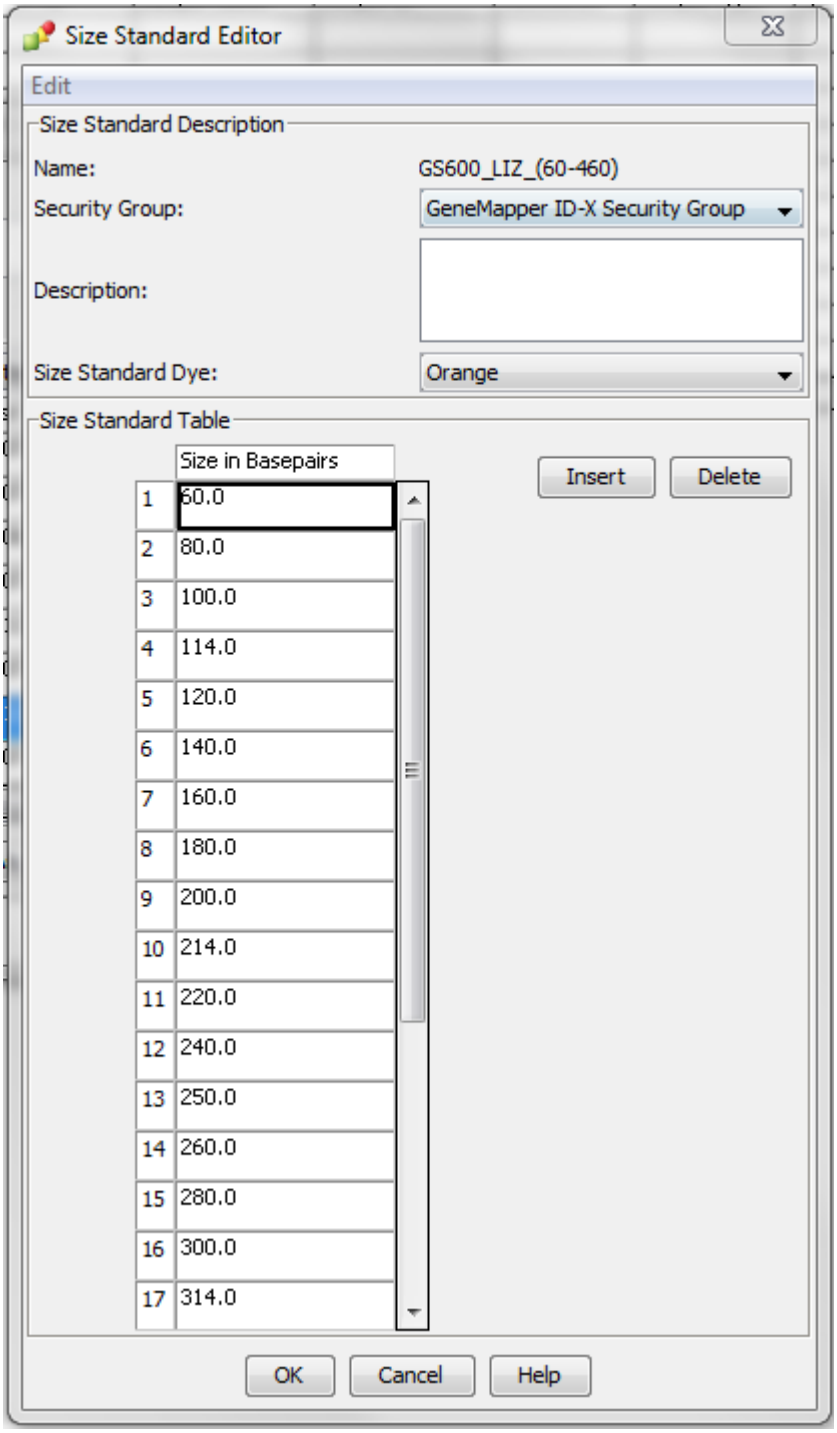
Create a size standard definition file

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

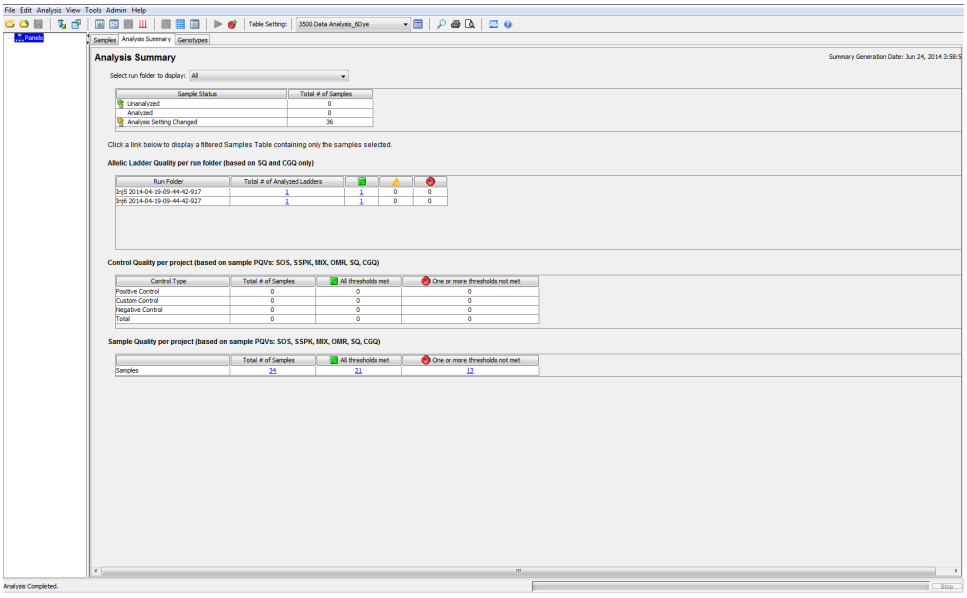
1. In the **Project** window, select **Edit ▸ Add Samples to Project**, then navigate to the disk or directory that contains the sample files.

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

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	Select **Yfiler_Plus_AnalysisMethod** (or the name of the analysis method you created).
Panel	Select **Yfiler_Plus_panels**.
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 Yfiler™ Plus PCR Amplification Kit was originally validated with the GeneScan™–600 LIZ™ Size Standard v2.0. If you use a different size standard, perform the appropriate internal validation studies to support the use of this size standard with the Yfiler™ Plus PCR Amplification 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 123 for a list of available documents.

5 Experiments and results

Importance of validation

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

Experiment conditions

We conducted developmental validation experiments according to the updated and revised guidelines from the Scientific Working Group on DNA Analysis Methods (SWGDAM, December 2012). Based on these guidelines, we conducted experiments that comply with guidelines 2.0 and 3.0 and its associated subsections. This DNA methodology is not novel. (Moretti *et al.*, 2001; Frank *et al.*, 2001; Wallin *et al., 2002*; and Holt *et al.*, 2000).

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

Laboratory requirements for internal validation

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

Developmental validation

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

SWGDAM guideline 2.2.1

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

SWGDAM guideline 3.9.2

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

PCR components

We examined the concentration of each component of the kit. We established that the concentration of each component was within the range where data indicated that the amplification met the required performance criteria for specificity, sensitivity, and reproducibility. For example, blood and buccal samples on treated-paper substrates or swab-sample lysates were amplified in the presence of varying concentrations of magnesium chloride, and the results were analyzed on a 3500xL Genetic Analyzer.

The performance of the multiplex is most robust within ±20% of the optimal magnesium chloride concentration.

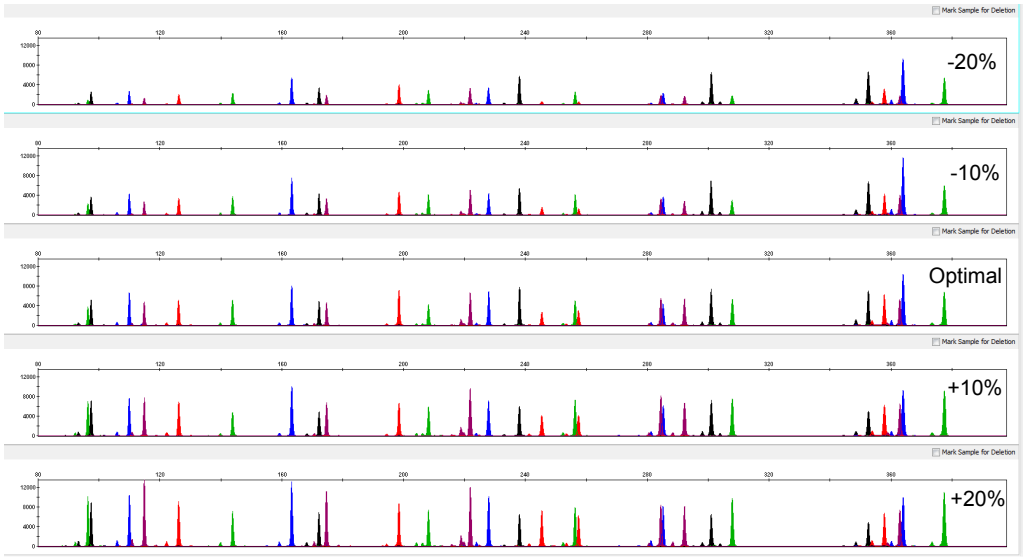


Figure 5 Electropherogram obtained from the amplification of a mixture of 1 ng of male 007 DNA and 1 µg of female 9947 DNA with varying concentrations of $MgCl_2$ analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 13,000 RFU).

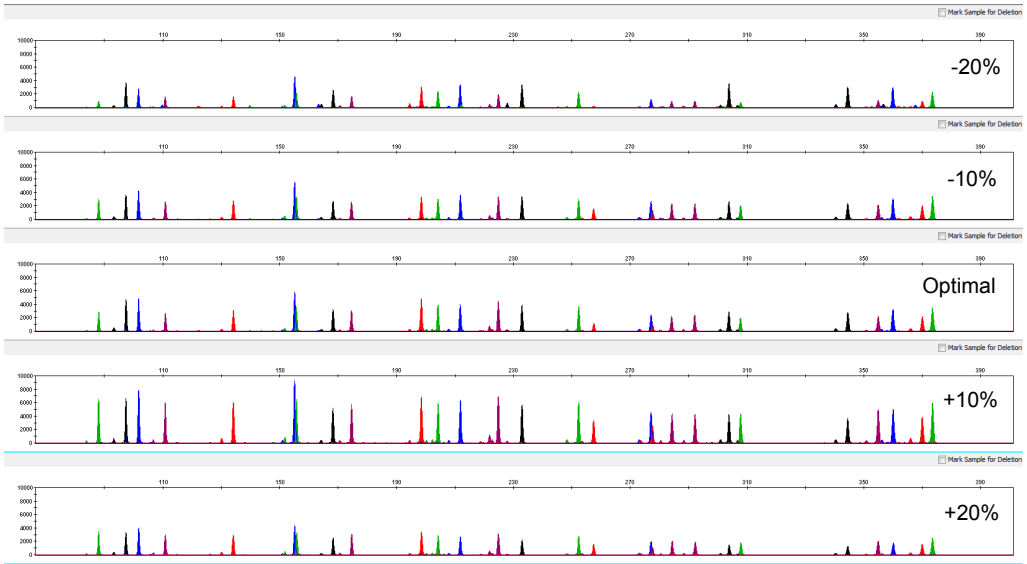


Figure 6 Electropherogram obtained from the amplification of a blood sample onFTA™ card amplified with the Yfiler™ Plus kit in the presence of varying concentrations of $MgCl_2$ and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 10,000 RFU).

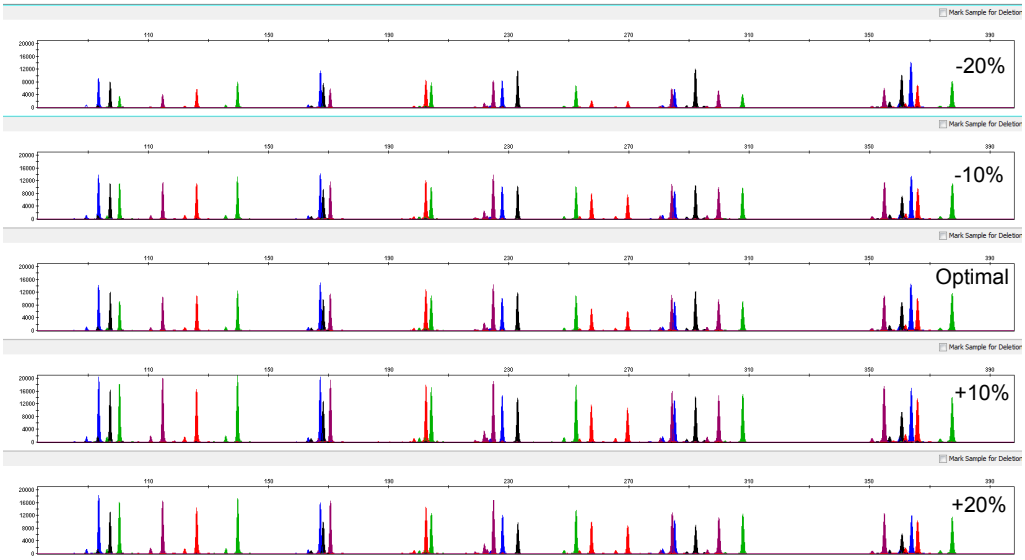


Figure 7 Electropherograms obtained from the amplification of a buccal sample on FTA™ card amplified with the Yfiler™ Plus kit in the presence of varying concentrations of $MgCl_2$ and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 20,000 RFU).

Thermal cycling temperatures

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

The denaturation temperatures tested were 93°C, 94°C, and 95°C, all for 4-second hold times on the Veriti™ Thermal Cycler. The annealing temperatures tested were 60.5, 61, 61.5, 62, and 62.5°C, for 1-minute hold times in the Veriti™ Thermal Cycler. The PCR products were analyzed using the 3500xL Genetic Analyzer.

No preferential amplification was observed in the denaturation temperature experiments. Of the tested annealing temperatures, 61°C, 61.5°C, and 62°C produced robust profiles with no significant cross reactivity to 1 µg of female DNA. At 62.5°C, the yield of most loci was reduced. This poses no problem with routine thermal cycler

calibration and when following the recommended amplification protocol. Preferential amplification was not observed at the standard annealing temperature of 61.5°C.

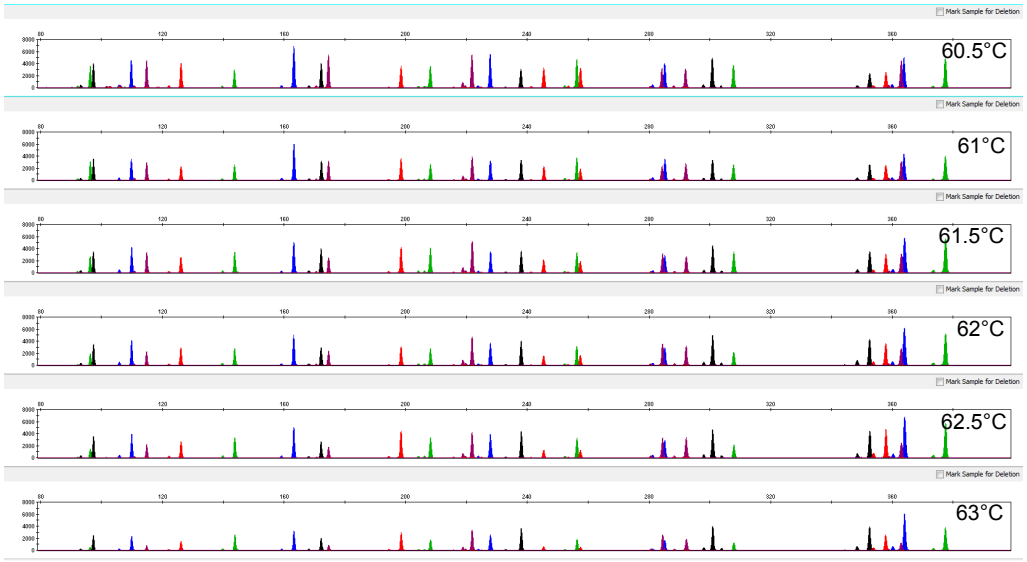


Figure 8 Electropherogram obtained from the amplification of a mixture of 1 ng of male 007 DNA and 1 µg of female 9947 DNA with varying annealing temperatures analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 8,000 RFU).

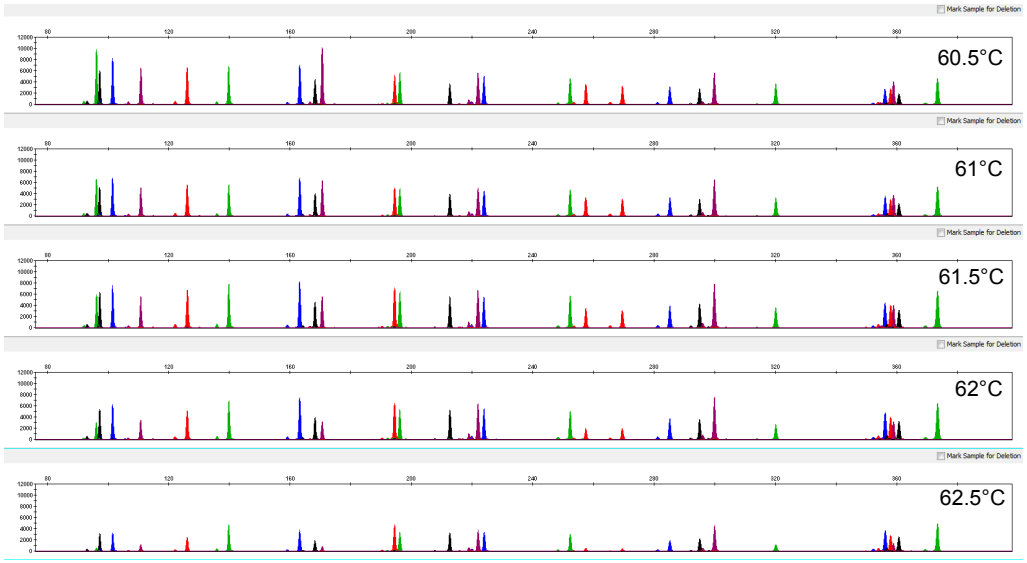


Figure 9 Electropherogram obtained from the amplification of a mixture of a blood sample on an FTA™ card with varying annealing temperatures analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 12,000 RFU).

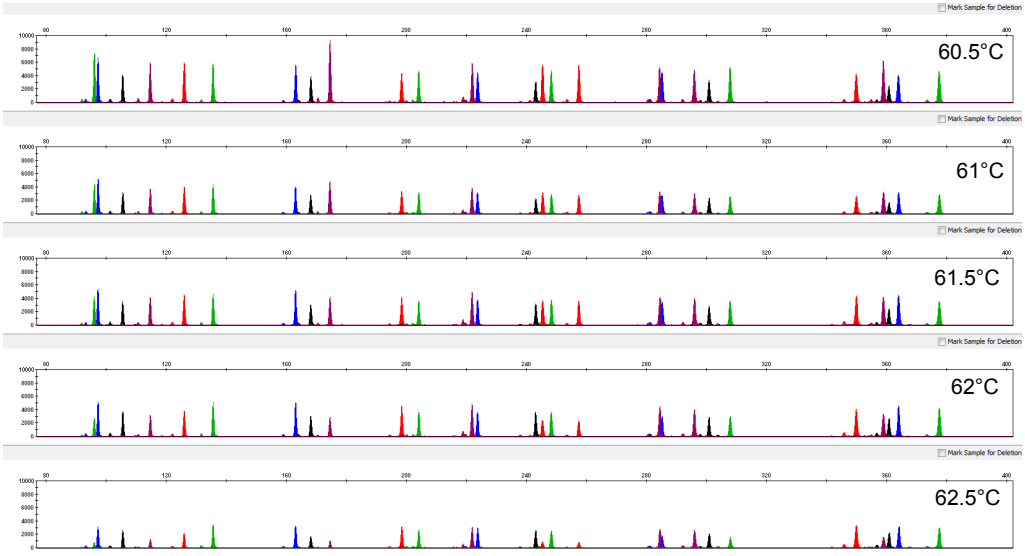


Figure 10 Electropherogram obtained from the amplification of a mixture of a buccal sample on an FTA™ card with varying annealing temperatures analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 10,000 RFU).

Accuracy, precision, and reproducibility

SWGDAM guideline 3.5

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

Accuracy observation

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

Figure 11 shows the size differences that are typically observed between sample alleles and allelic ladder alleles on the 3500xL Genetic Analyzer with POP-4™ Polymer. The X-axis represents the nominal nucleotide sizes for the Yfiler™ Plus Allelic Ladder. The dashed lines parallel to the X-axis represent the ±0.5-nt windows. The Y-axis represents the deviation of each sample allele size from the corresponding

Yfiler™ Plus Allelic Ladder allele size. All sample alleles are within ±0.5 nt from a corresponding allele in the Yfiler™ Plus Allelic Ladder.

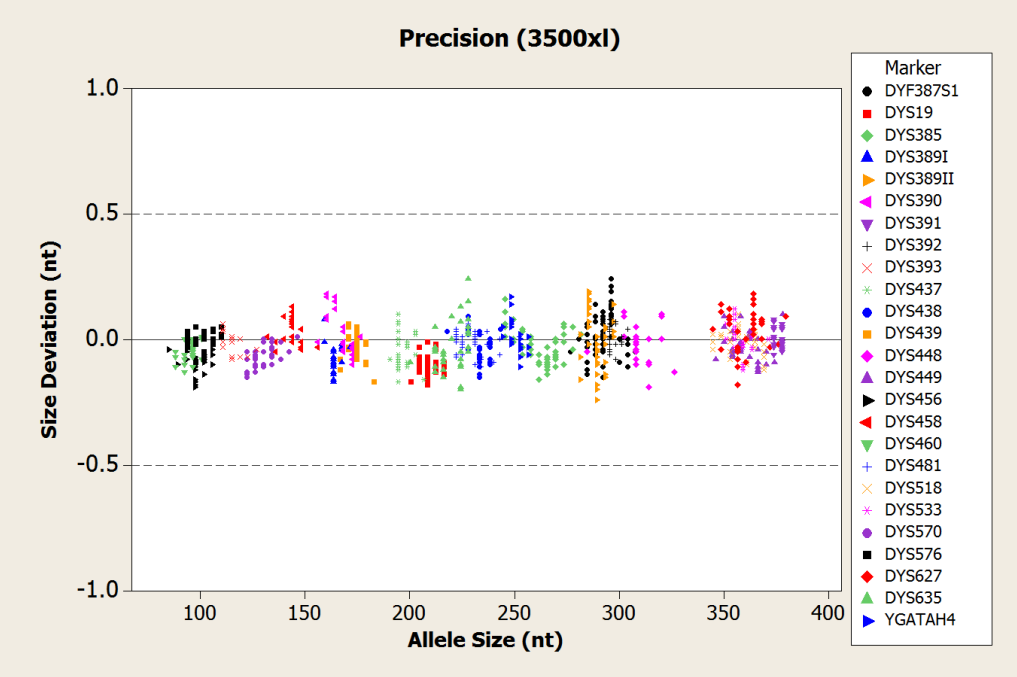


Figure 11 Size deviation of 78 samples analyzed on the 3500xL Genetic Analyzer

Precision and size window description

Sizing precision enables the determination of accurate and reliable genotypes. The recommended method for genotyping is to use a ±0.5-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

To view a table of typical precision results, see Table 9.

Extra peaks in the electropherogram

Causes of extra peaks

Peaks other than the target alleles may be detected on the electropherogram. Causes for the appearance of extra peaks include stutter products, incomplete 3′ A nucleotide addition (at the n-1 position), dye artifacts, and mixed DNA samples (see DNA Advisory Board (DAB) Standard 8.1.2.2).

Extra peaks: Stutter

Stutter definition

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

Contact HID Support for more information on plus stutter.

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

Stutter observations

Peak heights were measured for amplified samples at the loci used in the kit. All data were generated on the 3500xL Genetic Analyzer. Some conclusions from these measurements and observations are:

- For each locus, the stutter percentage generally increases with allele length.
- Smaller alleles display a lower level of stutter relative to the longer alleles within each locus.
- Each allele within a locus displays a consistent stutter percentage.
- Peaks in the stutter position that are above the stutter filter percentage specified in the software are not filtered (stutter filter percentage is calculated as the mean stutter for the locus plus three standard deviations). Peaks in the stutter position that have not been filtered and remain labeled can be further evaluated.
- The measurement of stutter percentage for allele peaks that are off-scale may be unusually high due to artificial truncation of the main allele peak.

Marker-specific plus stutter observed in the population study with the Yfiler™ Plus PCR Amplification Kit is shown in Figure 12 through Figure 20 and listed in Table 3.

Additional marker-specific plus stutter observed in the population study with the Yfiler™ Plus PCR Amplification Kit is listed in "Stutter percentage filter settings provided with GeneMapper™ ID-X Software" on page 75.

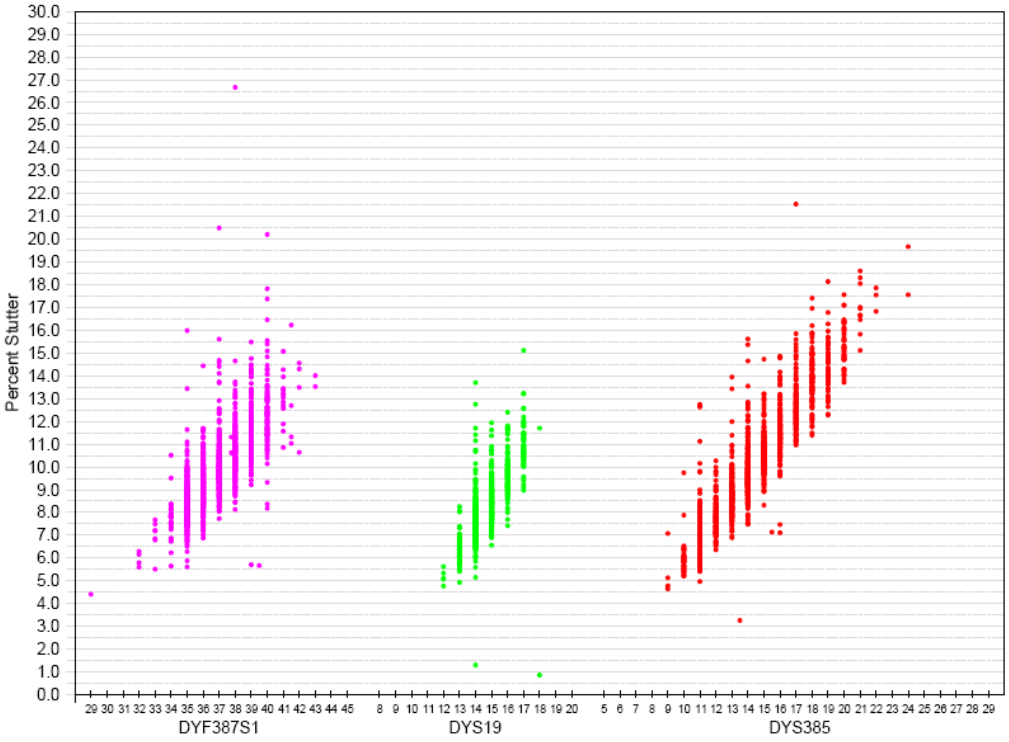


Figure 12 Minus stutter percentages for the DYF387S1, DYS19, and DYS385 a/b loci. (Blue, green, black, red, and purple colors indicate loci labeled with 6-FAM™, VIC™, NED™, TAZ™, and SID™ dyes, respectively.)

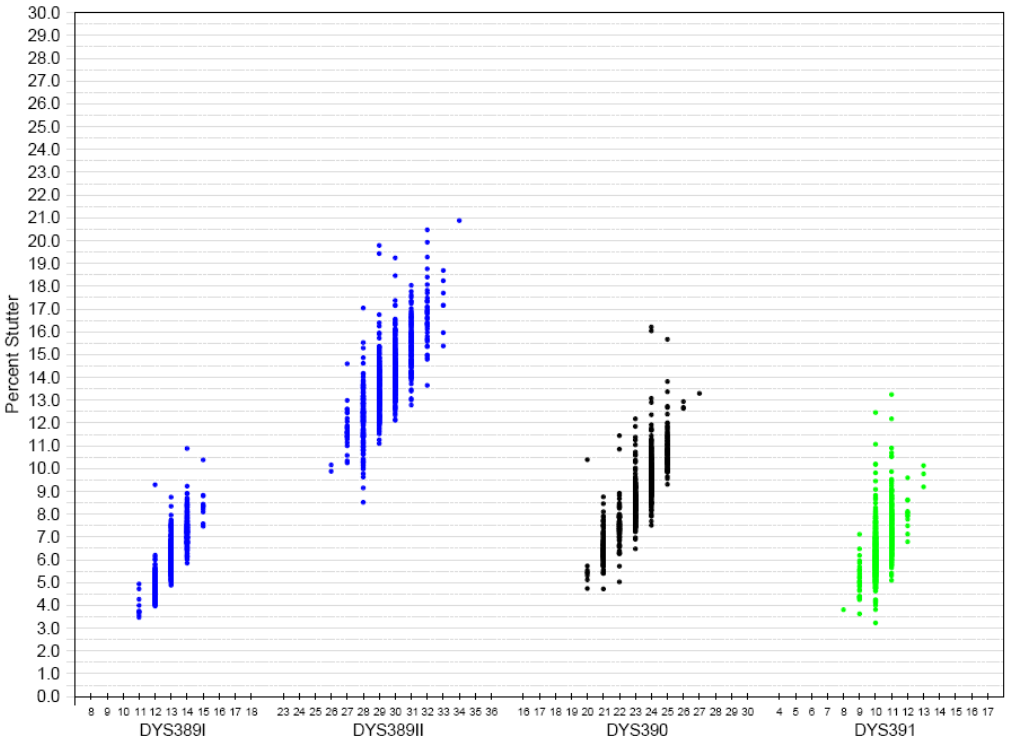


Figure 13 Minus stutter percentages for the DYS389I, DYS389II, DYS390, and DYS391 loci. (Blue, green, black, red, and purple colors indicate loci labeled 6-FAM™, VIC™, NED™, TAZ™, and SID™ dyes, respectively.)

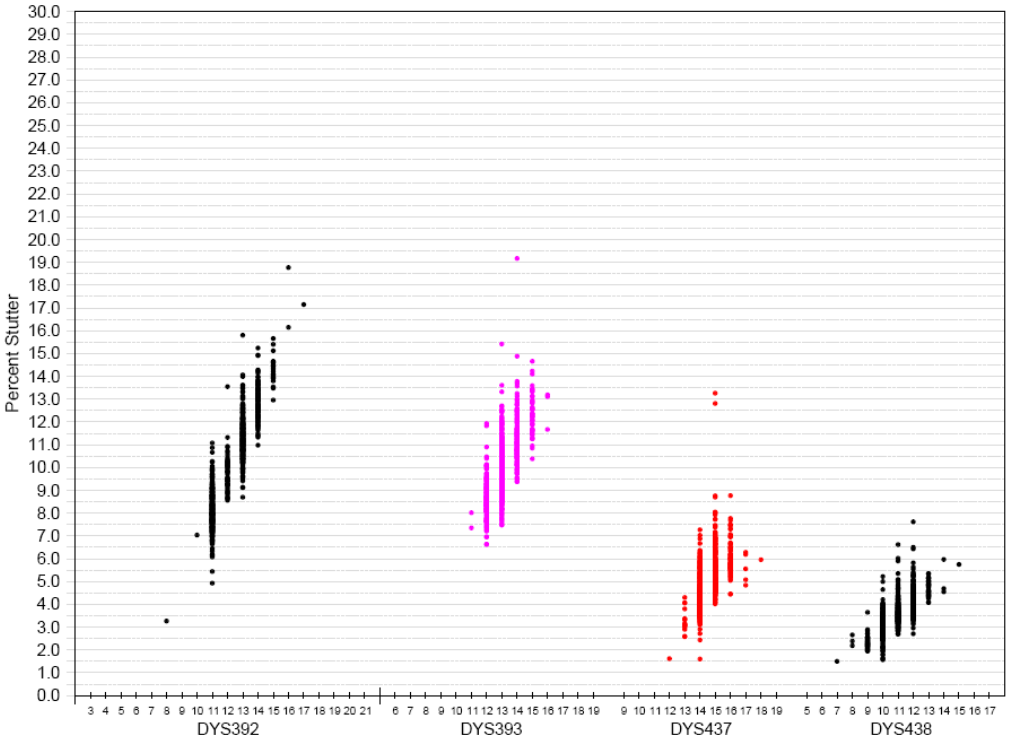


Figure 14 Minus stutter percentages for the DYS392, DYS393, DYS437, and DYS438 loci. (Blue, green, black, red, and purple colors indicate loci labeled 6-FAM™, VIC™, NED™, TAZ™, and SID™ dyes, respectively.)

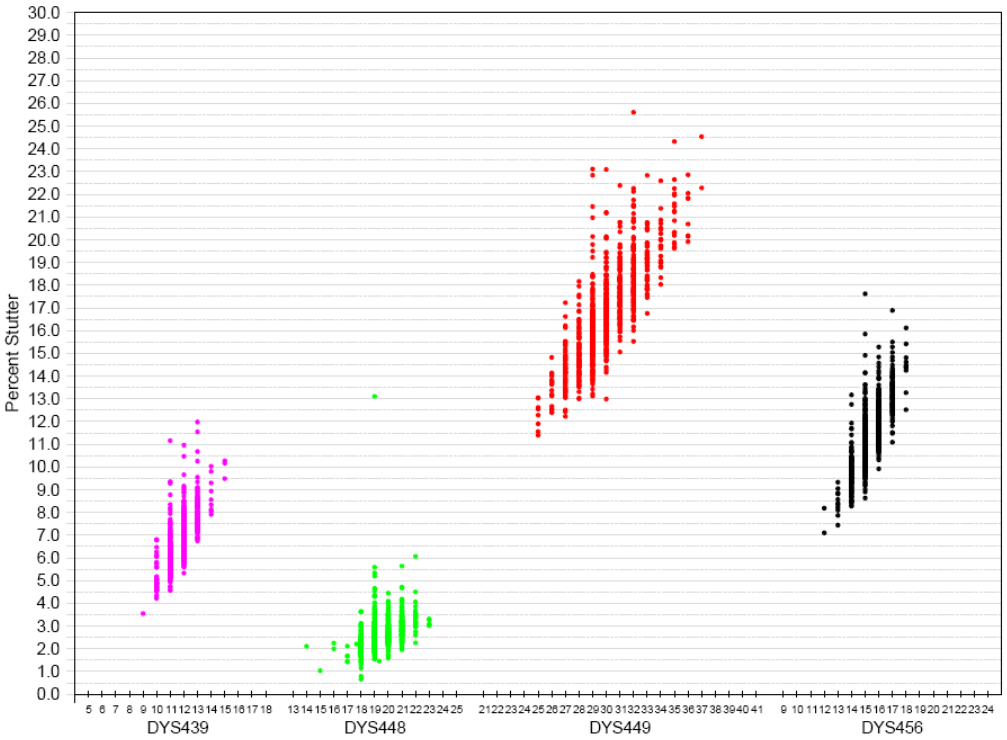


Figure 15 Minus stutter percentages for the DYS439, DYS448, DYS449, and DYS456 loci. (Blue, green, black, red, and purple colors indicate loci labeled 6-FAM™, VIC™, NED™, TAZ™, and SID™ dyes, respectively.)

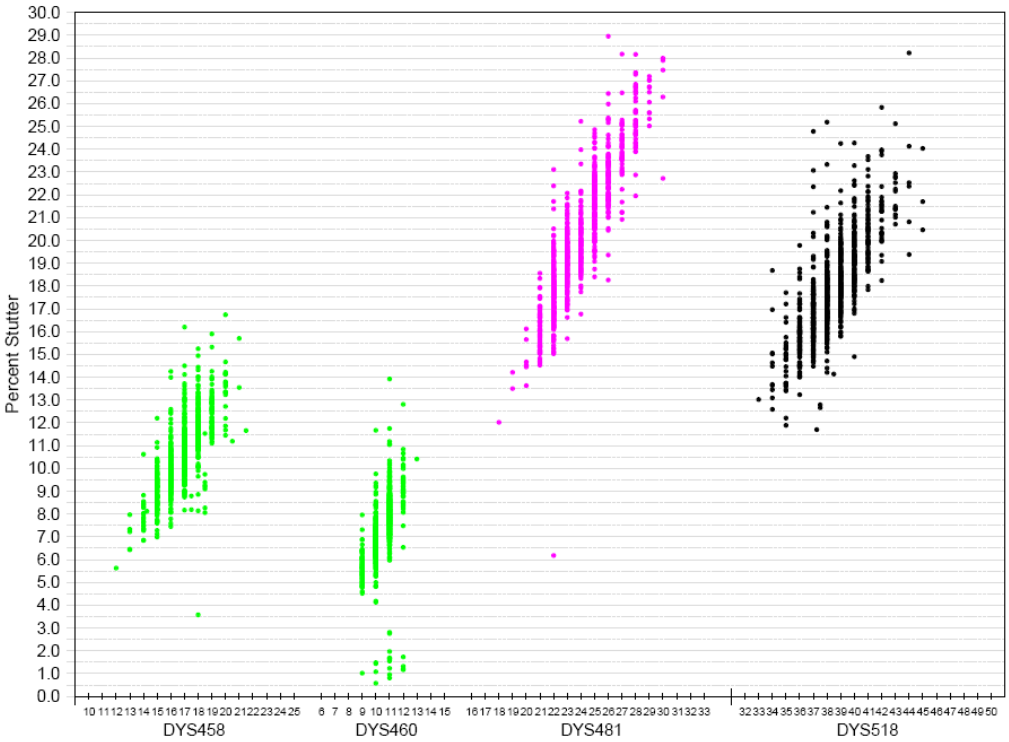


Figure 16 Minus stutter percentages for the DYS458, DYS460, DYS481, and DYS518 loci. (Blue, green, black, red, and purple colors indicate loci labeled 6-FAM™, VIC™, NED™, TAZ™, and SID™ dyes, respectively.)

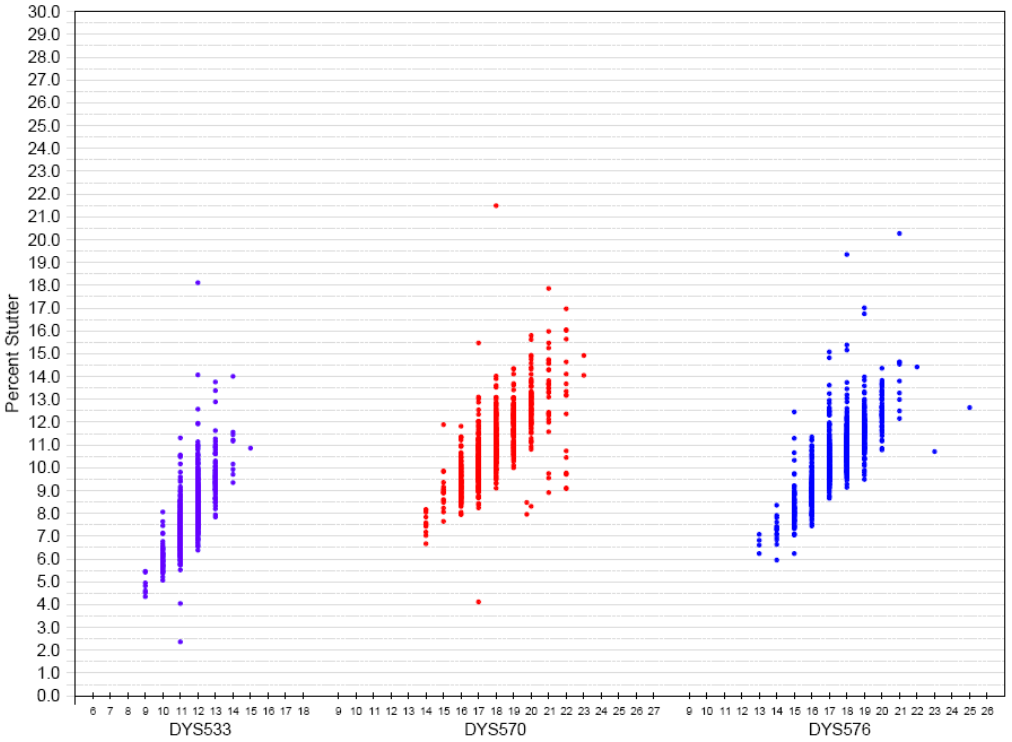


Figure 17 Minus stutter percentages for the DYS533, DYS570, and DYS576 loci. (Blue, green, black, red, and purple colors indicate loci labeled 6-FAM™, VIC™, NED™, TAZ™, and SID™ dyes, respectively.)

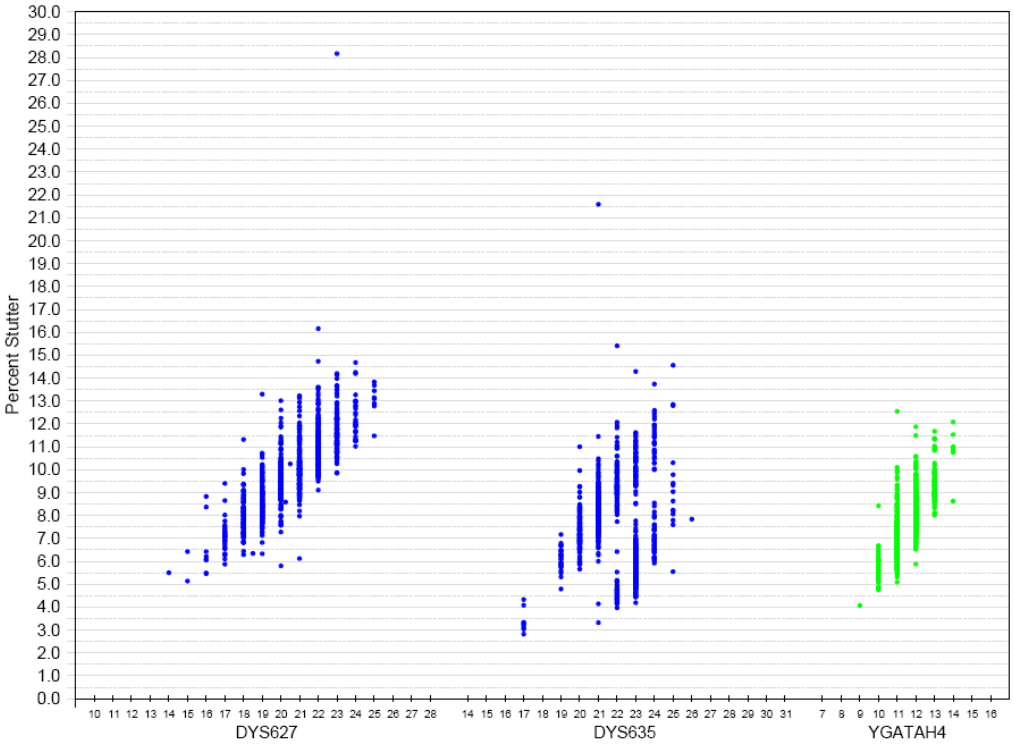


Figure 18 Minus stutter percentages for the DYS627, DYS635, and YGATAH4 loci. (Blue, green, black, red, and purple colors indicate loci labeled 6-FAM™, VIC™, NED™, TAZ™, and SID™ dyes, respectively.)

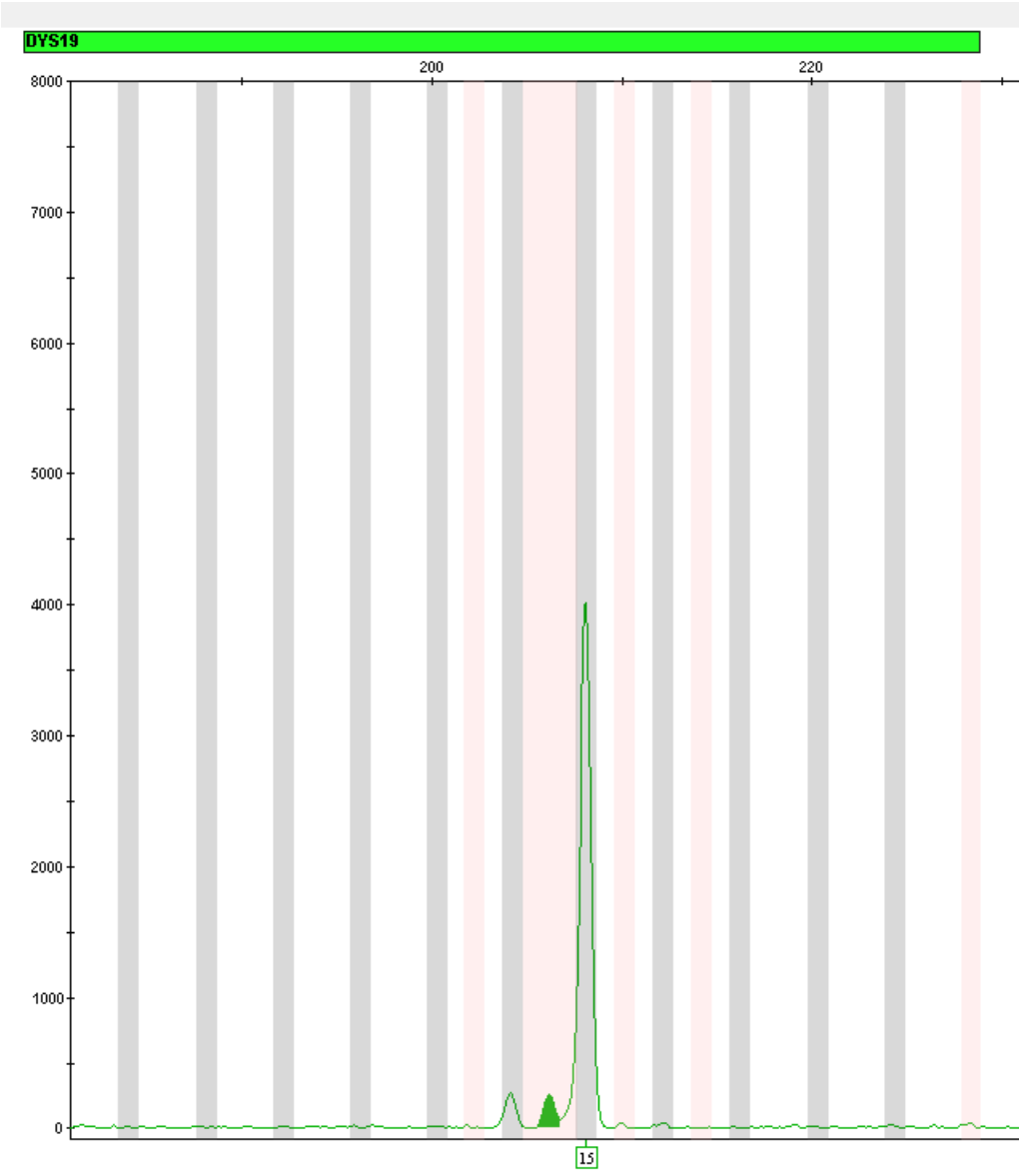


Figure 19 Example of reproducible 2-nt stutters in the DYS19 loci.

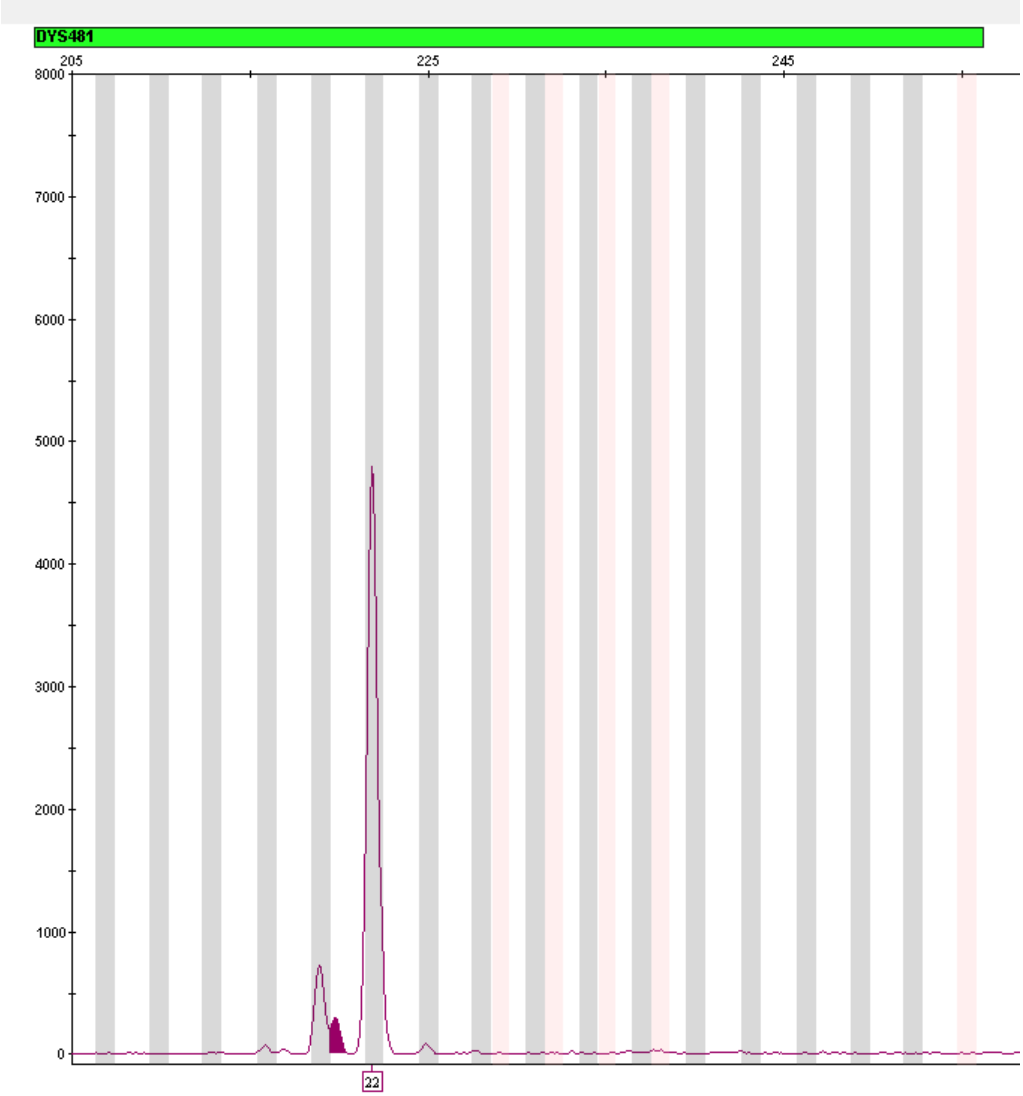


Figure 20 Example of reproducible 2-nt stutters in the DYS481 loci.

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

The settings in Table 3 were derived using the data that is shown earlier in this section. The proportion of the stutter product relative to the main allele (stutter percent) is measured by dividing the height of the stutter peak by the height of the main allele peak.

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

Table 3 Marker-specific % minus stutter for the Yfiler™ Plus kit loci

Locus	% Minus stutter (Mean + 3 standard deviations)[1]	% Mean stutter	% Minimum stutter	% Maximum stutter	2 nt % (Mean + 3 standard deviations)[1]
DYS576	15.15	10.44	5.95	20.27	[2]
DYS389I	9.16	6.08	3.47	10.88	[2]
DYS635	13.38	7.54	2.82	21.59	[2]
DYS389II	18.79	13.88	9.15	20.88	[2]
DYS627	15.18	9.90	5.14	28.17	2.71
DYS460	11.65	7.32	0.58	13.92	[2]
DYS458	15.31	10.58	3.58	16.74	[2]
DYS19	12.68	8.27	0.86	15.12	10.10
YGATAH4	11.53	7.75	4.07	22.14	[2]
DYS448	4.68	2.62	0.66	13.11	[2]
DYS391	9.99	6.58	3.23	13.25	[2]
DYS456	15.36	11.34	7.10	17.62	[2]
DYS390	13.58	8.74	4.72	16.21	[2]
DYS438	5.86	3.59	1.50	7.62	[2]
DYS392	16.94	10.27	3.27	31.35	[2]
DYS518	25.50	18.07	11.70	46.29	[2]
DYS570	15.65	10.88	4.12	21.49	[2]
DYS437	8.13	4.83	1.60	13.26	[2]
DYS385 a/b	18.32	10.02	3.26	21.54	[2]

Locus	% Minus stutter (Mean + 3 standard deviations)[1]	% Mean stutter	% Minimum stutter	% Maximum stutter	2 nt % (Mean + 3 standard deviations)[1]
DYS449	23.24	16.80	11.40	25.61	[2]
DYS393	14.07	9.97	6.63	19.17	[2]
DYS439	9.89	6.74	3.55	11.98	[2]
DYS481	28.55	19.98	12.02	28.95	9.55
DYF387S1	15.71	9.96	4.41	30.78	[2]
DYS533	12.00	7.94	2.37	18.11	1.88

[1] These percentages are used as stutter filters in GeneMapper ID-X Yfiler_Plus_Stutter.txt
[2] Undetermined

Table 4 Marker-specific % plus stutter for the Yfiler™ Plus kit loci

Locus	% Plus stutter (Mean + 3 standard deviations)[1]	% Mean stutter	% Minimum stutter	% Maximum stutter	2 nt % (Mean + 3 standard deviations)[1]
DYS576	3.38	1.15	0.42	8.45	[2]
DYS389I	3.45	0.81	0.23	8.94	[2]
DYS635	3.30	1.06	0.26	6.94	[2]
DYS389II	3.73	1.01	0.33	9.35	[2]
DYS627	2.62	0.95	0.26	6.40	[2]
DYS460	4.27	1.17	0.34	24.71	[2]
DYS458	2.52	0.95	0.34	5.86	[2]
DYS19	3.72	1.26	0.43	14.81	3.42
YGATAH4	2.27	0.94	0.33	4.55	[2]
DYS448	2.29	0.76	0.25	3.40	[2]
DYS391	3.41	0.98	0.33	9.02	[2]
DYS456	3.74	2.03	0.73	7.09	[2]
DYS390	3.51	1.19	0.35	3.36	[2]
DYS438	2.76	0.97	0.35	2.87	[2]
DYS392	11.00	6.32	1.91	16.65	[2]
DYS518	4.85	1.78	0.69	13.46	[2]

Locus	% Plus stutter (Mean + 3 standard deviations)[1]	% Mean stutter	% Minimum stutter	% Maximum stutter	2 nt % (Mean + 3 standard deviations)[1]
DYS570	2.88	1.15	0.45	10.50	[2]
DYS437	1.65	0.70	0.23	2.28	[2]
DYS385 a/b	3.70	1.12	0.19	13.17	[2]
DYS449	4.20	1.50	0.57	12.83	[2]
DYS393	4.95	2.07	0.59	22.21	[2]
DYS439	3.39	1.24	0.43	14.17	[2]
DYS481	5.59	2.59	0.96	21.03	[2]
DYF387S1	[2]	[2]	[2]	[2]	[2]
DYS533	4.60	1.71	0.70	5.73	[2]

[1] These percentages are used as stutter filters in GeneMapper ID-X Yfiler_Plus_Stutter.txt
[2] Undetermined

Extra peaks: Addition of 3' A nucleotide

3′ A nucleotide addition definition

Many DNA polymerases can catalyze the addition of a single nucleotide (predominantly adenosine) to the 3′ ends of double-stranded PCR products (Clark, 1988; Magnuson *et al*., 1996). This nontemplate addition results in a PCR product that is one nucleotide longer than the actual target sequence. The PCR product with the extra nucleotide is referred to as the "+A" form.

3′ A observations

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

The Yfiler™ Plus PCR Amplification Kit includes two main design features that promote maximum +A addition:

- The primer sequences have been optimized to encourage +A addition.
- The PCR chemistry allows complete +A addition with a short final incubation at The new, highly robust PCR chemistry allows complete +A addition with a short final incubation at 60°C for 22 minutes. .

This final extension step gives the DNA polymerase additional time to complete +A addition to all double-stranded PCR products. Figure 21 shows examples of incomplete and normal +A addition. Final extension incubation for longer than the recommended time can result in double +A addition, in which two nontemplate

adenosine residues are added to the PCR product. Double +A addition can cause "shoulders" on the right side of main allele peaks, and is therefore to be avoided.

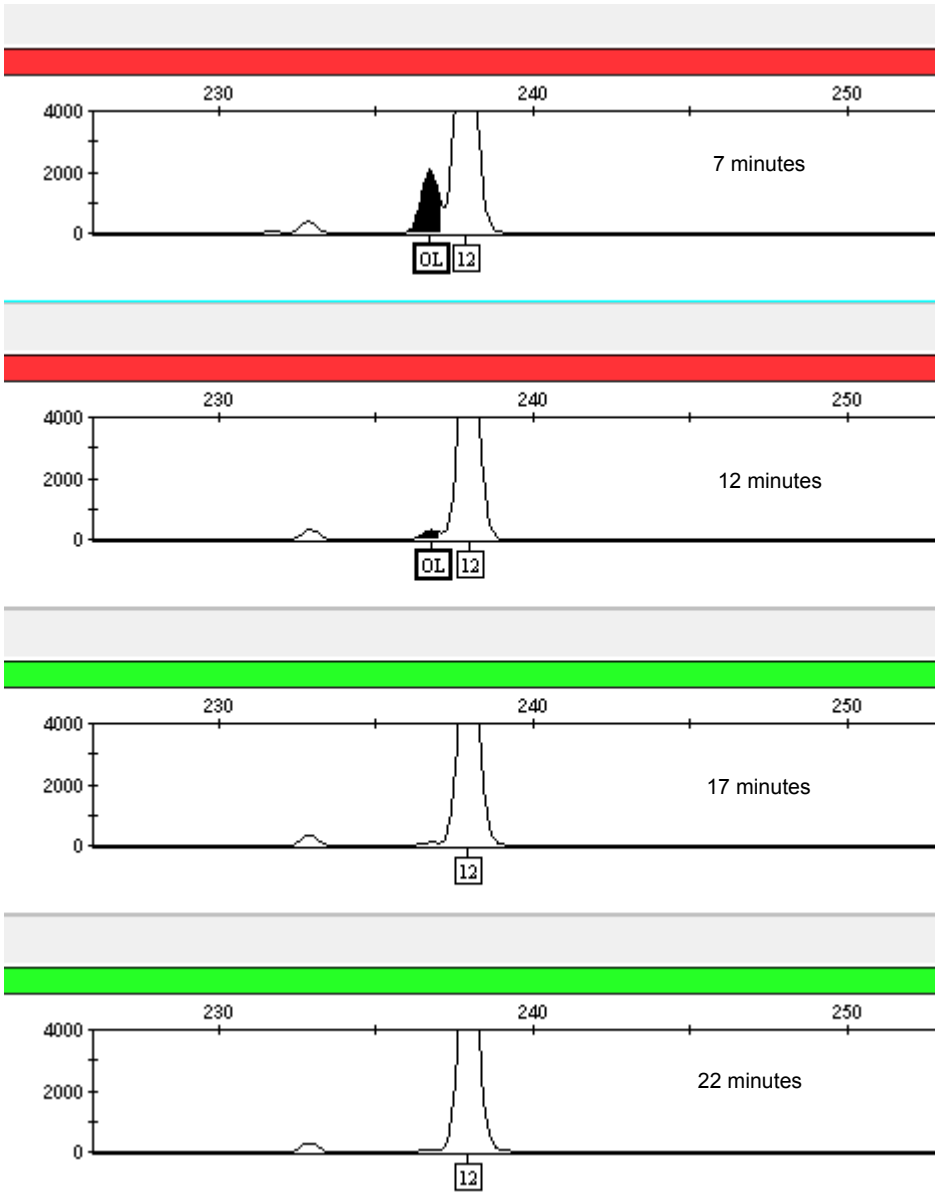


Figure 21 Omitting the final extension step results in shoulders on main allele peaks due to incomplete A nucleotide addition. Examples shown are the smaller amplicons of FAM™, NED™, and SID™ dye channel data from a 3500xL Genetic Analyzer using the Yfiler™ Plus PCR Amplification Kit.

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

Extra peaks: Artifacts

Artifact definition

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

Artifact observation

Additional reproducible DNA-dependent artifacts have been characterized and recorded on Table 5. It is important to consider possible noise and artifacts when interpreting data from the Yfiler™ Plus kit on the 3500/3500xL and 3130/3130*xl* Genetic Analyzers.

Table 5 DNA-dependent artifacts

Artifact	Color	Size	Comment
FAM270	Blue	270–271	Minor cross-reactive product observed with female DNA in excess of 2 µg.
FAM280	Blue	280–281	Minor cross-reactive product observed with female DNA in excess of 2 µg.
FAM348	Blue	348–349	Specific to cell-line derived kit Control DNA.
DYS391 (n–10)	Green	n – 10 nt	Specific to DYS391. Minor cross-reactive product observed with male DNA in excess of 1.0 ng.
TAZ140	Red	139–140	Minor cross-reactive product observed with female DNA in excess of 2 µg.
TAZ144	Red	144–145	Minor cross-reactive product observed with female DNA in excess of 2 µg.
TAZ225–260	Red	225–260	Multiple minor cross-reactive products observed with female DNA in excess of 2 µg.
TAZ412	Red	412–413	Cross-reactive product observed with female DNA in excess of 100 ng. Occurs outside of the read region. Does not impact interpretation.
VIC70	Green	70	Sporadic PCR artifact. Occurs outside of the VIC™ read region. Does not impact interpretation.
DYS437 (n–5)	Red	n – 5 nt	Specific to DYS437. Sporadic, non-target specific artifact.
DYS437 (n–12)	Red	n – 12 nt	Specific to DYS437. Sporadic, non-target specific artifact.
DYS437 (n–16)	Red	n – 12 nt	Specific to DYS437. Sporadic, non-target specific artifact.

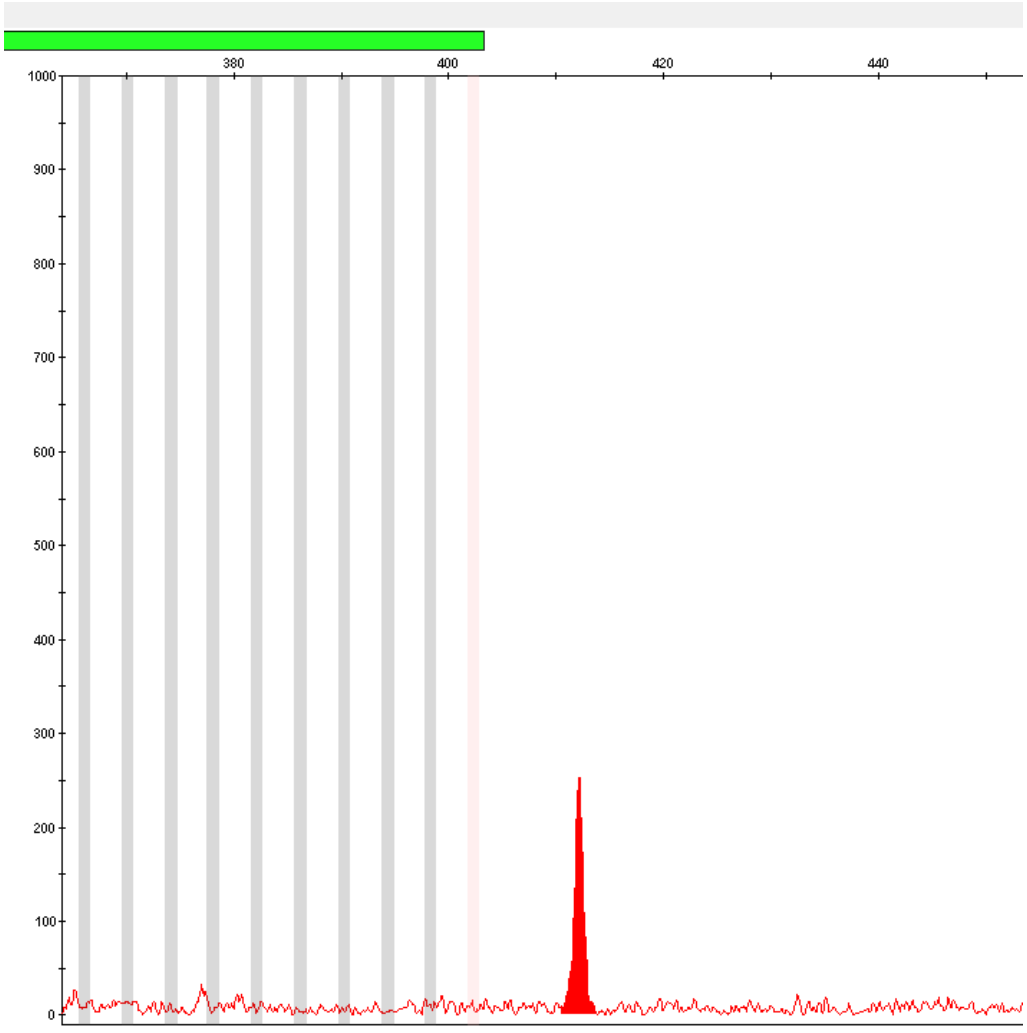


Figure 22 Example of the TAZ412 reproducible artifact in data produced on the 3500/3500xL Genetic Analyzer.

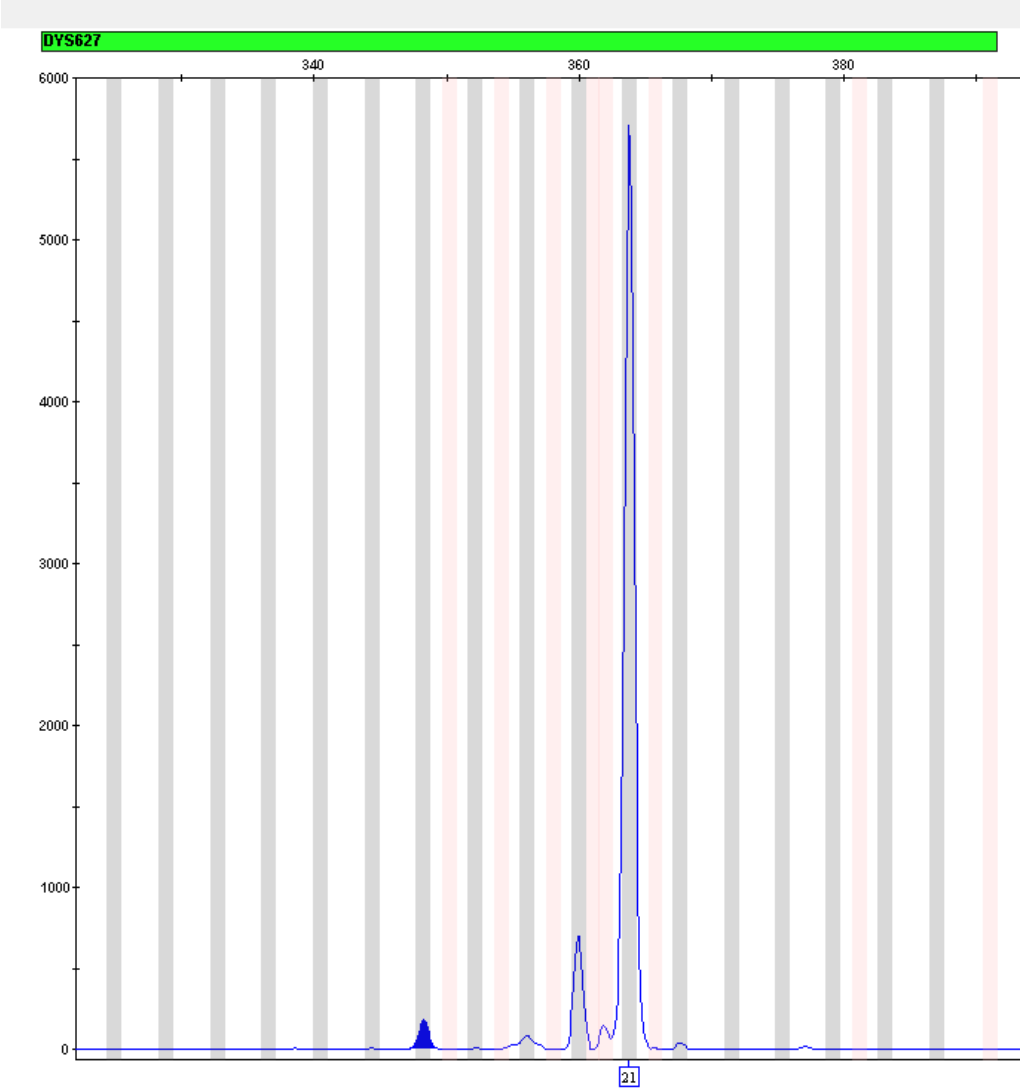


Figure 23 Example of the FAM348 reproducible artifact in data produced on the 3500/3500xL Genetic Analyzer.

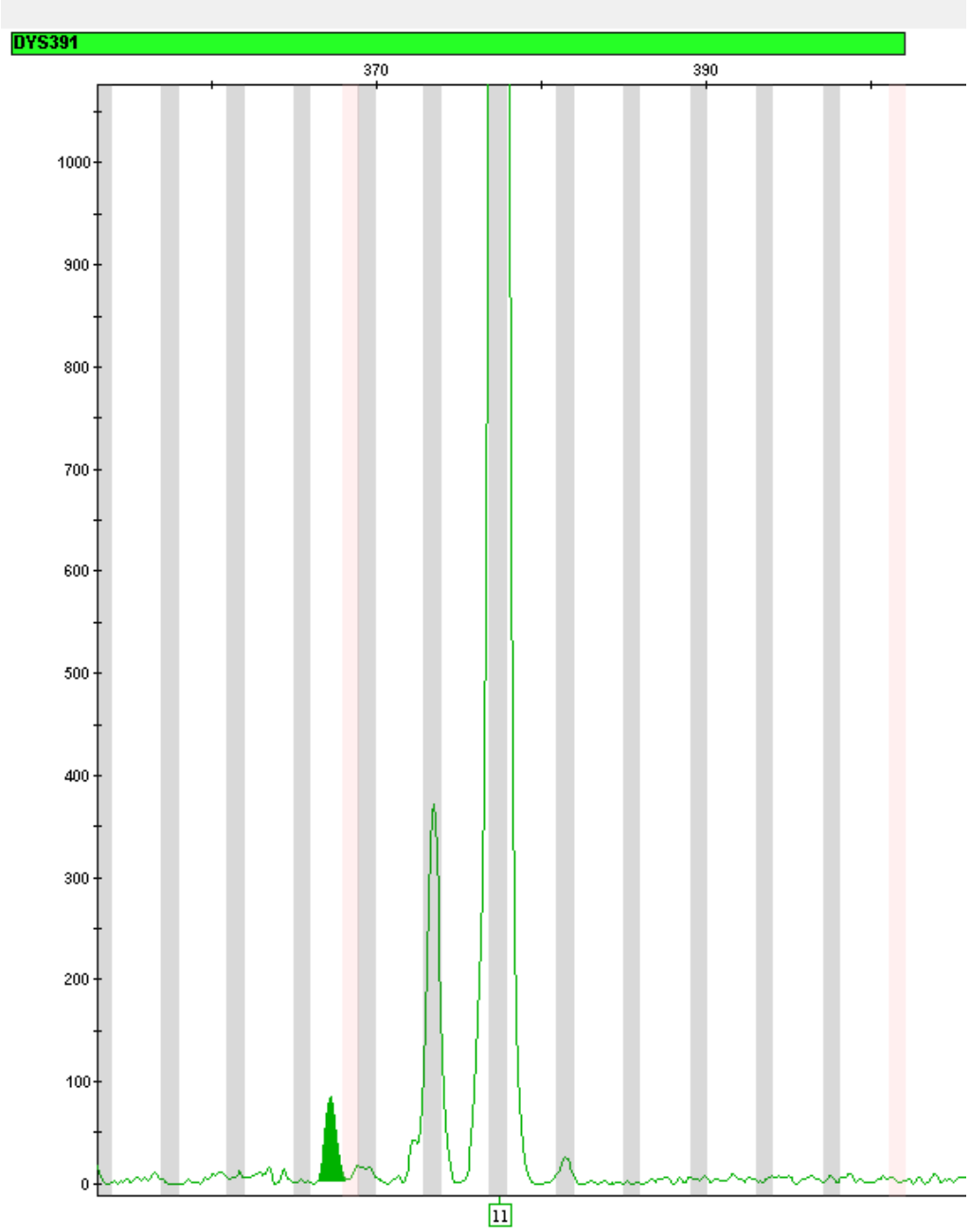


Figure 24 Example of the DYS391 (n–10) reproducible artifact in data produced on the 3500/3500xL Genetic Analyzer.

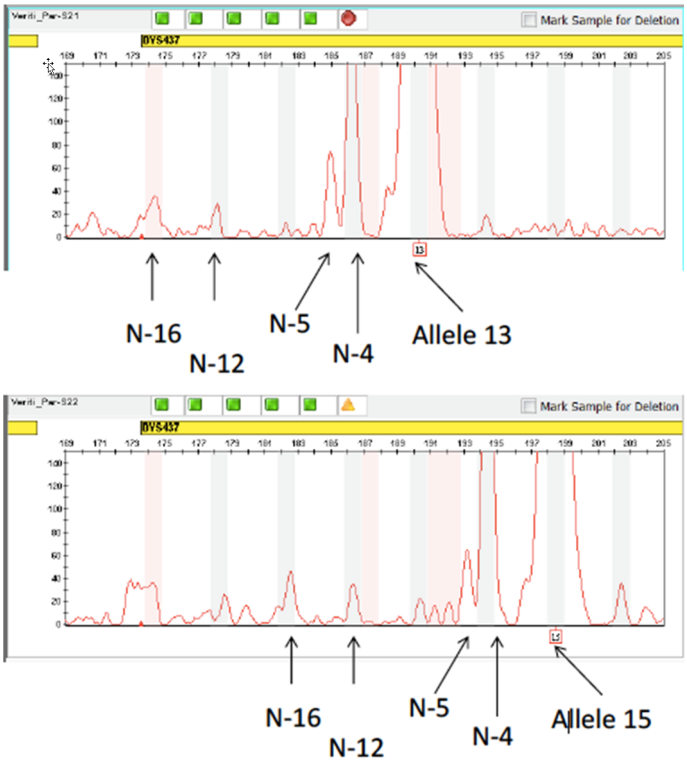


Figure 25 Examples of the DYS437 artifacts in data produced on the 3500/3500xL Genetic Analyzer

Characterization of loci

SWGDAM guideline 3.1

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

Loci in this kit

This section describes basic characteristics of the 27 Y-STR loci that are amplified with the Yfiler™ Plus PCR Amplification Kit. Most of these loci have been extensively characterized by other laboratories.

Nature of polymorphisms

DYS392 and DYS481 are trinucleotide repeats, DYS438 is a pentanucleotide repeat and DYS448 is a hexanucleotide repeat. Their allele differences result from differences in the number of repeat units 3-bp, 5-bp, and 6-bp respectively. The remaining Yfiler™ Plus PCR Amplification Kit loci are tetranucleotide short tandem repeat (STR) loci. The length differences among alleles of these particular loci result from differences in the number of 4-bp repeat units.

We have sequenced all the alleles in the Yfiler™ Plus PCR Amplification Kit Allelic Ladder. In addition, other groups in the scientific community have sequenced alleles at some of these loci (Redd *et al.*, 2002; **www.cstl.nist.gov/biotech/strbase/y_strs.htm**). Among the various sources of sequence data on the loci, there is consensus on the repeat patterns and structure of the STRs (Mulero *et al.*, 2014; Gusmao *et al.*, 2006).

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 Yfiler™ Plus PCR Amplification Kit loci have been mapped, and the location on the Y-chromosome is known based on the nucleotide sequence of the Y-chromosome. The Genbank accession numbers for representative sequences are: DYS19 (X77751, AC017019), DYS385 (AC022486, Z93950), DYS389 (AC011289, AF140635), DYS390 (AC011289), DYS391 (G09613, AC011302), DYS392 (G09867, AC06152), DYS393 (G09601, AC06152), DYS437 (AC002992), DYS438 (AC002531), DYS439 (AC002992), DYS448 (AC025227.6), DYS456 (AC010106.2), DYS458 (AC010902.4), DYS635 (G42676, AC011751), DYS635 (G42673), DYS449 (AC051663), DYS481 (FJ828747.1), DYS533 (AC053516), DYS570 (AC012068), DYS576 (AC010104), DYS518 (FJ828760) and DYS627 (BV208976).

Species specificity

SWGDAM Guideline 3.2

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

Nonhuman studies

The Yfiler™ Plus PCR Amplification Kit provides the required specificity for detecting human alleles. Species specificity testing was performed to ensure that there is no cross-reactivity with nonhuman DNA that may be present in forensic casework samples.

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

- Primates: gorilla, chimpanzee, and macaque (1.0 ng each)
- Non-primates: mouse, dog, pig, rat, sheep, horse, chicken, and cow (10.0 ng each)
- Microorganisms: *Candida albicans*, *Neisseria gonorrhoeae*, *Escherichia coli* 0157:H7, *Bacillus subtilis*, *Staphylococcus aureus*, and *Lactobacillus rhamnosus* (5 ng each)

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

The chimpanzee and gorilla DNA samples produced partial profiles in the 100–330 base pair region.

The remaining species tested did not yield reproducible detectable products.

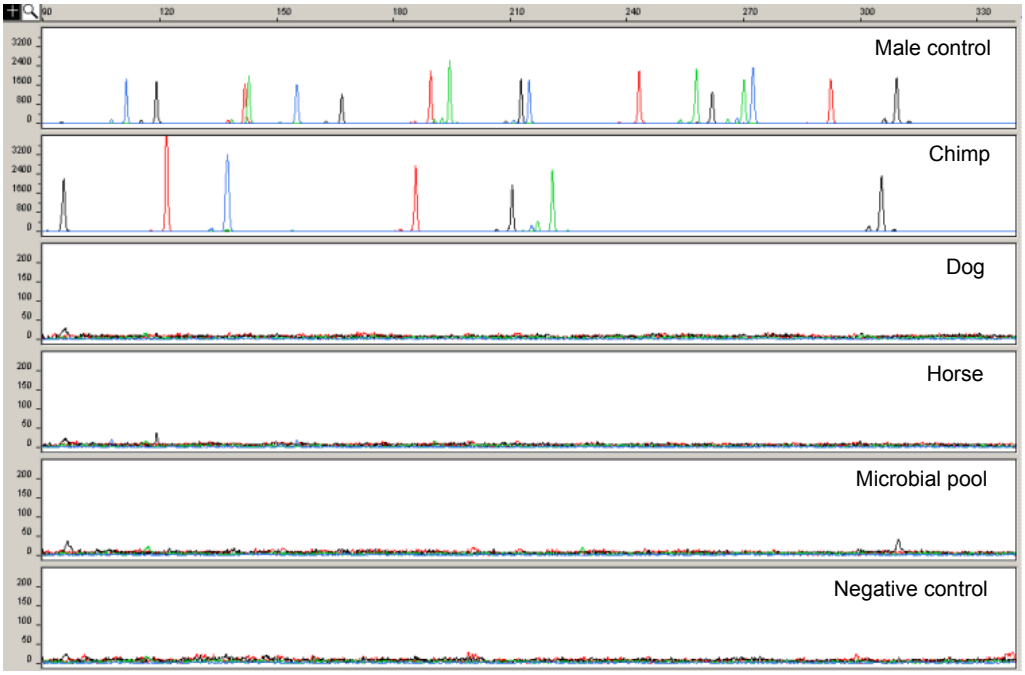


Figure 26 Representative electropherograms from a species specificity study including positive and negative control.

Sensitivity

SWGDAM guideline 3.3

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

Sample collection factors that can affect DNA quantity

The Yfiler™ Plus PCR Amplification Kit has been optimized at 25 µL PCR reaction volume to overcome the PCR inhibition expected when amplifying:

- Blood samples that are obtained directly from unpurified 1.2 mm treated paper discs
- Buccal cells that are obtained directly from unpurified 1.2 mm treated paper discs (with the addition of Prep-n-Go™ Buffer)
- Buccal swab sample lysate is prepared using Prep-n-Go™ Buffer

Depending on the following conditions, DNA quantities present on the 1.2 mm disc may vary from laboratory to laboratory:

- Volume of blood that is spotted onto the treated paper
- Collecting devices that are used
- Collection methods that are applied
- Swab-to-paper transfer protocol that is used

It is essential to optimize the PCR conditions for types of blood samples and spotting protocol. See "Direct amplification: Optimize PCR cycle number" on page 21.

Effect of DNA quantity on results

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

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

  Off-scale data is a problem because:

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

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

- Incomplete +A nucleotide addition.

To ensure minimal occurrence of offscale data when using the Yfiler™ Plus PCR Amplification Kit , optimize PCR cycle number according to instructions in the Perform PCR chapter.

When the total number of allele copies added to the PCR is extremely low, unbalanced amplification of the alleles may occur because of stochastic fluctuation.

Sensitivity observation

Figure 27 shows the results of amplification of different input DNA amounts. The optimal amount of input male DNA added to the Yfiler™ Plus kit is 0.5–1.0 ng for 30 cycles of amplification when using extracted and purified DNA. The final DNA concentration should be 0.05–0.10 ng/µL, so that 0.5–1.0 ng of male DNA is added to the 10-µL PCR reaction. If the sample contains degraded DNA, amplification of additional DNA can be beneficial.

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

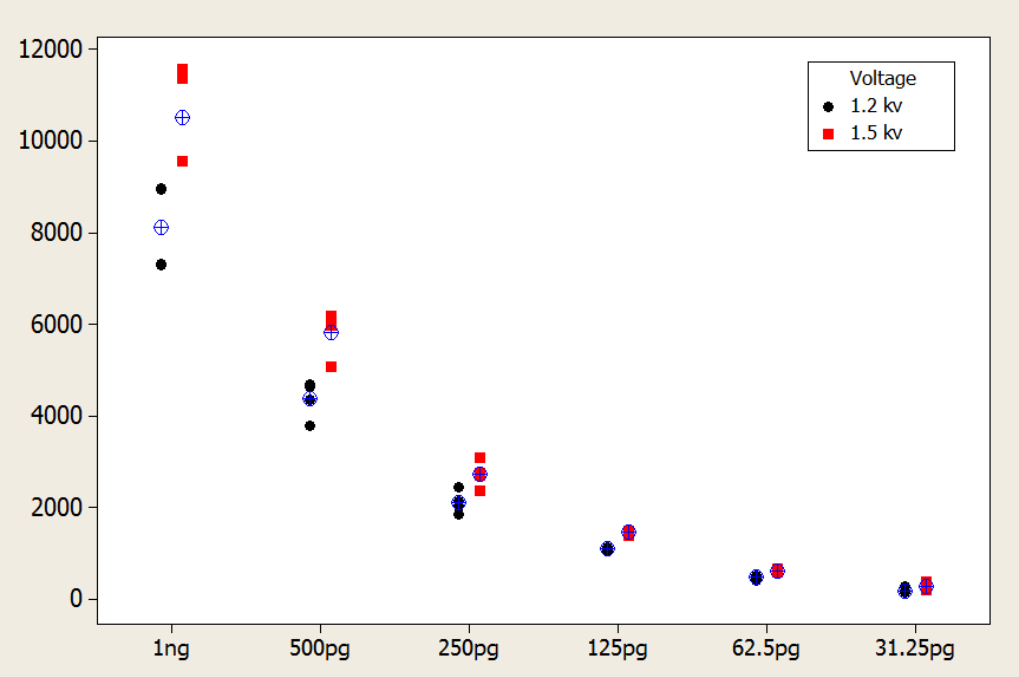


Figure 27 Effect of amplifying 1 ng, 500 pg, 250 pg, 125 pg, 62 pg, and 31 pg of male control DNA 007 using two voltage conditions. Data analyzed using the 3500xL Genetic Analyzer. The Y-axis scale is 0 to 12,000 RFUs.

Stability

SWGDAM guideline 3.4

"The ability to obtain results from DNA recovered from biological samples deposited on various substrates and subjected to various environmental and chemical insults should be evaluated. In most instances, assessment of the effects of these factors on new forensic DNA procedures is not required. However, if substrates and/or environmental and/or chemical insults could potentially affect the analytical process, then the process should be evaluated to determine the effects of such factors." (SWGDAM, December 2012)

Lack of amplification of some loci

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

Effect of inhibitors

Traces of humic acid can inhibit the PCR amplification of DNA evidence that is collected from soil. Amplification of 1.0 ng of DNA Control 007 in the presence of increasing amounts of humic acid was performed using the Yfiler™ Plus kit (Figure 28). The concentrations of humic acid tested were 0, 100, and 250 ng/µL. The same concentrations were tested with the Yfiler™ Plus kit for comparison. At 250 ng/µL, neither kit yielded amplified products.

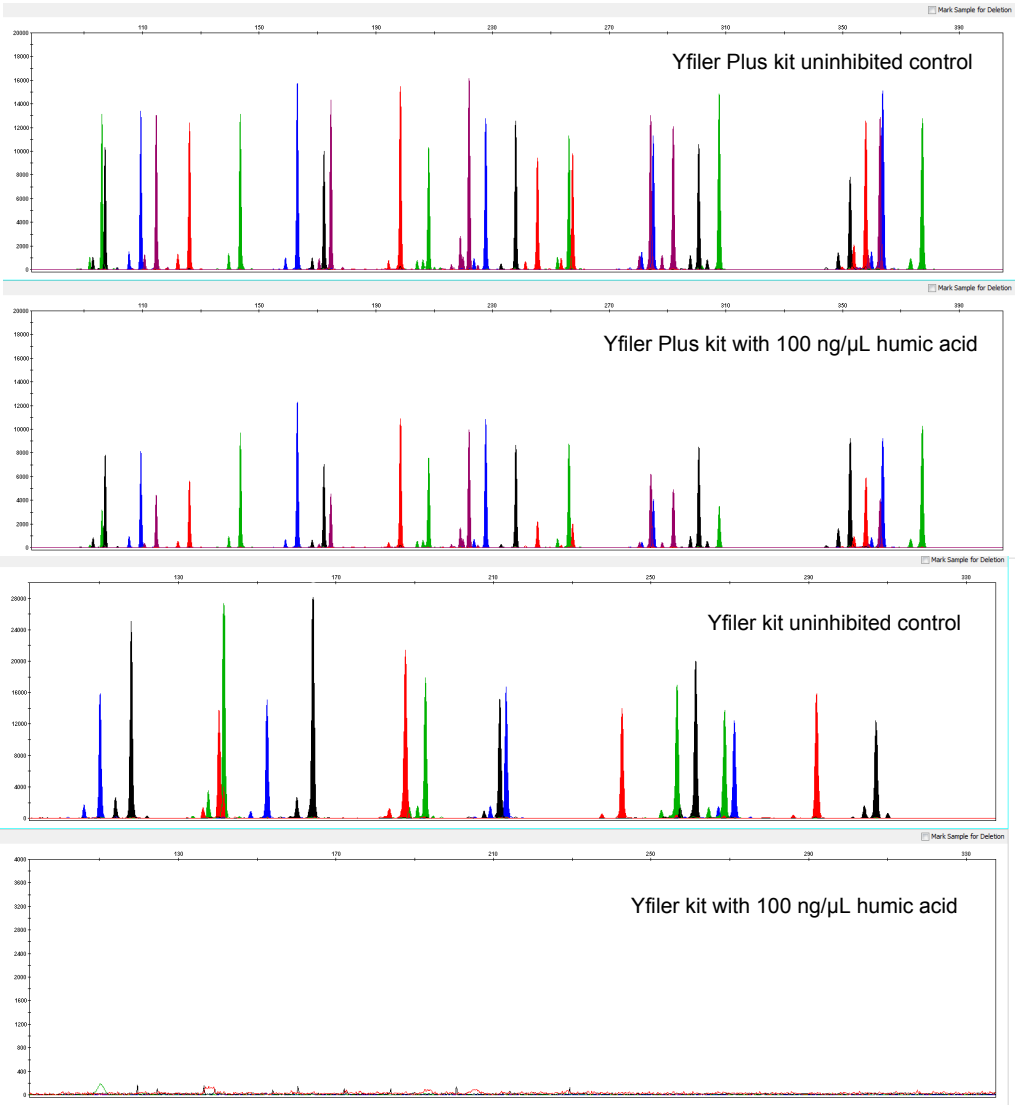


Figure 28 Electropherograms for the Yfiler™ Plus and AmpFℓSTR™ Yfiler™ kits show the improved performance of the Yfiler™ Plus kit in the presence of humic acid compared to the Yfiler™ kit. The Y-axis scale is 0 to 20,000 RFUs for the top two panels, 0 to 30,000 RFUs for the third panel, and 0 to 4,000 RFUs for the bottom panel.

Degraded DNA

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

Degraded DNA was prepared to examine the potential for preferential amplification of loci. High molecular weight DNA was incubated with the enzyme DNase I for varying amounts of time. The DNA was examined by agarose gel analysis to determine the average size of the DNA fragments at each time point.

2 ng of degraded DNA (or 1 ng undegraded DNA) was amplified using the Yfiler™ Plus kit . As the DNA became increasingly degraded, the loci became undetectable according to size. The loci failed to robustly amplify in the order of decreasing size as the extent of degradation progressed (Figure 29).

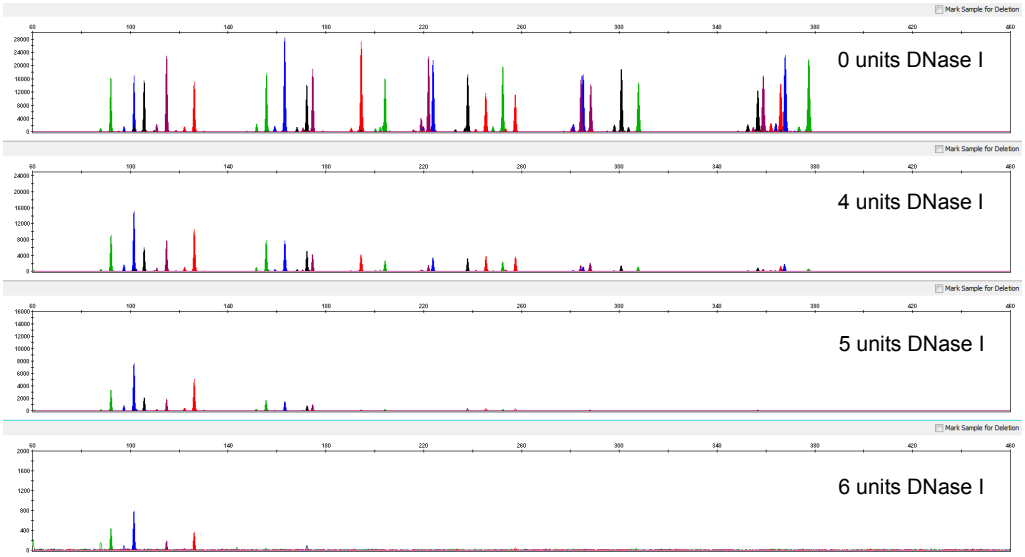


Figure 29 Amplification of A3121 DNA samples sonicated and incubated with increasing doses of DNase I. Panels 1, 2, 3, and 4 correspond to 0, 4, 5, and 6 units of DNase I. Note that the y-axis scale is magnified for more degraded samples, which generate lower peak heights.

Mixture studies

SWGDAM guideline 3.8

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

Male/female mixture studies

Evidence samples that contain body fluids and/or tissues originating from more than one individual are an integral component of forensic casework. Therefore it is essential to ensure that the DNA typing system is able to detect DNA mixtures. In the case of Y-STRs, the female DNA component is not amplified by the Y-chromosome-specific primers. Male/female mixture studies were performed up to a ratio of 1:4,000 using three different female DNAs. The amount of female DNA was kept constant at 1 µg and the amount of male control DNA was changed. The female DNA did not cause any interference with the interpretation of the male Y-STR profile as shown in Figure 30.

Low-level artifacts with female DNA have been occasionally observed in the FAM™ (270–280 bp) and TAZ™ (225–260 bp) dye. In general, these artifacts peaks should not affect interpretation due to their morphology and intensity.

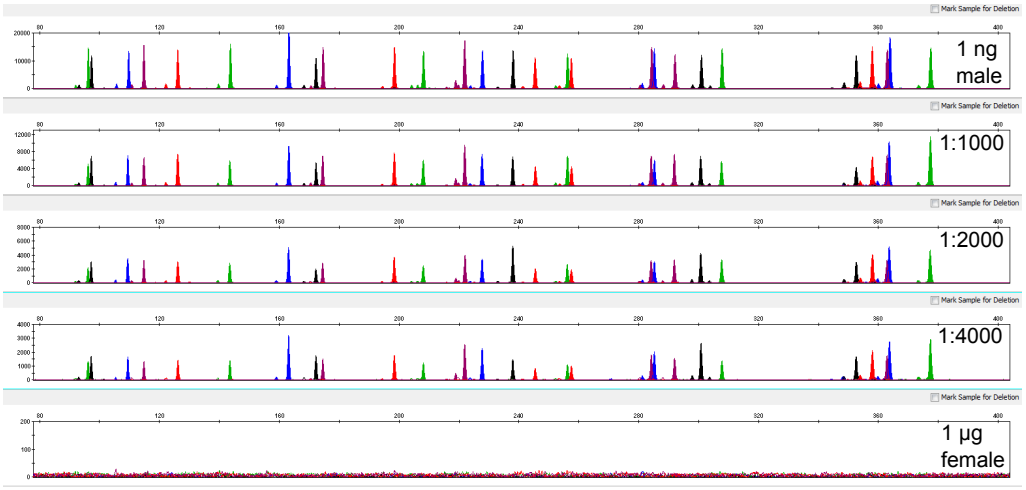


Figure 30 Amplification of male Control DNA 007 in the presence of female DNA 9947A. Profiles shown in the panels from top to bottom: 1 ng of male DNA, 1 ng male DNA with 1 µg female DNA, 500 pg male DNA with 1 µg female DNA, 250 pg male DNA with 1 µg female DNA, 1 µg female DNA. The Y-axis scale is magnified for lower input amounts of male DNA samples, which generate lower peak heights (Y-axis scale is 0 to 200 RFUs for the 1-µg female input).

Male/male mixture studies

Forensic samples may contain body fluids or tissues originating from more than one male. Mixtures of two male DNA samples were examined at various ratios (1:1 to 1:15). The total amount of genomic input DNA mixed at each ratio was 1 ng.

Table 6 Haplotypes of samples in the figure that follows this table.

Locus	Sample A	Sample B
DYS576	15	19
DYS389I	14	13
DYS635	21	24
DYS389II	31	29
DYS627	21	21
DYS460	10	11
DYS458	17	17
DYS19	15	15
YGATAH4	12	13
DYS448	21	19
DYS391	10	11

Locus	Sample A	Sample B
DYS456	13	15
DYS390	21	24
DYS438	12	12
DYS392	11	13
DYS518	38	37
DYS570	19	17
DYS437	14	15
DYS385 a/b	16, 19	11, 14
DYS449	29	30
DYS393	14	13
DYS439	11	12
DYS481	27	22
DYF387S1	36, 39	35, 37
DYS533	11	13

A representative electropherogram of 1-ng total male/male DNA mixture studies is shown in Figure 31. The limit of detection is when the minor component is present at approximately one-tenth of the concentration of the major component. The limit of detection for the minor component is influenced by the combination of genotypes in the mixture.

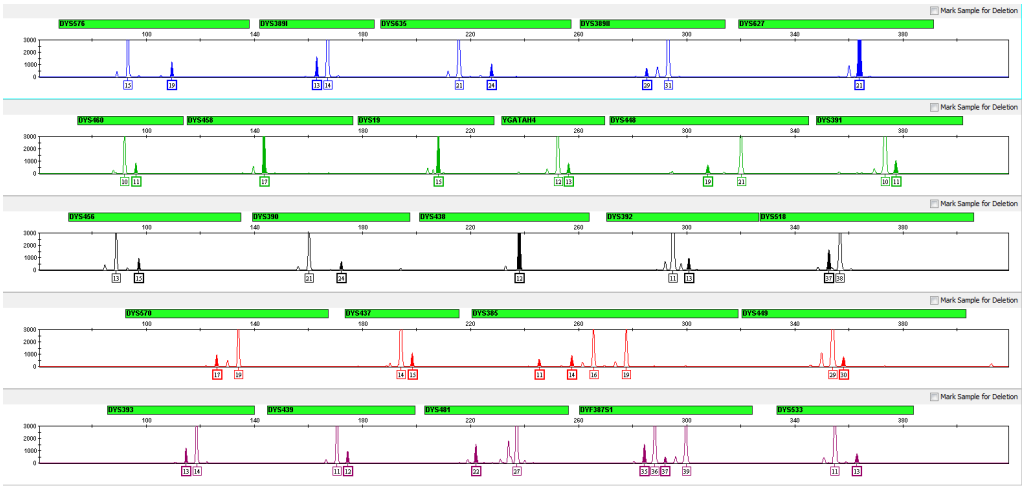


Figure 31 Mixtures of two male DNA samples (1:8 ratio, 125 pg: 875 pg) 1-ng input DNA. The alleles attributable to the minor component, even when the major component shares an allele, are highlighted.

Population data

SWGDAM YSTR guideline 10.1

"The laboratory should establish guidelines for the number of Y-STR loci used for searches of

population databases." (SWGDAM, January 2014)

Population data overview

All Y-STR loci analyzed in commercial kits are physically linked on the Y-chromosome. Due to the lack of recombination, the entire Y-chromosome haplotype must be treated as a single locus. Haplotype frequencies are estimated using the counting method. The counting method involves searching a given haplotype against a database to determine the number of times the haplotype was observed in that database. The frequency of the haplotype in the database is then estimated by dividing the count by the number of haplotypes searched. (SWGDAM, January 2014)

Population samples used in these studies

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

- African-American—557 samples
- U.S. Asian—340 samples
- U.S. Caucasian—533 samples
- U.S. Hispanic—391 samples

DNA was extracted with a 6100 Nucleic Acid Prep Station.

The Yfiler™ Plus PCR Amplification Kit contains loci for which extensive population data are available.

In addition to the haplotypes that we observed and recorded in our databases, other alleles have been published or reported to us by other laboratories (see the STRBase at **www.cstl.nist.gov/div831/strbase**, see the YHARD at **www.yhrd.org**, and see the U.S. Y-STR database at **www.usystrdatabase.org**).

Gene diversity values

Table 7 shows the Yfiler™ Plus kit gene diversity in three populations, which are listed as percentages.

Table 7 Yfiler™ Plus kit Gene diversity values across four different U.S. populations

Locus	African-American (n = 557)	U.S. Caucasian (n = 533)	U.S. Hispanic (n = 391)	U.S. Asian (n = 340)
DYS576	0.807	0.768	0.769	0.799
DYS389I	0.504	0.527	0.567	0.679
DYS635	0.716	0.646	0.713	0.786
DYS389II	0.746	0.676	0.729	0.770
DYS627	0.838	0.842	0.853	0.812

Locus	African-American (n = 557)	U.S. Caucasian (n = 533)	U.S. Hispanic (n = 391)	U.S. Asian (n = 340)
DYS460	0.573	0.537	0.571	0.675
DYS458	0.750	0.766	0.800	0.820
DYS19	0.726	0.459	0.632	0.703
YGATAH4	0.590	0.585	0.580	0.606
DYS448	0.707	0.583	0.697	0.755
DYS391	0.445	0.540	0.561	0.437
DYS456	0.615	0.737	0.700	0.603
DYS390	0.646	0.684	0.656	0.699
DYS438	0.551	0.581	0.688	0.547
DYS392	0.445	0.592	0.664	0.710
DYS518	0.843	0.806	0.807	0.867
DYS570	0.806	0.738	0.799	0.820
DYS437	0.504	0.577	0.592	0.476
DYS385 a/b	0.942	0.854	0.904	0.973
DYS449	0.857	0.783	0.818	0.882
DYS393	0.587	0.363	0.442	0.662
DYS439	0.629	0.625	0.682	0.669
DYS481	0.857	0.724	0.790	0.821
DYF387S1	0.941	0.874	0.913	0.945
DYS533	0.598	0.576	0.591	0.644

Gene diversity (D) = $\frac{n(1-\Sigma p_i^2)}{n-1}$ where n = sample size, pi = allele frequency (Johnson *et al.*, 2003).

Analyze the population data

In addition to the alleles that were observed and recorded in the Thermo Fisher Scientific databases, other known alleles have been published or reported to us by other laboratories. Some of these alleles occur at a low frequency and include several microvariants (Furedi *et al.*, 1999; Schoske *et al.*, 2004).

Discriminatory capacity of haplotypes

Table 8 shows the discriminatory capacity (DC) and the number of unique haplotypes (UH) for each Y-STR marker combination listed. The discriminatory capacity was determined by dividing the number of different haplotypes by the number of samples in that population (Schoske *et al.*, 2004). A unique haplotype is defined as one that occurs only once in a given population. The number of unique haplotypes is usually less than the number of different haplotypes in any given population.

Table 8 Discriminatory capacity and number of unique haplotypes for four U.S. populations

Y-STR marker combination	African-American (n = 557)		U.S. Caucasian (n = 533)		U.S. Hispanic (n = 391)		U.S. Asian (n = 340)	
	DC (%)	UH	DC (%)	UH	DC (%)	UH	DC (%)	UH
Yfiler™	98.2	547	95.7	510	95.9	375	91.5	311
Yfiler™ Plus	99.6	555	98.5	525	98.0	383	94.4	321

Mutation rate

The most accurate method of estimating Y-STR mutation rates is the direct observation of transmission between father and son. A large-scale Y-STR analysis of mutation rates was performed with 2,000 DNA-confirmed father-son pairs and encompassed the Yfiler™ Plus marker set (Ballantyne *et al.*, 2010, 2012, and 2014).

A

Table of Precision Results

Table of typical precision results

Table 9 Example of precision results of seven injections of the Yfiler™ Plus Allelic Ladder run on the 3130*xl*, 3500, and 3500xL

Allele	3130*xl*		3500		3500xL	
	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean
DYF387S1						
30	264.16–264.33	0.038–0.055	264.90–264.93	0.045–0.063	264.85–264.89	0.038–0.057
31	268.02–268.20	0.035–0.054	268.81–268.83	0.052–0.064	268.74–268.78	0.045–0.059
32	271.91–272.10	0.031–0.056	272.69–272.72	0.042–0.053	272.63–272.68	0.040–0.058
33	275.87–276.03	0.035–0.054	276.65–276.69	0.045–0.068	276.58–276.63	0.046–0.054
34	279.72–279.90	0.038–0.055	280.54–280.58	0.049–0.072	280.47–280.51	0.049–0.062
35	283.52–283.71	0.044–0.062	284.33–284.37	0.037–0.063	284.26–284.30	0.049–0.057
36	287.35–287.55	0.043–0.054	288.17–288.22	0.049–0.066	288.12–288.16	0.042–0.057
37	291.25–291.44	0.034–0.055	292.10–292.11	0.044–0.075	292.01–292.06	0.042–0.062
38	295.03–295.22	0.043–0.065	295.89–295.91	0.047–0.067	295.79–295.85	0.037–0.062
39	299.06–299.26	0.045–0.055	299.93–299.96	0.064–0.068	299.85–299.90	0.046–0.063
40	302.75–302.95	0.039–0.054	303.61–303.65	0.048–0.073	303.52–303.60	0.047–0.065
41	306.64–306.86	0.035–0.066	307.56–307.60	0.038–0.087	307.48–307.53	0.050–0.062
42	310.49–310.70	0.047–0.060	311.44–311.47	0.062–0.080	311.35–311.41	0.046–0.066
43	314.40–314.62	0.041–0.073	315.40–315.43	0.046–0.096	315.30–315.37	0.045–0.070
44	318.47–318.69	0.039–0.062	319.50–319.52	0.048–0.089	319.40–319.47	0.047–0.071
DYS19						
9	183.90–183.94	0.023–0.030	184.04–184.06	0.028–0.048	183.99–184.01	0.026–0.039
10	188.07–188.11	0.022–0.036	188.20–188.22	0.026–0.035	188.16–188.18	0.026–0.040
11	192.11–192.13	0.026–0.036	192.26–192.28	0.026–0.040	192.22–192.24	0.025–0.037

Allele	3130*xl*		3500		3500xL	
	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean
12	196.10–196.13	0.018–0.039	196.24–196.27	0.032–0.046	196.21–196.23	0.032–0.041
13	200.17–200.21	0.019–0.034	200.30–200.33	0.029–0.046	200.28–200.29	0.037–0.043
14	204.09–204.13	0.020–0.036	204.24–204.27	0.027–0.038	204.21–204.21	0.029–0.037
15	208.05–208.09	0.018–0.028	208.22–208.24	0.029–0.043	208.16–208.18	0.027–0.038
16	212.03–212.05	0.023–0.032	212.20–212.26	0.015–0.053	212.15–212.16	0.024–0.046
17	216.07–216.10	0.023–0.032	216.22–216.25	0.016–0.052	216.18–216.20	0.029–0.044
18	220.14–220.19	0.020–0.034	220.30–220.34	0.033–0.046	220.27–220.29	0.020–0.043
19	224.15–224.20	0.024–0.035	224.34–224.35	0.040–0.058	224.30–224.31	0.036–0.047
DYS385 a/b						
6	225.25–225.31	0.029–0.041	225.10–225.12	0.022–0.050	225.07–225.09	0.036–0.048
7	229.25–229.33	0.027–0.041	229.12–229.14	0.028–0.044	229.08–229.11	0.042–0.047
8	233.37–233.44	0.026–0.042	233.21–233.24	0.026–0.045	233.19–233.21	0.038–0.048
9	237.40–237.47	0.028–0.037	237.24–237.26	0.023–0.066	237.22–237.25	0.033–0.046
10	241.50–241.57	0.026–0.049	241.31–241.34	0.035–0.052	241.30–241.31	0.023–0.038
11	245.53–245.61	0.024–0.040	245.37–245.38	0.022–0.046	245.34–245.37	0.031–0.046
12	249.71–249.81	0.027–0.038	249.53–249.55	0.005–0.034	249.51–249.54	0.034–0.043
13	253.74–253.84	0.025–0.037	253.55–253.57	0.021–0.040	253.55–253.57	0.030–0.046
14	257.69–257.79	0.027–0.047	257.50–257.52	0.031–0.047	257.51–257.52	0.028–0.041
15	261.66–261.75	0.023–0.039	261.46–261.49	0.031–0.040	261.48–261.50	0.033–0.043
16	265.67–265.76	0.027–0.041	265.50–265.51	0.018–0.044	265.50–265.52	0.030–0.046
17	269.72–269.82	0.022–0.047	269.54–269.58	0.022–0.042	269.55–269.57	0.036–0.046
18	273.73–273.83	0.026–0.045	273.54–273.58	0.027–0.055	273.54–273.57	0.029–0.047
19	277.83–277.93	0.025–0.046	277.64–277.66	0.040–0.058	277.65–277.67	0.031–0.042
20	281.85–281.95	0.025–0.044	281.66–281.70	0.035–0.044	281.67–281.69	0.033–0.044
21	285.85–285.94	0.029–0.044	285.65–285.69	0.036–0.043	285.67–285.68	0.030–0.043
22	289.82–289.93	0.029–0.052	289.63–289.65	0.037–0.050	289.63–289.66	0.030–0.040
23	293.78–293.89	0.031–0.046	293.58–293.62	0.030–0.040	293.58–293.60	0.032–0.047
24	297.79–297.89	0.029–0.042	297.57–297.60	0.037–0.065	297.56–297.60	0.034–0.050
25	301.75–301.85	0.025–0.052	301.56–301.58	0.038–0.045	301.55–301.57	0.020–0.050

Allele	3130*xl*		3500		3500xL	
	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean
26	305.71–305.82	0.034–0.039	305.51–305.53	0.018–0.054	305.51–305.53	0.026–0.039
27	309.71–309.82	0.036–0.044	309.50–309.55	0.028–0.042	309.52–309.54	0.032–0.044
28	313.78–313.87	0.032–0.051	313.53–313.58	0.005–0.051	313.55–313.58	0.043–0.049
DYS389I						
9	146.74–146.78	0.025–0.034	146.98–147.01	0.019–0.044	146.81–146.84	0.025–0.042
10	150.83–150.87	0.026–0.033	151.06–151.09	0.019–0.039	150.90–150.91	0.032–0.041
11	154.86–154.91	0.024–0.031	155.09–155.11	0.030–0.042	154.92–154.94	0.034–0.038
12	158.98–159.04	0.027–0.036	159.22–159.24	0.007–0.030	159.04–159.07	0.021–0.035
13	163.19–163.26	0.020–0.031	163.41–163.43	0.028–0.045	163.25–163.27	0.026–0.038
14	167.12–167.19	0.018–0.034	167.33–167.35	0.015–0.030	167.18–167.19	0.027–0.033
15	171.17–171.21	0.017–0.040	171.34–171.37	0.022–0.039	171.21–171.22	0.021–0.038
16	175.19–175.24	0.020–0.025	175.37–175.40	0.020–0.046	175.23–175.24	0.026–0.039
17	179.23–179.28	0.013–0.034	179.40–179.41	0.005–0.032	179.25–179.27	0.041–0.045
DYS389II						
24	265.02–265.11	0.025–0.032	265.16–265.20	0.023–0.060	265.12–265.12	0.030–0.044
25	269.11–269.20	0.024–0.040	269.23–269.25	0.030–0.052	269.17–269.18	0.030–0.042
26	273.08–273.17	0.021–0.043	273.23–273.24	0.026–0.040	273.15–273.17	0.028–0.042
27	277.22–277.32	0.024–0.039	277.36–277.39	0.036–0.054	277.29–277.31	0.030–0.042
28	281.15–281.25	0.020–0.048	281.29–281.32	0.032–0.047	281.21–281.22	0.034–0.043
29	285.01–285.10	0.019–0.041	285.14–285.16	0.029–0.043	285.07–285.09	0.028–0.041
30	289.17–289.26	0.026–0.047	289.31–289.33	0.027–0.039	289.23–289.25	0.034–0.039
31	293.15–293.24	0.023–0.043	293.27–293.31	0.037–0.040	293.19–293.21	0.023–0.037
32	297.02–297.11	0.027–0.035	297.14–297.15	0.029–0.059	297.04–297.07	0.028–0.044
33	300.97–301.06	0.025–0.041	301.10–301.13	0.039–0.049	301.02–301.03	0.037–0.043
34	304.81–304.89	0.024–0.035	304.93–304.96	0.025–0.060	304.85–304.86	0.031–0.038
35	308.89–308.97	0.027–0.041	309.01–309.03	0.036–0.046	308.93–308.94	0.040–0.050
DYS390						
17	144.14–144.23	0.026–0.044	144.14–144.19	0.038–0.042	144.09–144.11	0.029–0.039
18	148.04–148.12	0.020–0.038	148.06–148.10	0.021–0.051	147.98–148.01	0.035–0.043

Allele	3130*xl*		3500		3500xL	
	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean
19	151.96–152.04	0.026–0.037	151.99–152.02	0.026–0.043	151.92–151.93	0.029–0.039
20	156.15–156.25	0.027–0.046	156.16–156.20	0.026–0.041	156.09–156.12	0.025–0.038
21	160.16–160.24	0.031–0.039	160.17–160.18	0.000–0.043	160.09–160.10	0.025–0.037
22	164.21–164.30	0.024–0.039	164.21–164.24	0.020–0.061	164.15–164.17	0.030–0.041
23	168.34–168.42	0.026–0.039	168.31–168.34	0.030–0.048	168.25–168.28	0.032–0.044
24	172.34–172.42	0.024–0.038	172.30–172.32	0.032–0.055	172.25–172.27	0.025–0.033
25	176.33–176.41	0.024–0.034	176.30–176.33	0.029–0.044	176.24–176.26	0.027–0.037
26	180.35–180.44	0.023–0.036	180.33–180.34	0.032–0.041	180.27–180.29	0.026–0.041
27	184.33–184.42	0.019–0.030	184.32–184.34	0.040–0.052	184.26–184.28	0.031–0.044
28	188.44–188.53	0.026–0.041	188.41–188.43	0.032–0.050	188.36–188.38	0.029–0.043
29	192.49–192.58	0.027–0.042	192.46–192.48	0.022–0.047	192.41–192.44	0.034–0.042
DYS391						
5	352.78–352.85	0.024–0.035	353.42–353.45	0.038–0.057	353.34–353.36	0.034–0.049
6	356.84–356.91	0.032–0.042	357.46–357.51	0.032–0.054	357.39–357.42	0.036–0.051
7	360.69–360.78	0.030–0.036	361.40–361.43	0.026–0.057	361.32–361.35	0.040–0.049
8	364.75–364.85	0.034–0.045	365.47–365.49	0.019–0.035	365.40–365.43	0.033–0.057
9	368.74–368.85	0.023–0.042	369.47–369.50	0.024–0.060	369.39–369.43	0.033–0.046
10	372.75–372.87	0.020–0.040	373.47–373.48	0.036–0.076	373.40–373.44	0.032–0.050
11	376.75–376.87	0.029–0.044	377.47–377.47	0.019–0.047	377.38–377.42	0.037–0.044
12	380.81–380.90	0.031–0.052	381.44–381.48	0.018–0.050	381.36–381.39	0.028–0.049
13	384.93–385.02	0.029–0.045	385.56–385.60	0.020–0.063	385.47–385.50	0.037–0.042
14	388.98–389.07	0.024–0.038	389.60–389.62	0.023–0.050	389.50–389.54	0.028–0.036
15	393.02–393.08	0.026–0.046	393.63–393.67	0.024–0.043	393.55–393.57	0.033–0.045
16	397.05–397.12	0.031–0.042	397.66–397.70	0.022–0.057	397.58–397.61	0.032–0.047
DYS392						
4	273.67–273.77	0.028–0.036	274.35–274.38	0.045–0.056	274.25–274.28	0.039–0.047
5	276.63–276.74	0.027–0.044	277.36–277.39	0.048–0.063	277.25–277.28	0.030–0.048
6	279.61–279.73	0.021–0.038	280.32–280.35	0.033–0.051	280.22–280.25	0.045–0.054
7	282.64–282.75	0.023–0.032	283.35–283.39	0.042–0.056	283.26–283.29	0.037–0.048

Allele	3130*xl*		3500		3500xL	
	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean
8	285.54–285.66	0.026–0.030	286.23–286.25	0.050–0.065	286.12–286.16	0.032–0.048
9	288.52–288.60	0.027–0.036	289.12–289.14	0.040–0.057	289.00–289.04	0.034–0.044
10	291.25–291.38	0.026–0.040	291.97–292.00	0.049–0.065	291.87–291.89	0.034–0.052
11	294.30–294.42	0.026–0.039	295.04–295.05	0.051–0.072	294.91–294.94	0.030–0.055
12	297.26–297.38	0.026–0.038	297.96–297.99	0.036–0.058	297.84–297.88	0.035–0.058
13	300.19–300.30	0.031–0.045	300.89–300.90	0.037–0.067	300.77–300.80	0.038–0.047
14	303.01–303.13	0.027–0.036	303.73–303.74	0.048–0.061	303.59–303.63	0.036–0.050
15	306.00–306.12	0.028–0.040	306.70–306.74	0.043–0.070	306.59–306.63	0.033–0.052
16	309.03–309.15	0.026–0.044	309.71–309.73	0.041–0.073	309.58–309.62	0.038–0.053
17	311.93–312.06	0.027–0.044	312.67–312.70	0.027–0.064	312.54–312.58	0.027–0.047
18	314.96–315.09	0.029–0.040	315.75–315.77	0.019–0.060	315.61–315.65	0.042–0.051
19	318.11–318.23	0.022–0.035	318.88–318.90	0.028–0.056	318.75–318.78	0.035–0.047
20	321.21–321.35	0.022–0.039	321.97–322.00	0.049–0.063	321.84–321.88	0.027–0.058
DYS393						
7	90.33–90.36	0.021–0.032	90.35–90.39	0.027–0.037	90.23–90.25	0.029–0.037
8	94.35–94.36	0.024–0.037	94.37–94.42	0.020–0.032	94.27–94.28	0.033–0.041
9	98.51–98.53	0.020–0.030	98.53–98.56	0.028–0.039	98.43–98.44	0.040–0.044
10	102.63–102.64	0.014–0.029	102.67–102.70	0.021–0.052	102.56–102.57	0.033–0.040
11	106.89–106.90	0.019–0.027	106.94–106.96	0.026–0.037	106.82–106.84	0.029–0.037
12	110.79–110.81	0.024–0.030	110.85–110.88	0.039–0.056	110.74–110.76	0.031–0.041
13	114.80–114.81	0.017–0.028	114.87–114.91	0.020–0.041	114.76–114.78	0.026–0.035
14	118.72–118.74	0.021–0.029	118.79–118.81	0.033–0.043	118.67–118.69	0.010–0.032
15	122.51–122.53	0.024–0.036	122.61–122.62	0.031–0.044	122.48–122.49	0.030–0.041
16	126.56–126.59	0.017–0.029	126.66–126.68	0.029–0.046	126.54–126.56	0.036–0.041
17	130.54–130.55	0.015–0.033	130.62–130.66	0.023–0.036	130.51–130.53	0.027–0.041
18	134.51–134.54	0.019–0.029	134.61–134.64	0.025–0.038	134.52–134.53	0.030–0.043
DYS437						
10	177.93–177.98	0.027–0.035	178.26–178.28	0.031–0.053	178.20–178.22	0.030–0.047
11	181.97–182.01	0.026–0.036	182.29–182.32	0.030–0.045	182.24–182.26	0.040–0.046

Allele	3130*xl*		3500		3500xL	
	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean
12	186.02–186.07	0.021–0.041	186.35–186.38	0.027–0.041	186.31–186.32	0.031–0.046
13	189.98–190.02	0.020–0.039	190.28–190.31	0.020–0.051	190.24–190.26	0.033–0.043
14	194.03–194.07	0.023–0.041	194.34–194.37	0.028–0.049	194.29–194.31	0.041–0.044
15	198.15–198.20	0.025–0.031	198.49–198.51	0.017–0.040	198.46–198.47	0.035–0.046
16	202.13–202.19	0.030–0.037	202.47–202.50	0.026–0.047	202.44–202.45	0.035–0.041
17	206.08–206.12	0.023–0.034	206.42–206.45	0.026–0.056	206.36–206.38	0.033–0.040
18	210.03–210.07	0.022–0.036	210.38–210.41	0.031–0.054	210.33–210.35	0.038–0.045
DYS438						
6	207.14–207.22	0.024–0.032	207.69–207.73	0.044–0.059	207.67–207.68	0.033–0.040
7	212.16–212.22	0.025–0.032	212.70–212.71	0.030–0.049	212.66–212.68	0.030–0.054
8	217.23–217.31	0.022–0.038	217.78–217.82	0.029–0.050	217.76–217.77	0.032–0.038
9	222.32–222.41	0.023–0.032	222.87–222.90	0.023–0.064	222.86–222.87	0.030–0.040
10	227.38–227.47	0.027–0.030	227.91–227.94	0.016–0.068	227.90–227.93	0.036–0.054
11	232.43–232.52	0.032–0.037	232.96–232.98	0.031–0.065	232.95–232.98	0.032–0.046
12	237.48–237.58	0.021–0.038	238.03–238.04	0.035–0.047	238.00–238.03	0.032–0.049
13	242.64–242.71	0.024–0.033	243.14–243.17	0.027–0.052	243.14–243.16	0.025–0.037
14	247.82–247.87	0.022–0.031	248.30–248.32	0.040–0.050	248.28–248.31	0.033–0.046
15	252.87–252.90	0.022–0.037	253.31–253.33	0.027–0.056	253.31–253.34	0.024–0.041
16	257.80–257.85	0.014–0.029	258.24–258.26	0.021–0.041	258.24–258.27	0.034–0.039
DYS439						
6	149.93–150.01	0.016–0.027	150.28–150.31	0.021–0.046	150.23–150.24	0.028–0.039
7	154.05–154.12	0.020–0.036	154.38–154.41	0.033–0.055	154.32–154.34	0.034–0.041
8	158.15–158.23	0.031–0.036	158.51–158.52	0.031–0.052	158.43–158.46	0.025–0.046
9	162.21–162.30	0.024–0.034	162.54–162.59	0.036–0.046	162.49–162.50	0.036–0.043
10	166.33–166.41	0.023–0.029	166.65–166.69	0.033–0.039	166.60–166.62	0.026–0.044
11	170.27–170.36	0.022–0.032	170.60–170.64	0.039–0.054	170.56–170.59	0.027–0.045
12	174.30–174.39	0.020–0.032	174.64–174.67	0.029–0.050	174.58–174.61	0.028–0.045
13	178.38–178.47	0.027–0.047	178.72–178.75	0.032–0.052	178.66–178.70	0.041–0.046
14	182.44–182.52	0.029–0.035	182.78–182.81	0.035–0.051	182.74–182.76	0.025–0.045

Allele	3130*xl*		3500		3500xL	
	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean
15	186.45–186.53	0.024–0.033	186.79–186.82	0.029–0.052	186.76–186.78	0.036–0.044
16	190.51–190.60	0.025–0.042	190.86–190.88	0.026–0.048	190.83–190.85	0.037–0.047
17	194.58–194.66	0.024–0.040	194.93–194.96	0.027–0.054	194.90–194.93	0.034–0.047
DYS448						
14	277.79–277.88	0.032–0.045	278.38–278.41	0.033–0.059	278.39–278.41	0.040–0.063
15	283.72–283.80	0.021–0.044	284.33–284.34	0.030–0.065	284.31–284.34	0.034–0.048
16	289.61–289.69	0.033–0.043	290.22–290.25	0.031–0.062	290.21–290.24	0.031–0.050
17	295.50–295.59	0.031–0.045	296.12–296.14	0.039–0.060	296.11–296.13	0.039–0.059
18	301.34–301.43	0.036–0.043	301.98–302.00	0.040–0.061	301.95–302.00	0.040–0.066
19	307.17–307.28	0.035–0.043	307.85–307.88	0.042–0.061	307.83–307.87	0.037–0.050
20	313.18–313.29	0.038–0.048	313.87–313.91	0.051–0.079	313.86–313.90	0.040–0.060
21	319.33–319.45	0.036–0.049	320.06–320.09	0.035–0.052	320.06–320.10	0.039–0.066
22	325.50–325.63	0.041–0.053	326.21–326.23	0.038–0.062	326.22–326.26	0.049–0.060
23	331.46–331.61	0.036–0.056	332.16–332.19	0.039–0.070	332.16–332.20	0.045–0.066
24	337.39–337.53	0.038–0.051	338.10–338.14	0.024–0.077	338.11–338.15	0.040–0.060
DYS449						
22	325.50–325.57	0.027–0.062	325.58–325.61	0.037–0.049	325.63–325.67	0.035–0.049
23	329.59–329.64	0.027–0.064	329.65–329.67	0.028–0.054	329.71–329.73	0.039–0.060
24	333.63–333.66	0.025–0.062	333.66–333.72	0.025–0.041	333.75–333.76	0.032–0.045
25	337.66–337.69	0.031–0.058	337.68–337.72	0.033–0.045	337.76–337.78	0.027–0.040
26	341.69–341.75	0.029–0.052	341.72–341.77	0.025–0.042	341.79–341.81	0.039–0.051
27	345.76–345.87	0.029–0.050	345.80–345.84	0.031–0.049	345.87–345.88	0.032–0.043
28	349.82–349.93	0.028–0.061	349.87–349.89	0.018–0.051	349.93–349.94	0.029–0.039
29	353.87–353.98	0.025–0.053	353.92–353.95	0.020–0.060	353.98–353.99	0.034–0.044
30	357.92–358.03	0.028–0.060	357.97–358.00	0.027–0.042	358.03–358.06	0.035–0.050
31	361.97–362.04	0.036–0.045	362.01–362.04	0.021–0.040	362.07–362.09	0.032–0.059
32	365.98–366.06	0.034–0.042	366.01–366.04	0.038–0.055	366.08–366.10	0.035–0.041
33	369.99–370.07	0.029–0.042	370.00–370.04	0.038–0.048	370.08–370.11	0.030–0.047
34	373.99–374.09	0.025–0.044	374.02–374.04	0.027–0.061	374.09–374.12	0.033–0.053

Allele	3130*xl*		3500		3500xL	
	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean
35	377.99–378.08	0.032–0.047	378.01–378.06	0.040–0.055	378.10–378.11	0.039–0.047
36	382.03–382.12	0.034–0.043	382.03–382.08	0.027–0.053	382.12–382.13	0.031–0.051
37	386.08–386.18	0.022–0.043	386.09–386.12	0.025–0.045	386.17–386.19	0.034–0.048
38	390.12–390.22	0.020–0.042	390.14–390.16	0.025–0.044	390.21–390.23	0.036–0.044
39	394.14–394.28	0.030–0.043	394.18–394.20	0.029–0.049	394.24–394.26	0.037–0.043
40	398.17–398.31	0.029–0.053	398.21–398.25	0.024–0.047	398.27–398.29	0.030–0.038
DYS456						
10	76.25–76.30	0.033–0.043	76.25–76.28	0.044–0.050	76.08–76.10	0.031–0.042
11	80.51–80.56	0.016–0.036	80.54–80.56	0.025–0.047	80.40–80.42	0.026–0.037
12	84.72–84.78	0.023–0.035	84.74–84.77	0.009–0.043	84.63–84.64	0.028–0.035
13	88.92–88.97	0.020–0.036	88.95–88.99	0.021–0.035	88.84–88.85	0.024–0.036
14	93.11–93.16	0.022–0.036	93.13–93.17	0.024–0.047	93.03–93.05	0.028–0.039
15	97.28–97.34	0.024–0.037	97.30–97.35	0.027–0.038	97.23–97.24	0.021–0.035
16	101.47–101.53	0.022–0.031	101.52–101.55	0.010–0.033	101.42–101.43	0.034–0.040
17	105.65–105.72	0.021–0.030	105.73–105.75	0.025–0.038	105.62–105.64	0.023–0.035
18	109.81–109.88	0.022–0.037	109.89–109.92	0.023–0.056	109.77–109.80	0.027–0.038
19	113.92–113.98	0.029–0.032	114.01–114.04	0.028–0.060	113.89–113.91	0.032–0.038
20	117.90–117.95	0.022–0.035	118.01–118.02	0.026–0.044	117.88–117.88	0.022–0.029
21	121.86–121.93	0.022–0.036	121.97–122.01	0.040–0.044	121.84–121.86	0.036–0.042
22	125.85–125.93	0.027–0.033	125.98–126.00	0.032–0.048	125.85–125.87	0.032–0.038
23	129.87–129.94	0.029–0.033	130.00–130.04	0.042–0.058	129.88–129.89	0.032–0.040
24	133.89–133.96	0.026–0.033	134.04–134.05	0.030–0.041	133.92–133.94	0.024–0.044
DYS458						
11	119.84–119.98	0.027–0.034	120.25–120.28	0.039–0.065	120.11–120.13	0.039–0.041
12	123.66–123.81	0.026–0.038	124.10–124.13	0.042–0.056	123.96–123.98	0.026–0.040
13	127.52–127.66	0.025–0.041	127.97–128.01	0.035–0.053	127.82–127.85	0.027–0.040
14	131.37–131.53	0.033–0.038	131.84–131.90	0.040–0.069	131.70–131.74	0.024–0.041
15	135.26–135.41	0.020–0.043	135.77–135.80	0.035–0.059	135.62–135.65	0.030–0.038
16	139.17–139.32	0.027–0.046	139.68–139.72	0.041–0.051	139.56–139.58	0.028–0.044

Allele	3130*xl*		3500		3500xL	
	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean
17	143.06–143.24	0.030–0.044	143.61–143.64	0.028–0.049	143.47–143.52	0.030–0.040
18	147.14–147.31	0.030–0.045	147.70–147.72	0.033–0.054	147.56–147.59	0.035–0.047
19	151.22–151.39	0.029–0.050	151.77–151.83	0.040–0.060	151.65–151.68	0.034–0.043
20	155.22–155.39	0.028–0.047	155.79–155.82	0.031–0.062	155.65–155.68	0.029–0.049
21	159.12–159.30	0.028–0.049	159.71–159.73	0.028–0.041	159.57–159.61	0.027–0.042
22	163.07–163.26	0.027–0.050	163.65–163.69	0.039–0.063	163.52–163.55	0.035–0.048
23	166.99–167.18	0.019–0.060	167.58–167.61	0.028–0.059	167.45–167.48	0.030–0.042
24	170.92–171.11	0.028–0.064	171.52–171.54	0.042–0.063	171.38–171.42	0.033–0.044
DYS460						
7	79.58–79.61	0.031–0.038	79.58–79.60	0.053–0.060	79.38–79.40	0.023–0.039
8	83.76–83.80	0.021–0.040	83.75–83.78	0.042–0.058	83.58–83.60	0.026–0.037
9	87.96–87.98	0.025–0.037	87.92–87.97	0.027–0.035	87.77–87.79	0.029–0.038
10	92.12–92.15	0.024–0.042	92.10–92.13	0.023–0.043	91.95–91.97	0.031–0.044
11	96.29–96.31	0.028–0.041	96.26–96.30	0.030–0.049	96.11–96.13	0.030–0.040
12	100.46–100.48	0.023–0.045	100.44–100.48	0.039–0.048	100.28–100.30	0.034–0.042
13	104.65–104.67	0.028–0.035	104.64–104.67	0.030–0.038	104.48–104.50	0.025–0.039
14	108.80–108.84	0.028–0.039	108.79–108.82	0.025–0.040	108.63–108.66	0.027–0.033
DYS481						
17	206.82–206.84	0.018–0.034	206.89–206.93	0.023–0.040	206.96–206.97	0.025–0.038
18	209.80–209.81	0.018–0.032	209.86–209.88	0.027–0.044	209.92–209.93	0.030–0.036
19	212.76–212.78	0.026–0.036	212.86–212.87	0.009–0.050	212.89–212.91	0.037–0.045
20	215.78–215.80	0.021–0.035	215.86–215.88	0.016–0.042	215.91–215.94	0.027–0.042
21	218.85–218.87	0.018–0.033	218.91–218.94	0.010–0.032	218.98–218.99	0.029–0.040
22	221.88–221.90	0.024–0.039	221.93–221.96	0.020–0.042	222.00–222.02	0.038–0.048
23	224.88–224.90	0.023–0.033	224.94–224.97	0.025–0.037	225.02–225.03	0.039–0.049
24	227.88–227.90	0.021–0.032	227.95–227.97	0.037–0.055	228.03–228.04	0.037–0.043
25	230.90–230.92	0.017–0.034	230.96–230.99	0.035–0.054	231.03–231.04	0.030–0.049
26	233.91–233.93	0.022–0.033	233.97–233.98	0.027–0.049	234.04–234.06	0.032–0.042
27	236.91–236.93	0.018–0.034	236.98–237.00	0.023–0.053	237.06–237.07	0.033–0.037

Allele	3130*xl*		3500		3500xL	
	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean
28	239.94–239.95	0.023–0.033	239.99–240.01	0.000–0.048	240.07–240.08	0.026–0.048
29	243.03–243.05	0.023–0.031	243.08–243.10	0.026–0.046	243.15–243.16	0.030–0.034
30	246.11–246.13	0.023–0.031	246.14–246.16	0.025–0.052	246.21–246.23	0.029–0.040
31	249.17–249.21	0.023–0.032	249.18–249.21	0.009–0.038	249.27–249.28	0.030–0.038
32	252.15–252.20	0.025–0.033	252.19–252.20	0.020–0.045	252.27–252.28	0.029–0.039
DYS518						
32	332.35–332.38	0.025–0.054	332.32–332.35	0.027–0.045	332.32–332.35	0.038–0.046
33	336.40–336.41	0.029–0.050	336.35–336.38	0.024–0.045	336.36–336.39	0.030–0.050
34	340.43–340.48	0.026–0.047	340.36–340.40	0.005–0.044	340.39–340.41	0.005–0.041
35	344.52–344.59	0.025–0.051	344.45–344.49	0.032–0.051	344.48–344.50	0.035–0.052
36	348.60–348.70	0.024–0.047	348.54–348.57	0.011–0.044	348.56–348.59	0.027–0.042
37	352.67–352.76	0.025–0.059	352.60–352.64	0.033–0.053	352.63–352.65	0.029–0.042
38	356.74–356.82	0.026–0.054	356.69–356.71	0.038–0.049	356.70–356.73	0.038–0.054
39	360.82–360.88	0.025–0.044	360.73–360.77	0.036–0.049	360.77–360.79	0.035–0.058
40	364.83–364.91	0.021–0.043	364.76–364.78	0.026–0.046	364.80–364.82	0.030–0.041
41	368.87–368.92	0.021–0.036	368.78–368.80	0.032–0.059	368.82–368.84	0.035–0.046
42	372.87–372.94	0.030–0.038	372.78–372.83	0.033–0.043	372.83–372.85	0.030–0.041
43	376.91–376.98	0.026–0.043	376.81–376.83	0.026–0.057	376.84–376.88	0.038–0.053
44	380.95–381.01	0.024–0.034	380.83–380.86	0.041–0.052	380.88–380.89	0.030–0.049
45	385.00–385.07	0.024–0.038	384.89–384.92	0.019–0.045	384.93–384.95	0.029–0.046
46	389.05–389.14	0.027–0.035	388.96–388.97	0.026–0.054	388.99–389.00	0.038–0.048
47	393.09–393.19	0.023–0.043	393.00–393.02	0.033–0.049	393.04–393.06	0.025–0.053
48	397.12–397.24	0.034–0.047	397.04–397.08	0.028–0.054	397.08–397.12	0.031–0.052
49	401.16–401.26	0.025–0.039	401.08–401.10	0.018–0.041	401.10–401.13	0.031–0.051
DYS533						
7	338.37–338.42	0.028–0.035	338.61–338.63	0.036–0.049	338.55–338.56	0.020–0.036
8	342.44–342.48	0.024–0.040	342.64–342.69	0.027–0.043	342.61–342.62	0.040–0.046
9	346.54–346.56	0.026–0.038	346.72–346.77	0.028–0.044	346.68–346.70	0.032–0.047
10	350.61–350.63	0.026–0.039	350.82–350.85	0.026–0.053	350.75–350.76	0.033–0.048

Allele	3130*xl*		3500		3500xL	
	Standard deviation	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**	**Mean**
11	354.68–354.69	0.024–0.035	354.87–354.91	0.024–0.037	354.81–354.83	0.028–0.051
12	358.73–358.77	0.026–0.035	358.93–358.97	0.015–0.059	358.88–358.90	0.029–0.046
13	362.76–362.78	0.023–0.035	362.96–362.99	0.018–0.043	362.90–362.93	0.028–0.045
14	366.76–366.79	0.021–0.035	366.95–366.99	0.029–0.048	366.92–366.95	0.034–0.043
15	370.78–370.82	0.022–0.029	370.99–371.01	0.032–0.064	370.94–370.97	0.031–0.048
16	374.79–374.82	0.033–0.034	374.99–375.02	0.030–0.047	374.93–374.96	0.035–0.047
17	378.80–378.83	0.025–0.039	379.01–379.03	0.010–0.045	378.94–378.97	0.041–0.049
DYS570						
10	97.99–98.02	0.021–0.034	98.02–98.04	0.026–0.044	97.95–97.96	0.035–0.043
11	102.12–102.15	0.024–0.034	102.13–102.16	0.023–0.041	102.08–102.09	0.028–0.034
12	106.23–106.27	0.021–0.029	106.26–106.29	0.032–0.046	106.20–106.21	0.026–0.036
13	110.33–110.35	0.022–0.033	110.35–110.37	0.032–0.044	110.28–110.30	0.028–0.043
14	114.36–114.38	0.018–0.030	114.40–114.41	0.024–0.038	114.32–114.34	0.017–0.037
15	118.29–118.32	0.021–0.030	118.32–118.34	0.022–0.037	118.23–118.25	0.035–0.038
16	122.21–122.24	0.023–0.041	122.23–122.26	0.032–0.041	122.15–122.17	0.027–0.036
17	126.18–126.21	0.026–0.041	126.21–126.22	0.030–0.042	126.13–126.14	0.027–0.042
18	130.16–130.19	0.021–0.041	130.18–130.20	0.015–0.039	130.11–130.12	0.028–0.034
19	134.15–134.19	0.027–0.038	134.18–134.21	0.025–0.038	134.11–134.13	0.026–0.041
20	138.19–138.23	0.026–0.043	138.20–138.25	0.027–0.046	138.14–138.16	0.027–0.036
21	142.27–142.30	0.024–0.033	142.29–142.31	0.024–0.037	142.23–142.25	0.034–0.040
22	146.38–146.42	0.021–0.028	146.39–146.42	0.020–0.033	146.34–146.35	0.028–0.038
23	150.50–150.55	0.027–0.031	150.49–150.51	0.035–0.046	150.45–150.47	0.025–0.039
24	154.62–154.67	0.023–0.040	154.61–154.64	0.027–0.039	154.57–154.60	0.027–0.036
25	158.75–158.81	0.024–0.038	158.73–158.75	0.034–0.038	158.69–158.70	0.016–0.029
26	162.84–162.88	0.027–0.042	162.80–162.82	0.019–0.042	162.76–162.79	0.031–0.040
DYS576						
10	72.26–72.37	0.028–0.050	72.79–72.82	0.041–0.073	72.45–72.47	0.038–0.045
11	76.42–76.55	0.027–0.043	77.02–77.03	0.059–0.066	76.69–76.71	0.028–0.047
12	80.56–80.68	0.020–0.038	81.18–81.20	0.039–0.064	80.88–80.89	0.036–0.040

Allele	3130*xl*		3500		3500xL	
	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean
13	84.65–84.77	0.021–0.033	85.27–85.30	0.040–0.052	84.98–85.01	0.031–0.037
14	88.71–88.86	0.029–0.041	89.35–89.38	0.040–0.068	89.07–89.10	0.029–0.040
15	92.79–92.93	0.026–0.043	93.42–93.47	0.039–0.057	93.17–93.19	0.024–0.040
16	96.84–96.99	0.029–0.039	97.49–97.54	0.041–0.055	97.24–97.26	0.029–0.040
17	100.90–101.05	0.021–0.045	101.59–101.63	0.027–0.051	101.34–101.35	0.038–0.039
18	104.98–105.14	0.029–0.040	105.69–105.72	0.038–0.069	105.41–105.44	0.026–0.037
19	109.02–109.19	0.026–0.053	109.73–109.77	0.033–0.071	109.47–109.49	0.025–0.043
20	113.02–113.20	0.025–0.050	113.76–113.79	0.037–0.080	113.49–113.50	0.034–0.040
21	116.93–117.09	0.029–0.042	117.65–117.67	0.033–0.065	117.38–117.40	0.031–0.040
22	120.77–120.95	0.025–0.042	121.51–121.54	0.042–0.074	121.23–121.27	0.026–0.041
23	124.66–124.83	0.029–0.042	125.43–125.46	0.041–0.081	125.14–125.17	0.030–0.039
24	128.56–128.74	0.030–0.050	129.36–129.38	0.062–0.069	129.07–129.09	0.028–0.040
25	132.47–132.66	0.025–0.042	133.28–133.32	0.035–0.072	132.99–133.04	0.029–0.036
DYS627						
11	323.89–324.05	0.049–0.059	325.01–325.03	0.048–0.094	324.93–324.98	0.052–0.066
12	327.79–327.94	0.043–0.066	328.92–328.97	0.038–0.095	328.84–328.89	0.058–0.074
13	331.66–331.84	0.033–0.053	332.81–332.85	0.053–0.080	332.73–332.77	0.048–0.070
14	335.53–335.71	0.046–0.062	336.67–336.72	0.045–0.086	336.61–336.65	0.055–0.063
15	339.38–339.53	0.040–0.056	340.53–340.57	0.054–0.086	340.45–340.50	0.044–0.070
16	343.29–343.42	0.040–0.055	344.42–344.47	0.025–0.103	344.35–344.42	0.048–0.056
17	347.21–347.32	0.040–0.060	348.36–348.40	0.050–0.101	348.28–348.33	0.047–0.059
18	351.11–351.22	0.037–0.061	352.27–352.31	0.052–0.097	352.19–352.24	0.050–0.071
19	355.07–355.22	0.047–0.062	356.26–356.29	0.050–0.098	356.17–356.23	0.046–0.063
20	358.90–359.06	0.047–0.060	360.11–360.17	0.048–0.114	360.02–360.10	0.053–0.072
21	362.69–362.85	0.038–0.064	363.92–363.94	0.059–0.092	363.83–363.90	0.053–0.066
22	366.53–366.69	0.049–0.070	367.77–367.80	0.040–0.095	367.69–367.75	0.051–0.067
23	370.36–370.53	0.045–0.064	371.62–371.66	0.044–0.075	371.53–371.61	0.054–0.064
24	374.21–374.37	0.054–0.069	375.47–375.52	0.074–0.077	375.39–375.46	0.062–0.070
25	378.05–378.21	0.059–0.074	379.32–379.36	0.055–0.067	379.23–379.32	0.058–0.067

Allele	3130*xl*		3500		3500xL	
	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean
26	381.90–382.06	0.062–0.080	383.22–383.27	0.059–0.086	383.12–383.20	0.056–0.074
27	385.79–385.94	0.064–0.079	387.10–387.14	0.069–0.094	387.02–387.10	0.046–0.073
DYS635						
15	191.34–191.39	0.026–0.034	191.90–191.92	0.036–0.052	191.76–191.77	0.034–0.046
16	195.40–195.44	0.024–0.038	195.95–195.98	0.030–0.049	195.82–195.83	0.033–0.047
17	199.45–199.49	0.025–0.036	200.00–200.01	0.000–0.056	199.87–199.88	0.042–0.046
18	203.42–203.46	0.020–0.036	203.96–203.98	0.021–0.048	203.82–203.83	0.031–0.044
19	207.37–207.41	0.021–0.038	207.92–207.94	0.022–0.040	207.77–207.79	0.030–0.040
20	211.35–211.38	0.027–0.036	211.89–211.91	0.017–0.060	211.75–211.77	0.034–0.041
21	215.41–215.44	0.022–0.034	215.96–215.99	0.016–0.059	215.82–215.83	0.030–0.052
22	219.43–219.48	0.027–0.039	219.99–220.01	0.000–0.048	219.85–219.87	0.045–0.046
23	223.44–223.49	0.023–0.037	224.00–224.02	0.033–0.061	223.86–223.89	0.038–0.053
24	227.31–227.38	0.024–0.036	227.88–227.92	0.034–0.051	227.76–227.77	0.034–0.043
25	231.45–231.52	0.023–0.036	232.03–232.06	0.034–0.064	231.91–231.93	0.037–0.050
26	235.35–235.42	0.021–0.030	235.93–235.95	0.039–0.053	235.81–235.84	0.032–0.042
27	239.49–239.55	0.032–0.036	240.07–240.09	0.000–0.058	239.95–239.97	0.043–0.046
28	243.60–243.65	0.026–0.038	244.17–244.19	0.023–0.035	244.05–244.09	0.031–0.048
29	247.70–247.75	0.022–0.032	248.24–248.27	0.017–0.039	248.14–248.17	0.038–0.046
30	251.73–251.77	0.021–0.037	252.25–252.29	0.020–0.052	252.14–252.18	0.032–0.045
YGATAH4						
8	235.91–235.96	0.022–0.032	236.18–236.22	0.031–0.055	236.15–236.16	0.030–0.047
9	239.92–239.96	0.023–0.036	240.19–240.22	0.031–0.065	240.16–240.18	0.028–0.055
10	244.04–244.09	0.023–0.030	244.31–244.34	0.033–0.047	244.27–244.28	0.030–0.046
11	248.14–248.15	0.018–0.033	248.39–248.41	0.041–0.052	248.35–248.36	0.032–0.047
12	252.15–252.17	0.020–0.034	252.37–252.39	0.020–0.040	252.35–252.37	0.031–0.040
13	256.07–256.10	0.024–0.039	256.31–256.33	0.029–0.039	256.28–256.30	0.027–0.042
14	259.97–260.00	0.015–0.032	260.24–260.27	0.000–0.049	260.19–260.23	0.032–0.047
15	263.94–263.99	0.019–0.042	264.22–264.25	0.019–0.040	264.20–264.22	0.034–0.045

B

Troubleshooting

Observation	Possible cause	Recommended action
Faint or no signal from both the DNA Control 007 and the DNA test samples at all loci	The incorrect volume of Master Mix or Primer Set was used.	Use the correct volume of Master Mix or Primer Set.
	The DNA Polymerase was not activated.	Repeat the amplification with an initial hold at 95°C for 1 minute.
	The Master Mix was not vortexed thoroughly before aliquoting.	Vortex the Master Mix thoroughly.
	The Primer Set was exposed to too much light.	Replace the Primer Set and store it protected from light.
	Evaporation.	Ensure that the plate is properly sealed with film and that a compression pad was used with the GeneAmp™ PCR System 9700. (A compression pad should not be used with other validated thermal cyclers.)
	The thermal cycler malfunctioned.	See the thermal cycler user manual and check the instrument calibration.
	Incorrect thermal cycler conditions were used.	Use correct thermal cycler conditions.
	A MicroAmp™ base was used with a tray/retainer set and tubes in GeneAmp™ PCR System 9700.	Remove the MicroAmp™ base.
	The tubes or plate were not seated tightly in the thermal cycler during amplification.	Push the tubes or plate firmly into the block after first cycle.
	The wrong PCR reaction tubes or plate were used.	Use MicroAmp™ Reaction Tubes with Caps or the MicroAmp™ Optical 96-well Reaction Plate for the GeneAmp™ PCR System 9700 or Veriti™ Thermal Cycler.
	Insufficient PCR product was electrokinetically injected.	Use correct genetic analyzer settings.
	Degraded formamide was used.	Check the storage of formamide. Do not thaw and refreeze multiple times. Try Hi-Di™ Formamide.
Positive signal from DNA Control 007 but partial or no signal from DNA test samples	The quantity of test DNA sample is below the assay sensitivity.	Quantify DNA and (when possible) add 1.0 ng of DNA. For low concentration samples, add up to 15 µL of the DNA sample to the reaction mix, or consider using the 30-cycle protocol.
	The test sample contains a high concentration of PCR inhibitor (for example, heme	Quantify the DNA, then use the minimum necessary volume of test sample DNA.

Observation	Possible cause	Recommended action
Positive signal from DNA Control 007 but partial or no signal from DNA test samples	compounds, certain dyes).	Wash the sample in a Centricon™-100 centrifugal filter unit.
	The test sample DNA is severely degraded.	Use the Quantifiler™ HP DNA Quantification Kit or the Quantifiler™ Trio DNA Quantification Kit to evaluate sample quality during the quantification step. If DNA is degraded, reamplify with an increased amount of DNA or use the AmpFℓSTR™ MiniFiler™ PCR Amplification Kit.
	The test sample was diluted in the wrong buffer (for example, a TE buffer with an incorrect EDTA concentration).	Redilute DNA using low-TE buffer (with 0.1 mM EDTA).
More than two alleles present at a locus	Secondary gene duplication at DYS385 a/b, and/or DYF387S1.	Some samples may exhibit uneven peak height ratios at these markers due to either the stochastic effects of the PCR or a secondary duplication event in one of the alleles. We recommend that allele calls be made based on peaks that are present (conservative approach) unless additional evidence is gathered to conclusively demonstrate that a secondary duplication event has taken place.
	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.	Interpret the results according to your laboratory procedure. See Chapter 5, "Experiments and results"
	The test sample contained mixed DNA.	Interpret the results according to your laboratory procedure. See Chapter 5, "Experiments and results"
	Incomplete 3´ A base addition (n–1 nt position) occured.	Include the final extension step of 60°C for 22 minutes in the PCR.
	The signal exceeds the dynamic range of the instrument and is causing signal "pull-up" into adjacent channels.	Ensure the cycle number is optimized. Use fewer PCR cycles or interpret the off-scale data according to your laboratory procedure.
		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.

Observation	Possible cause	Recommended action
More than two alleles present at a locus		3130 instruments: Depending on instrument sensitivity, DNA input amounts ≥1 ng amplified at 29 cycles can demonstrate off-scale peak heights for homozygous peaks. For more information, go to **thermofisher.com**, then search for the technical note *Evaluation of a decreased cycle number (28 cycles) and decreased injection time (5 seconds) when the GlobalFiler™ PCR Amplification Kit is run on a 3130 xl Genetic Analyzer*, or contact your local Human Identification representative.
	Poor spectral separation occurred.	Perform a spectral calibration.
		Confirm that Filter Set J6 modules are installed and used for analysis.
	Too much DNA was present in the reaction.	Use recommended amount of template DNA (1.0 ng).
	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.
Some but not all loci visible on electropherogram of DNA Test Samples	The punched disc you used was too large.	Use a 1.2 mm disc.
	Insufficient lysis of the swab head occurred.	Ensure the swab heads are incubated for 20 minutes in 400 µL of Prep-n-Go™ Buffer.
	The PCR reaction volume you used is lower than the volume required for the amplification.	Use the correct PCR reaction volume: 15 µL
STR profiles contain many off-scale alleles	The PCR cycle number used was too high.	Perform a sensitivity experiment to determine the optimal PCR cycle number based on the sample type.
	Blood samples: Too much liquid blood was spotted onto the paper substrate.	Spot <100 µL of liquid blood per sample area.
Poor peak height balance	Incorrect thermal cycler conditions were used.	Use correct thermal cycler conditions.

C Materials required but not supplied

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

STR kit

Item	Source
Yfiler™ Plus PCR Amplification Kit, 100-reaction kit	4484678
Yfiler™ Plus PCR Amplification Kit, 500-reaction kit	4482730
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

Sample preparation required materials

Treated paper substrate

Item	Source
Collection system:	
Whatman™ FTA™ Classic Cards	MLS
Whatman™ EasiCollect™ system	MLS
Sample preparation:	
Prep-n-Go™ Buffer (for use with untreated paper substrates)	4467079

Item	Source
Low-TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0)	Teknova T0223
Punch tool:	
Harris Micro-Punch™ tool, 1.2-mm	MLS
BSD600-Duet Semi-Automated Dried Sample Punch Instrument with a 1.2-mm punch head	Contact your local sales office.
BSD1000-GenePunch Automated Dried Sample Punch Instrument with a 1.2-mm punch head	

Untreated paper substrate

Item	Source
Collection system:	
903 paper	MLS
Punch tool:	
Harris Micro-Punch™ tool, 1.2-mm	MLS
BSD600-Duet Semi-Automated Dried Sample Punch Instrument with a 1.2-mm punch head	Contact your local sales office.
BSD1000-GenePunch Automated Dried Sample Punch Instrument with a 1.2-mm punch head	

Swab substrate

Item	Source
Collection system	
4N6FLOQSwabs™, regular tip	4473979
Sample preparation:	
Prep-n-Go™ Buffer (for use with buccal swab substrates)	4471406
Heated lysis protocol only: 1.5 mL tube format or 96-well deep-well plate format	
1.5 mL tube format	
1.5 mL tubes	MLS
Oven	VWR™ Scientific dry heat block or equivalent
96-well deep-well plate format	
PrepFiler™ 96-Well Processing Plates	4392904

Item	Source
Robbins Scientific™ Model 400 Hybridization Incubator or equivalent	MLS
Agilent™ Benchtop Rack for 200 µL Tubes/V Bottom Plates (metal) or equivalent **IMPORTANT!** Do not use a plastic plate adaptor.	Agilent Technologies 410094

Thermal cycler required materials

ProFlex™ PCR System

Item	Source
ProFlex™ 96-well PCR System	4484075
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
3500/3500xL Data Collection Software v3 (RUO)	A26287 [1]
3500/3500xL Data Collection Software v2 (RUO)	4475183[2]
HID Updater 3500 Data Collection Software v2	4480670
Anode buffer container (ABC)	4393927
Cathode buffer container (CBC)	4408256
POP-4™ Polymer (960 samples) for 3500/3500xL Genetic Analyzers	4393710
POP-4™ Polymer (384 samples) for 3500/3500xL Genetic Analyzers	4393715
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
Conditioning reagent	4393718
8-Capillary array, 36 cm for 3500 Genetic Analyzers	4404683
24-Capillary array, 36 cm for 3500xL Genetic Analyzers	4404687
96-well retainer & base set (Standard) 3500/3500xL Genetic Analyzers	4410228
8-Tube retainer & base set (Standard) for 3500/3500xL Genetic Analyzers	4410231
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.
[2] Contact your Thermo Fisher Scientific HID representative.

3130 Series Genetic Analyzer

Item	Source
3130 Data Collection Software v4	4475105
3130*xl* Data Collection Software-v4	4475126
3130/3730 Data Collection Software-v4 6-Dye Module v1	4480670
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3100/3130 *xl* Genetic Analyzer Capillary Array, 36-cm	4315931
POP-4™ Polymer for 3130/3130 *xl* Genetic Analyzers	4352755

Item	Source
Running Buffer, 10×	402824
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
MicroAmp™ Optical 96-Well Reaction Plate	N8010560

Analysis software required materials

GeneMapper™ *ID-X* Software

Item	Source
GeneMapper™ *ID-X* Software v1.5 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

D Plate layouts

Example PCR plate layout

The following layout is recommended for use with the sensitivity experiment in the Perform PCR chapter. Create 3 identical plates for amplification at 3 different cycle numbers.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Samp 1	Samp 8	Samp 15	Samp 22								
B	Samp 2	Samp 9	Samp 16	Samp 23								
C	Samp 3	Samp 10	Samp 17	Samp 24								
D	Samp 4	Samp 11	Samp 18	Samp 25								
E	Samp 5	Samp 12	Samp 19	Samp 26								
F	Samp 6	Samp 13	Samp 20	Neg ctrl								
G	Samp 7	Samp 14	Samp 21	007								
H												

Example electrophoresis plate layout

The following layout is recommended for use with the sensitivity experiment in the Perform PCR chapter.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Samp 1	Samp 8	Samp 15	Samp 22	Samp 1	Samp 8	Samp 15	Samp 22	Samp 1	Samp 8	Samp 15	Samp 22
B	Samp 2	Samp 9	Samp 16	Samp 23	Samp 2	Samp 9	Samp 16	Samp 23	Samp 2	Samp 9	Samp 16	Samp 23
C	Samp 3	Samp 10	Samp 17	Samp 24	Samp 3	Samp 10	Samp 17	Samp 24	Samp 3	Samp 10	Samp 17	Samp 24
D	Samp 4	Samp 11	Samp 18	Samp 25	Samp 4	Samp 11	Samp 18	Samp 25	Samp 4	Samp 11	Samp 18	Samp 25
E	Samp 5	Samp 12	Samp 19	Samp 26	Samp 5	Samp 12	Samp 19	Samp 26	Samp 5	Samp 12	Samp 19	Samp 26
F	Samp 6	Samp 13	Samp 20	Neg ctrl	Samp 6	Samp 13	Samp 20	Neg ctrl	Samp 6	Samp 13	Samp 20	Neg ctrl
G	Samp 7	Samp 14	Samp 21	007	Samp 7	Samp 14	Samp 21	007	Samp 7	Samp 14	Samp 21	007
H	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank	Allelic Ladder	CE Blank

Cycle 1 Cycle 2 Cycle 3

E

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 15 .

F

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 adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

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:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.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	
Yfiler™ Plus PCR Amplification Kit – PCR Setup – Treated and Untreated Paper Substrates Quick Reference	100030920
Yfiler™ Plus PCR Amplification Kit – PCR Setup – Extracted DNA Quick Reference	100030921
Yfiler™ Plus PCR Amplification Kit – PCR Setup – Swab Substrate Quick Reference	100030922
Yfiler™ Plus PCR Amplification Kit – PCR Amplification and CE Quick Reference	100030923
Thermal cyclers	
ProFlex™ PCR System User Guide	MAN0007697
Veriti™ Thermal Cycler User Guide	4375799
GeneAmp™ PCR System 9700 Base Module User Manual	4303481
3500 Series Genetic Analyzer	
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v1 User Guide	4401661
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v2 User Guide	4476988
HID Updater 3500 Data Collection Software v2.0 User Bulletin	NA
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 3.1 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
3130 Series Genetic Analyzer	
3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide	4352716
3130/3130xl Genetic Analyzers Using Data Collection Software v3.0 User Bulletin	4363787
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

Document title	Pub. No.
GeneMapper™ *ID-X* Software all versions	
GeneMapper™ ID-X Software Bin Overlap User Bulletin	100029546
GeneMapper™ *ID-X* Software v1.0	
GeneMapper™ ID-X Software v1.0 Getting Started Guide— Basic Features	4375574
GeneMapper™ ID-X Software v1.0 Quick Reference— Basic Features	4375670
GeneMapper™ ID-X Software v1.0 Installation Guide	4476603
GeneMapper™ ID-X Software v1.0 Administrator Guide	4376327
GeneMapper™ ID-X Software v1.0 Reference Guide	4375671
GeneMapper™ *ID-X* Software v1.1	
GeneMapper™ ID-X Software v1.1 Getting Started Guide— Mixture Analysis Tool	4396773
GeneMapper™ *ID-X* Software v1.2	
GeneMapper™ ID-X Software v1.2 Verification Experiments and Installation Procedures User Bulletin	4462639
GeneMapper™ ID-X Software v1.2 Quick Reference— Mixture Analysis Tool	4426482
GeneMapper™ ID-X Software v1.2 Reference Guide	4426481
GeneMapper™ *ID-X* Software v1.3	
GeneMapper™ ID-X Software v1.3 Verification Experiments and Installation Procedures User Bulletin	4470483
GeneMapper™ *ID-X* Software v1.4	
GeneMapper™ ID-X Software v1.4 New Features and Installation Procedures User Bulletin	4477684
GeneMapper™ *ID-X* Software v1.5	
GeneMapper™ ID-X Software v1.5 New Features and Verification User Bulletin	100031708
GeneMapper™ ID-X Software v1.5 Getting Started Guide— Basic Features	100031701
GeneMapper™ ID-X Software v1.5 Quick Reference— Basic Features	100031702
GeneMapper™ ID-X Software v1.5 Getting Started Guide— Mixture Analysis Tool	100031704
GeneMapper™ ID-X Software v1.5 Quick Reference— Mixture Analysis Tool	100031705
GeneMapper™ ID-X Software v1.5 Installation Guide	100031706
GeneMapper™ ID-X Software v1.5 Administrator Guide	100031703
GeneMapper™ ID-X Software v1.5 Reference Guide	100031707

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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