USER GUIDE

applied biosystems
life technologies

Yfiler® Plus PCR Amplification Kit

for use with:
100 reaction kit (Part no. 4484678)
500 reaction kit (Part no. 4482730)

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Revision B

life technologies

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Contents

About This Guide

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

Revision history

Revision	Date	Description
A	July 2014	New document.
B	October 2014	Add Chapter 5.

Purpose

The *Yfiler® Plus PCR Amplification Kit User Guide* provides information about the Life Technologies instruments, chemistries, and software associated with the Yfiler® Plus PCR Amplification Kit.

1 Overview

Product overview

Purpose

The 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 and direct PCR amplification from the following types of single-source samples:

- Blood and buccal samples on treated paper substrates without the need for sample purification.
- 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

Yfiler® Plus PCR Amplification Kit amplifies 27 Y-STR loci in a single PCR amplification reaction.

Product description

The Yfiler® Plus Kit contains all the necessary reagents for the amplification of human male-specific genomic DNA.

The reagents are designed for use with the following Life Technologies instruments:

- Applied Biosystems® 3500/3500xL Genetic Analyzer
- Applied Biosystems® 3130/3130*xl* Genetic Analyzer
- GeneAmp® PCR System 9700 with the Gold-plated Silver 96-Well Block
- GeneAmp® PCR System 9700 with the Silver 96-Well Block
- Veriti® 96-Well Thermal Cycler

Substrates

Possible substrates for use with this kit include:

- Treated paper: Copan NUCLEIC-CARD System or Whatman FTA® cards
- Untreated paper: 903 paper, Bode Buccal DNA Collector™

About the primers

Non-nucleotide linkers are used in primer synthesis for the DYS389I/II, DYS635, DYS627, DYS19, YGATAH4, DYS448, DYS391, DYS390, DYS438, DYS391, DYS390, DYS438, DYS392, DYS518, DYS437 and DYS449 loci. For these primers, non-nucleotide linkers are placed between the primers and the fluorescent dye during oligonucleotide synthesis (Butler, 2005, Grossman *et al.*, 1994, and Baron *et al.*, 1996). Non-nucleotide linkers enable reproducible positioning of the alleles to facilitate interlocus spacing. The combination of a six-dye fluorescent system and the inclusion of non-nucleotide linkers allows for simultaneous amplification and efficient separation of the 27 Y-STR loci during automated DNA fragment analysis.

Loci amplified by the kit

The following table shows the loci amplified and the corresponding fluorescent marker dyes. The Yfiler® Plus Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the allelic ladder and the haplotype of the DNA Control 007 are also listed in the table.

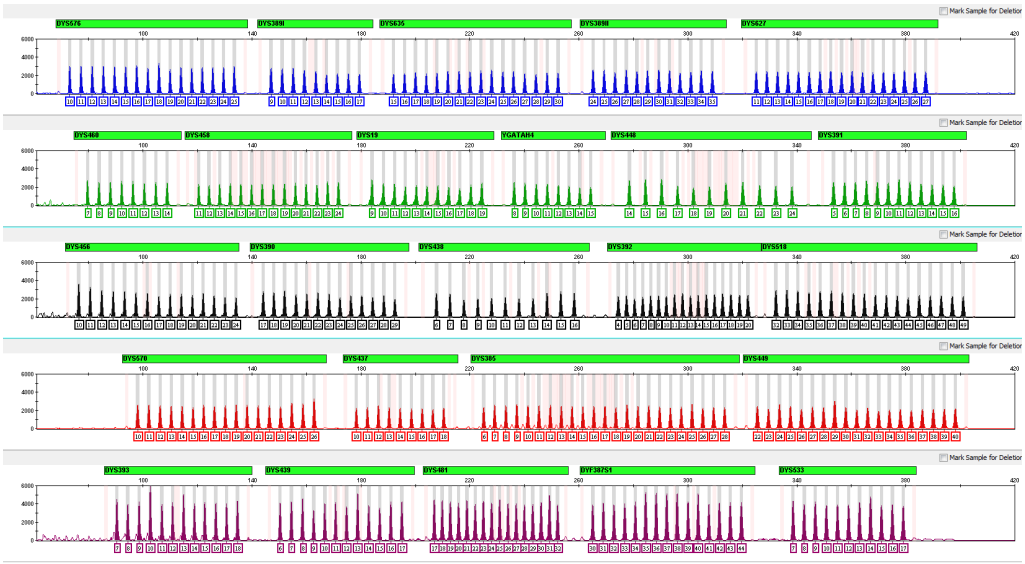
Table 1 Yfiler® Plus Kit

Locus designation	Alleles included in Yfiler® Plus Allelic Ladder	Dye label	Control DNA 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	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

Allelic ladder profile

Figure 1 shows the allelic ladder for the Yfiler® Plus Kit. See "Allelic ladder requirements" on page 33 for information on ensuring accurate genotyping.

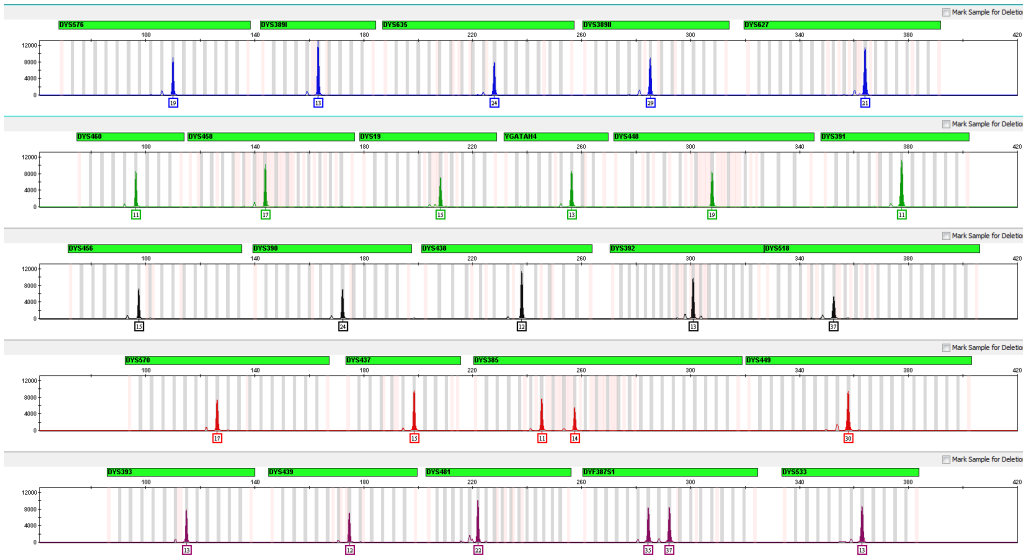
Figure 1 GeneMapper® *ID-X* Software v1.4 plot of the Yfiler® Plus Allelic Ladder



Control DNA 007 profile

Figure 2 shows amplification of Control DNA 007 using the Yfiler® Plus Kit.

Figure 2 1 ng of Control DNA 007 amplified with the Yfiler® Plus Kit and analyzed on the Applied Biosystems® 3500xL Genetic Analyzer



Workflow overview for casework samples

Prepare samples

Extract DNA


AutoMate *Express*™ System + PrepFiler® Express Kit

Quantify DNA

Quantifiler® Trio DNA Quantification Kit

Perform PCR

Prepare reactions

Yfiler® Plus PCR Amplification Kit

Perform PCR

GeneAmp® PCR System 9700 Cycler

Veriti® 96-Well Thermal Cycler

Perform electro-phoresis

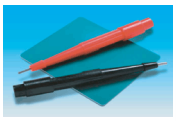
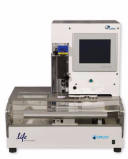
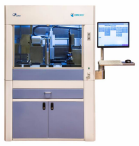
3130/3130*xl* Genetic Analyzer

3500/3500xL Genetic Analyzer

Analyze data

GeneMapper® *ID-X* v1.4

Workflow overview for database samples

<table>
<tr><td>Prepare samples</td><td>Obtain samples</td><td>Treated or untreated paper substrates</td><td>Obtain samples</td><td>Swab substrates</td></tr>
<tr><td></td><td>Prepare samples</td><td> 
Harris Manual Punch
  or 
CPA200™ or CPA300™ instrument</td><td>Prepare samples</td><td> 
Lyse in Prep-n-Go™ Buffer</td></tr>
<tr><td>Perform PCR</td><td>Prepare reactions</td><td colspan="3">Yfiler® Plus PCR Amplification Kit</td></tr>
<tr><td></td><td>Perform PCR</td><td> 
GeneAmp® PCR System 9700 Cycler</td><td colspan="2"> 
Veriti® 96-Well Thermal Cycler</td></tr>
<tr><td>Perform electro-phoresis</td><td></td><td> 
3130/3130xl Genetic Analyzer</td><td colspan="2"> 
3500/3500xL Genetic Analyzer</td></tr>
<tr><td>Analyze data</td><td colspan="4"> 
GeneMapper® ID-X v1.4</td></tr>
</table>

Instrument and software overview

This section provides information about the Data Collection Software versions required to run the Yfiler® Plus PCR Amplification Kit on specific instruments.

Data Collection and GeneMapper® *ID-X* Software

The Data Collection Software provides instructions to firmware running on the instrument and displays instrument status and raw data in real time. As the instrument measures sample fluorescence with its detection system, the Data Collection Software collects the data and stores it. The Data Collection Software stores information about each sample in a sample file (.fsa), which is then analyzed by the GeneMapper® *ID-X* Software.

Instrument and software compatibility

Table 2 Software specific to each instrument

Instrument	Operating system	Data Collection Software	Analysis software
3500/3500xL	• Windows® XP • Windows Vista®	3500 Series Data Collection Software v1.0 and v2.0	GeneMapper® *ID-X* Software v1.4 or higher
3130/3130*xl*	Windows® 7	4.0	

About multicomponent analysis

Life Technologies fluorescent multi-color dye technology allows the analysis of multiple loci, including loci that have alleles with overlapping size ranges. Alleles for overlapping loci are distinguished by labeling locus-specific primers with different colored dyes.

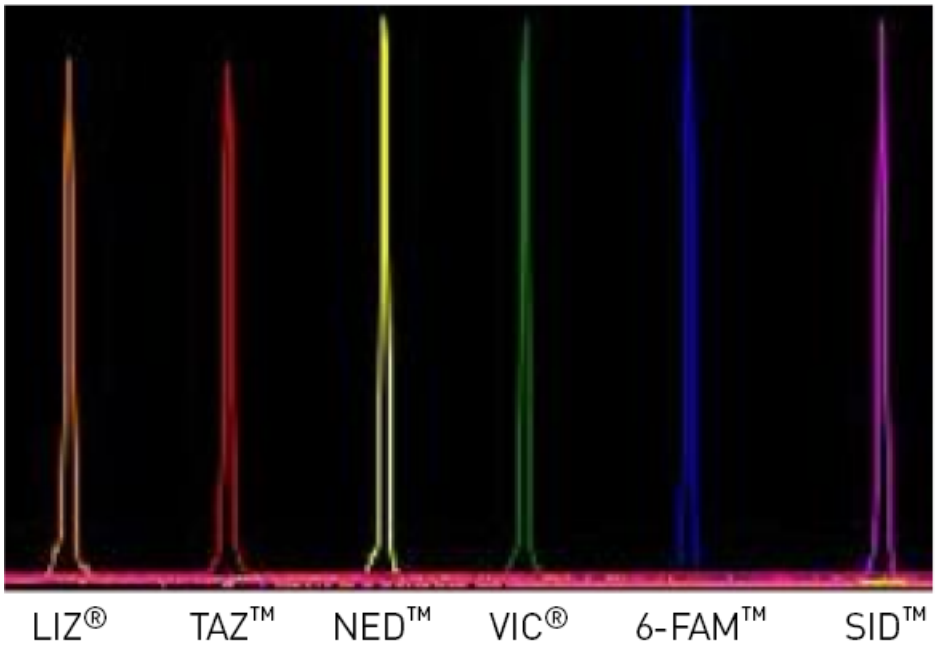
Multicomponent analysis is the process that separates the six different fluorescent dye colors into distinct spectral components. The five dyes used in the Yfiler® Plus Kit to label samples are 6-FAM™, VIC®, NED™, TAZ, and SID dyes. The sixth dye, LIZ® dye, is used to label the GeneScan™ 600 LIZ® Size Standard v2.0.

How multicomponent analysis works

Each of these fluorescent dyes emits its maximum fluorescence at a different wavelength. During data collection on the Life Technologies instruments, the fluorescence signals are separated by diffraction grating according to their wavelengths and projected onto a charge-coupled device (CCD) camera in a predictably spaced pattern. The 6-FAM™ dye emits at the shortest wavelength and it is displayed as blue, followed by the VIC® dye (green), NED™ dye (yellow), TAZ dye (red), SID dye (purple) and LIZ® dye (orange).

Although each of these dyes emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra between the dyes (Figure 3). The goal of multicomponent analysis is to correct for spectral overlap.

Figure 3 Spectral calibration of the six dyes used in the Yfiler® Plus Kit



Materials and equipment

Kit contents and storage

The Yfiler® Plus PCR Amplification Kit is available in two sizes:

- 100 reactions (Cat. no. 4484678) –This kit contains enough reagents for two sets of 50 reactions.
- 500 reactions (Cat. no. 4482730) – This kit contains enough reagents for two sets of 250 reactions.

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

Component	Description	100 reactions	500 reactions	Storage
Yfiler® Plus Master Mix	Contains enzyme, salts, dNTPs, bovine serum albumin, and 0.05% sodium azide in buffer and salt.	2 tubes, 0.5 mL/tube	4 tubes, 1.25 mL/tube	–15 to –25°C on receipt, 2 to 8°C after initial use
DNA Control 007	Contains 2.0 ng/µL human male genomic DNA in 0.05% sodium azide and buffer†. See Table 1 on page 10 for profile.	1 tube, 0.05 mL	2 tubes, 0.05 mL/tube	
Yfiler® Plus Primer Set	Contains locus-specific dye-labeled and unlabeled, forward and reverse primers to amplify human male DNA target.	2 tubes, 0.25 mL/tube	2 tubes, 1.25 mL/tube	–15 to –25°C on receipt, 2 to 8°C after initial use Store protected from light.
Yfiler® Plus Allelic Ladder	Contains amplified alleles. See Table 1 on page 10 for a list of alleles included in the allelic ladder.	2 tubes, 0.025 mL/tube	2 tubes, 0.05 mL/tube	

† The DNA Control 007 is included at a concentration appropriate to its intended use as an amplification control (i.e., to provide confirmation of the capability of the kit reagents to generate a profile of expected genotype). The DNA Control 007 is not designed to be used as a DNA quantitation control, and you may see variation from the labelled concentration when quantitating aliquots of the DNA Control 007.

Standards for samples

For the Yfiler® Plus Kit, the panel of standards needed for PCR amplification, PCR product sizing, and genotyping are:

- **DNA Control 007** – A positive control for evaluating the efficiency of the amplification step and STR genotyping using the Yfiler® Plus Allelic Ladder.
- **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 Yfiler® Plus Kit PCR products. Order the GeneScan™ 600 LIZ® Size Standard v2.0 (Part no. 4408399) separately.
- **Yfiler® Plus Allelic Ladder** – Allelic ladder developed by Life Technologies for accurate characterization of the alleles amplified by the Yfiler® Plus Kit. The Yfiler® Plus Allelic Ladder contains most of the alleles reported for the 27 loci. Refer to Table 1 on page 10 for a list of the alleles included in the Yfiler® Plus Allelic Ladder.

2 Perform PCR

Section 2.1 Amplification from extracted DNA

Required user-supplied reagents

In addition to the Yfiler® Plus Kit reagents, the use of low-TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) is recommended. You can prepare the buffer as described in the procedure below or order it from Teknova (Cat # T0223).

To prepare low-TE buffer:

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 and autoclave the solutions.
3. Store at room temperature.

DNA quantification

Importance of quantification

Quantifying the amount of DNA in a sample before amplification allows you to determine whether or not sufficient DNA is present to permit amplification and to calculate the optimum amount of DNA to add to the reaction. The optimum amount of DNA for the Yfiler® Plus Kit is 1.0 ng in a maximum input volume of 10 µL for 30 PCR cycles.

If too much DNA is added to the PCR reaction, then 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 are problematic 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, and it results in poor spectral separation ("pull-up").
- Incomplete A-nucleotide addition.

When the total number of allele copies added to the PCR is extremely low, allelic dropout can occur resulting in a partial profile.

Methods of quantifying DNA

Life Technologies provides several kits for quantifying DNA in samples. See the references cited in the following table for details about these kits.

Product	Description
Quantifiler® Human DNA Quantification Kit (Part no. 4343895) *and* Quantifiler® Y Human Male DNA Quantification Kit (Part no. 4343906) For more information, see *Quantifiler® Human DNA Quantification Kits User's Manual* (Pub no. 4344790)	**Properties:** The Quantifiler® Human and Quantifiler® Y Human Male Kits are highly specific for human DNA, and they individually detect total human or male DNA, respectively. The kits detect single-stranded and degraded DNA. **How they work:** The Quantifiler® DNA Quantification Kits consist of target-specific and internal control 5' nuclease assays. The Quantifiler® Human and Quantifiler® Y Human Male Kits contain different target-specific assays (human DNA or human male DNA, respectively) that each consist of two locus-specific PCR primers and one TaqMan® MGB probe labeled with FAM™ dye for detecting the amplified sequence. The kits each contain a separate internal PCR control (IPC) assay, which consists of an IPC template DNA (a synthetic sequence not found in nature), two primers for amplifying the IPC template, and one TaqMan® MGB probe labeled with VIC® dye for detecting the amplified IPC.
Quantifiler® Duo DNA Quantification Kit (Part no. 4387746) For more information, see *Quantifiler® Duo DNA Quantification Kit User's Manual* (Part no.4391294)	**Properties:** The Quantifiler® Duo Kit is highly specific for human DNA. This kit combines the detection of both total human and male DNA in one PCR reaction.The kit detects single-stranded and degraded DNA. **How it works:** The Quantifiler® Duo DNA Quantification Kit consists of target-specific and internal control 5' nuclease assays. The Quantifiler® Duo kit combines two human-specific assays in one PCR reaction (for total human DNA and human male DNA). The two human DNA specific assays each consist of two PCR primers and a TaqMan® probe. The TaqMan® probes for the human DNA and human male DNA assays are labeled with VIC® and FAM™ dyes, respectively. In addition, the kit contains an internal PCR control (IPC) assay similar in principle to that used in the other Quantifiler kits, but labeled with NED™ dye.
Quantifiler® HP DNA Quantification Kit (Cat. no. 4482911) For more information, see *Quantifiler HP and Trio DNA Quantification Kits User Guide* (Pub no. 4485354)	**Properties:** The Quantifiler® HP Kit is designed to quantify the total amount of amplifiable human DNA in a sample. **How it works:** The Quantifiler® HP DNA Quantification Kit uses multiple-copy target loci for improved detection sensitivity. The human-specific target loci (Small Autosomal, Large Autosomal, and Y-chromosome targets) each consist of multiple copies dispersed on various autosomal chromosomes (Small Autosomal and Large Autosomal). To maximize the consistency of quantification results, genomic targets were selected with conserved primer- and probe-binding sites within individual genomes and also with minimal copy number variability between different individuals and population groups. As a result, the detection sensitivity of the assay is improved over Quantifiler® Duo, Human, and Y Human Male DNA Quantification Kit assays. The primary quantification targets (Small Autosomal and Y) consist of relatively short amplicons (75 to 80 bases) to improve the detection of degraded DNA samples. In addition, the kit each contains a Large Autosomal target with a longer amplicon (>200 bases) to aid in determining if a DNA sample is degraded.

Product	Description
Quantifiler® Trio DNA Quantification Kit (Cat. no. 4482910) For more information, see *Quantifiler HP and Trio DNA Quantification Kits User Guide* (Pub no. 4485354)	**Properties:** The Quantifiler® Trio Kit is designed to simultaneously quantify the total amount of amplifiable human DNA and human male DNA in a sample. **How it works:** The Quantifiler® Trio DNA Quantification Kit uses multiple-copy target loci for improved detection sensitivity. The human-specific target loci (Small Autosomal, Large Autosomal, and Y-chromosome targets) each consist of multiple copies dispersed on various autosomal chromosomes (Small Autosomal and Large Autosomal), or multiple copies on the Y-chromosome. To maximize the consistency of quantification results, genomic targets were selected with conserved primer- and probe-binding sites within individual genomes and also with minimal copy number variability between different individuals and population groups. As a result, the detection sensitivity of the assay is improved over Quantifiler® Duo, Human, and Y Human Male DNA Quantification Kit assays. The primary quantification targets (Small Autosomal and Y) consist of relatively short amplicons (75 to 80 bases) to improve the detection of degraded DNA samples. In addition, kits each contain a Large Autosomal target with a longer amplicon (>200 bases) to aid in determining if a DNA sample is degraded.

Prepare the amplification kit reactions

1. Calculate the volume of each component needed to prepare the reactions, using the table below.

DNA sample	Volume per reaction
Yfiler® Plus Master Mix	10.0 µL
Yfiler® Plus Primer Set	5.0 µL

2. Prepare reagents. Include additional reactions in your calculations to provide excess volume for the loss that occurs during reagent transfers. Thaw the Master Mix and the Primer Set, then vortex all reagent tubes, including the enzyme, for 3 seconds and centrifuge briefly before opening the tubes.

   IMPORTANT! Thawing is required only during first use of the Primer Set and Master Mix. After first use, these reagents are stored at 2 to 8°C and, therefore, they do not require subsequent thawing. Do not refreeze these reagents.

3. Prepare the reaction mixture: Pipette the required volumes of components into an appropriately sized polypropylene tube.
4. Vortex the reaction mixture for 3 seconds, then centrifuge briefly.
5. Dispense 15 µL of the reaction mixture into each reaction well of a MicroAmp® Optical 96-Well Reaction Plate or each MicroAmp® tube.
6. Prepare the DNA samples:

DNA sample	To prepare...
Negative control	Add 10 µL of low-TE buffer (10mM Tris, 0.1mM EDTA, pH 8.0).
Test sample	Dilute a portion of the test DNA sample with low-TE buffer so that 1.0 ng of total DNA is in a final volume of 10 µL. Add 10 µL of the diluted sample to the reaction mix.
Positive control	Add 007 control DNA to a total amount of 1.0 ng.

The final reaction volume (sample or control plus reaction mixture) is 25 µL.

7. Seal the plate with MicroAmp® Clear Adhesive Film or MicroAmp® Optical Adhesive Film, or cap the tubes.
8. Centrifuge the tubes at 3000 rpm for about 20 seconds in a tabletop centrifuge (with plate holders if using 96-well plates).
9. Amplify the samples in a GeneAmp® PCR System 9700 with the silver or gold-plated silver 96-well block or a Veriti® 96-Well Thermal Cycler.

   Note: The Yfiler® Plus Kit is not validated for use with the GeneAmp PCR System 9700 with the aluminium 96-well block. Use of this thermal cycling platform may adversely affect performance of the Yfiler® Plus Kit.

Perform PCR

1. Program the thermal cycling conditions:
   - When using the GeneAmp® PCR System 9700 with either 96-well silver or gold-plated silver block, select the **9600 Emulation Mode**.
   - When using the Veriti® 96-Well Thermal Cycler, refer to the following document for instructions on how to configure the Veriti instrument to run in the 9600 Emulation Mode: *User Bulletin: Veriti® 96-Well Thermal Cycler AmpFℓSTR® Kit Validation* (Part no.4440754).

Initial incubation step	Denature	Anneal/ Extend	Final extension	Final hold
HOLD	CYCLE (30)		HOLD	HOLD
95°C 1 min	94°C 4 sec	61.5°C 1min	60°C 22 min	4°C ∞

2. Load the plate into the thermal cycler and close the heated cover.

IMPORTANT! If using the 9700 thermal cycler with silver or gold-plated silver block and adhesive clear film instead of caps to seal the plate wells, be sure to place a MicroAmp® compression pad (Part no. 4312639) on top of the plate to prevent evaporation during thermal cycling. The Veriti® Thermal Cycler does not require a compression pad.

3. Start the run.
4. On completion of the run, store the amplified DNA and protect from light.

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

IMPORTANT! Store the amplified products so that they are protected from light.

Section 2.2 Direct amplification of DNA

Optimize PCR cycle number

Before using the Yfiler® Plus Kit for the first time, perform a single initial sensitivity experiment to determine the appropriate cycle number to use during internal validation studies and operational use of the Yfiler® Plus Kit. This experiment 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 blood samples on untreated paper), perform separate sensitivity experiments for each sample type and substrate to be used for testing.

The Yfiler® Plus Kit is optimized to amplify unpurified:

- Single-source blood samples on treated paper
- Buccal samples on treated paper substrates without the need for sample purification
- Blood samples collected on untreated paper with the addition of Prep-n-Go™ Buffer
- Buccal samples collected on swab substrates and treated with Prep-n-Go™ Buffer

When amplifying single-source, unpurified samples using the Yfiler® Plus Kit, you should expect to see greater variation in peak height from sample to sample than is expected with purified samples. Careful optimization of the cycle number will help to minimize the impact of this variation.

Select samples and prepare plates

1. Select 20 of each sample and substrate type. Ensure the selected samples represent a "typical" range of samples analyzed in your laboratory.

   IMPORTANT! The number of samples recommended for this study has been chosen to allow you to complete electrophoresis using a single 96-well plate, thus minimizing the impact of run-to-run variation on the results.

2. Prepare the samples and the reactions as described in the protocols later in this chapter. Prepare sufficient PCR reagents to complete amplification of three replicate plates.
3. Create three identical PCR plates.

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 below:

Sample type	Substrate
	Treated or untreated paper
Blood	26, 27, 28, 29 cycles
Buccal	26, 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.

Determine optimum conditions

1. Run the PCR products on the appropriate CE platform using the recommended protocol; see Chapter 3, "Perform electrophoresis" on page 33.
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 should generate profiles with the following peak heights, with no instances of allelic dropout and minimal occurrence of off-scale allele peaks.

Instrument	Peak height
31xx	2500–4000 RFU
3500 Series	5000–12,000 RFU

Treated or untreated paper: prepare reactions

Sample prep guidelines

- Do not add water to the wells on the reaction plate before adding the punches. If your laboratory is experiencing static issues with the paper discs, you may prepare and dispense the 25 µL reaction mix into the wells of the reaction plate before adding the punches.
- 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 into the appropriate well on the reaction plate.
- For automated punching: Please refer to the User Guide of your automated or semi-automated disc punch instrument for proper guidance.
- For blood on untreated paper samples, add 2 µL of Prep-n-Go™ buffer on top of the 1.2-mm sample punch.

Prepare the reactions

1. Add samples to the reaction plate:

Well(s)	Add the following to wells of a MicroAmp® Optical 96-Well Reaction Plate	
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: The volumes of positive control are suggested amounts and may be adjusted if peak heights are too high or too low for your optimized cycle number.		

2. Calculate the volume of each component needed to prepare the reactions, using the table below.

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

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

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

3. Prepare reagents. Thaw the Master Mix and Primer Set, then vortex for 3 seconds. Centrifuge briefly before opening the tubes or bottles.

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

4. Pipet the required volumes of components into an appropriately sized polypropylene tube.
5. Vortex the reaction mix for 3 seconds, then centrifuge briefly.
6. Dispense 25 µL of the reaction mix into each reaction well of a MicroAmp® Optical 96-Well Reaction Plate.

7. Seal the plate with MicroAmp® Clear Adhesive Film or MicroAmp® Optical Adhesive Film.

   IMPORTANT! If using the 9700 thermal cycler with silver or gold-plated silver block and adhesive clear film instead of caps to seal the plate wells, place a MicroAmp® 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.

8. Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders.

Swab substrates: prepare reactions

Sample prep guidelines

- Detach each buccal swab head from the swab shaft before lysis.
- If 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)
  - 96-well deep-well plate (Part no. 4392904) with an oven and a metal plate adaptor (Robbins Scientific® Model 400 Hybridization Incubator or similar, Agilent® Benchtop Rack for 200 µl Tubes/V Bottom Plates (metal) Part no. 410094 or similar)

    IMPORTANT! Do not use a plastic plate adaptor.

- For optimum performance, lysis of a whole swab is recommended. To preserve the sample, evaluate lysis of a half swab.

Prepare the sample lysate (room temperature protocol)

1. Add 400 µL Prep-n-Go™ Buffer (Part no. 4471406) to 1.5 mL tubes or the appropriate wells of a 96-well deep-well plate (Part no. 4392904).
2. Into each tube or well, put the entire head of each swab and let stand for 20 minutes at room temperature (20 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. Proceed to "Prepare the reactions" on page 29 or see "Store the sample lysate" on page 31.

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, Part no. 4471406) to 1.5 mL tubes or the appropriate wells of a 96-well deep-well plate (Part no. 43929040).

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, then 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. Proceed to the next section to prepare the reactions or see "Store the sample lysate" on page 31.

Prepare the reactions

1. Add Prep-n-Go™ Buffer (Part no. 4471406) to the control wells in the reaction plate:

Well(s)	**Add the following to wells of a MicroAmp® Optical 96-Well Reaction Plate...**	
Negative control	3 µL of Prep-n-Go™ Buffer	
Positive control	• For 25 and 26 cycles	0 µL of Prep-n-Go™ Buffer
	• For 27 cycles	1 µL of Prep-n-Go™ Buffer
	• For 28 cycles	2 µL of Prep-n-Go™ Buffer

2. Prepare reagents:
   a. Thaw the Master Mix, and Primer Set, then vortex for 3 seconds and centrifuge briefly before opening the tubes or bottles.

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

3. Calculate the volume of each component needed to prepare the reactions using the table below.

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

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

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

4. Pipet the required volumes of components into an appropriately sized polypropylene tube.
5. Vortex the reaction mix for 3 seconds, then centrifuge briefly.
6. 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 and sample lysate or positive control).

   Note: For samples and controls, add 3 µL of lysate, or the 1-, 2-, or 3 µL of control DNA in addition to the 25 µL of reaction mix. There is no need to compensate for the volume of lysate/control.

7. Add samples to the reaction plate:

Well(s)	Add the following to wells of a MicroAmp® Optical 96-Well Reaction Plate...	
Test samples	3 µL of sample lysate	
Positive control	• For 25 and 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

Note: The volumes of positive control are suggested amounts and may be adjusted if peak heights are too high or too low for your optimized cycle number.

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

8. Seal the plate with MicroAmp® Clear Adhesive Film or MicroAmp® Optical Adhesive Film.

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 using the 9700 thermal cycler with silver or gold-plated silver block, place a MicroAmp® compression pad (Part no. 4312639) on top of the plate to additionally prevent evaporation during thermal cycling. The Veriti® Thermal Cycler does not require a compression pad.

9. Vortex the reaction mix at medium speed for 3 seconds.
10. Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders.
11. Amplify the samples in a Veriti® 96-well Thermal Cycler or PCR System 9700 with the silver or gold-plated silver 96-well block as described in "Perform PCR" on page 31.

Store the sample lysate

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

Store the sample lysate as needed:

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

These storage recommendations are preliminary pending the results of ongoing stability studies. 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

1. Program the thermal cycling conditions.
   - When using the GeneAmp® PCR System 9700 with either 96-well silver or gold-plated silver block, select the **9600 Emulation Mode**.
   - When using the Veriti® 96-Well Thermal Cycler, refer to the following document for instructions on how to configure the Veriti instrument to run in the 9600 Emulation Mode: *User Bulletin: Veriti® 96-Well Thermal Cycler AmpFℓSTR® Kit Validation* (Part no. 4440754).

Initial incubation step	Denature	Anneal/Extend	Final Extension	Final hold
HOLD	CYCLE (26–29)		HOLD	HOLD
95°C 1 min	94°C 4 sec	61.5°C 1 min	60.0°C 22 min	4°C ∞

2. Load the plate into the thermal cycler and close the heated cover.

IMPORTANT! If using the 9700 thermal cycler with silver or gold-plated silver block and adhesive clear film instead of caps to seal the plate wells, be sure to place a MicroAmp® 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. Start the run.

4. On completion of the run, store the amplified DNA and protect from light.

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

IMPORTANT! Store the amplified products so that they are protected from light.

3 Perform electrophoresis

Allelic ladder requirements

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

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

IMPORTANT! Variation in laboratory temperature can cause changes in fragment migration speed and sizing variation between both single- and multiple-capillary runs (with larger size variations seen between samples injected in multiple-capillary runs). We recommend the above frequency of allelic ladder injections, which should account for normal variation in run speed. However, during internal validation studies, 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, because size values obtained for the same sample can differ between instrument platforms because of different polymer matrices and electrophoretic conditions.

Section 3.1 3500/3500xL instruments

Set up the 3500/3500xL instruments for electrophoresis

Reagents and parts

Appendix C, "Ordering information" on page 109 lists the required materials 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.

Do not refreeze kit components after thawing.

Electrophoresis software setup and reference documents

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

Genetic Analyzer	Operating System	Data Collection Software	Additional software	Plate templates, assays, run modules, and conditions (installed with the HID Updater)	References
3500§	Windows Vista®	3500 Data Collection Software v2.0	HID Updater 3500 DC v2.0 (Part no. 4480670)	• Plate templates: 6dye_36_POP4 • Assays: GF+Norm_POP4 and GF_POP4 which contain instrument protocol HID36_POP4_J6_NT3200 with the following conditions: – Run module: HID36_POP4 – Injection conditions: 1.2 kV/16 sec† – Alternate injection conditions: 1.5 kV/16 sec‡ – Run conditions: 13 kV/1550 sec – Dye Set J6	*3500/3500xL Genetic Analyzer User Guide* (Pub. no. 4401661) *HID Updater 3500 Data Collection Software v2 Release Notes*
3500xL§				• Plate templates: 6dye_36_POP4_xl • Assays: GF+Norm_POP4_xl and GF_POP4_xl which contain instrument protocol HID36_POP4xl_J6_NT3200 with the following conditions: – Run module: HID36_POP4xl – Injection conditions: 1.2 kV/24 sec – Alternate injection conditions: 1.5 kV/24 sec†† – Run conditions: 13 kV/1550 sec – Dye Set J6	
3500§ 3500xL§	Windows® 7	3500 Data Collection Software v2.0	HID Updater 3500 DC v2.0 (Part no. 4480670)	Same as 3500 Data Collection Software v2.0 listed above	*3500/3500xL Genetic Analyzer User Guide* (Pub. no. 4476988) *HID Updater 3500 Data Collection Software v2 Release Notes*

† 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.

‡ 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.

§ We conducted validation studies for the Yfiler® Plus Kit using the 3130*xl*, 3500, or 3500xL configurations.

†† This kit was developed using two injection voltage conditions for the 3500xL instrument; 1.2 kV/24 sec and 1.5 kV/24 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

You can run 6-dye samples on 3500 Data Collection Software v1 or v2. Before running on either system for the first time, run the HID Updater 3500 DC v2.0 (Part no. 4480670). The HID Updater installs plate templates, assays, and instrument protocols that can be used to run Yfiler® Plus Kit samples. For more information, refer to the release notes provided with the Updater.

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

1. Shut down the 3500/3500xL Data Collection Software.
2. Download the updater from **www.lifetechnologies.com/support ▸ Software, Patches & Updates ▸ 3500 Series Genetic Analyzers for Human Identification ▸ HID Updater 3500 DC v2.0**.
3. Click on the **Read me** file to review the software release notes.
4. Click on the updater .exe file.
5. Follow the on-screen prompts.
6. Restart the computer.

Create a Yfiler Plus® assay

The Yfiler® Plus assay will have 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:

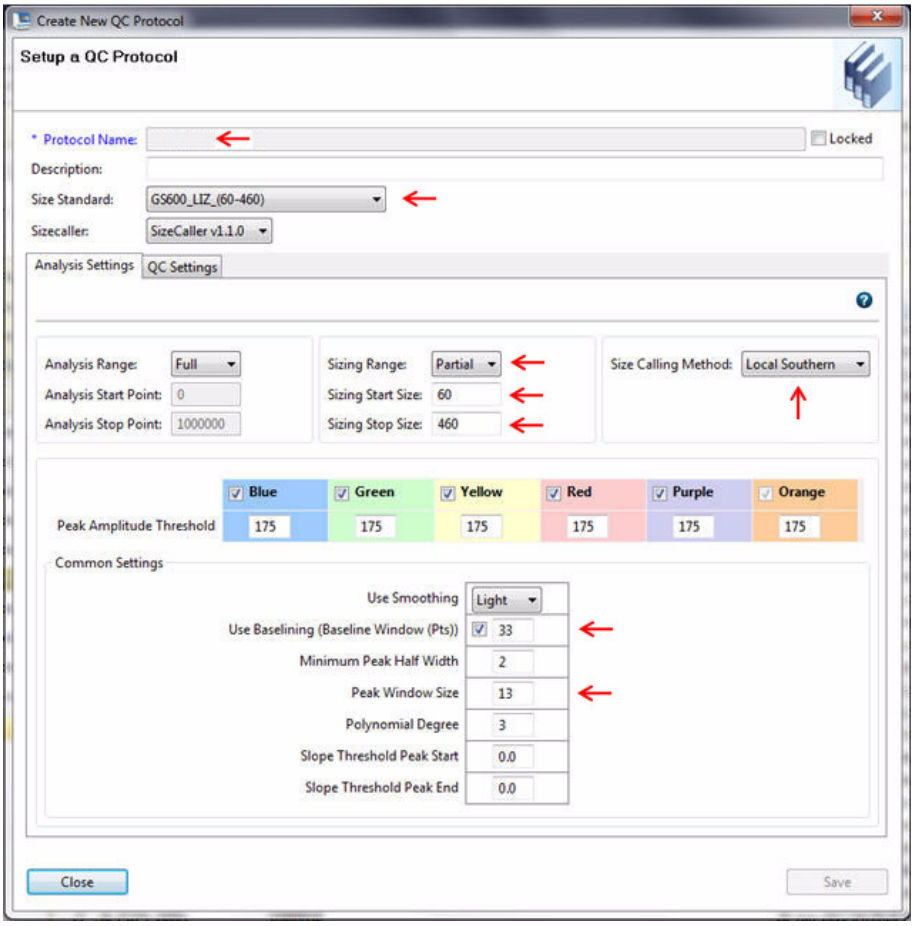
1. Access the Assays library by selecting the **Library** tab and then the **Assays** tab under Library Resources.
2. Select an existing assay (i.e. GF_POP4), click duplicate and give it a new name (i.e. YFP_POP4).
3. Select the new assay and then click **Edit** to open it.
4. Open the instrument protocol by clicking on **Edit** and modify the injection and run conditions specific to your instrument class and as shown on the table on page 35. Save the modified instrument protocol by clicking on **Save to Library** and give the protocol a new name.
5. Follow the instructions below to edit the QC protocol.

Modify 3500 QC protocol size-calling method

The Yfiler® Plus Kit has been validated with data that was analyzed using the Local Southern method (60–460 base pairs). The QC protocol provided in the HID assay installed by the HID Updater 3500 DC v2.0 is set for the 3rd Order Least Squares method. To use the Local Southern method for fragment sizing, edit the QC protocol:

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

2. Create a new QC protocol according to the figure:



   a. Name the new QC protocol according to your laboratory's standard convention.

   b. Set the following parameters:

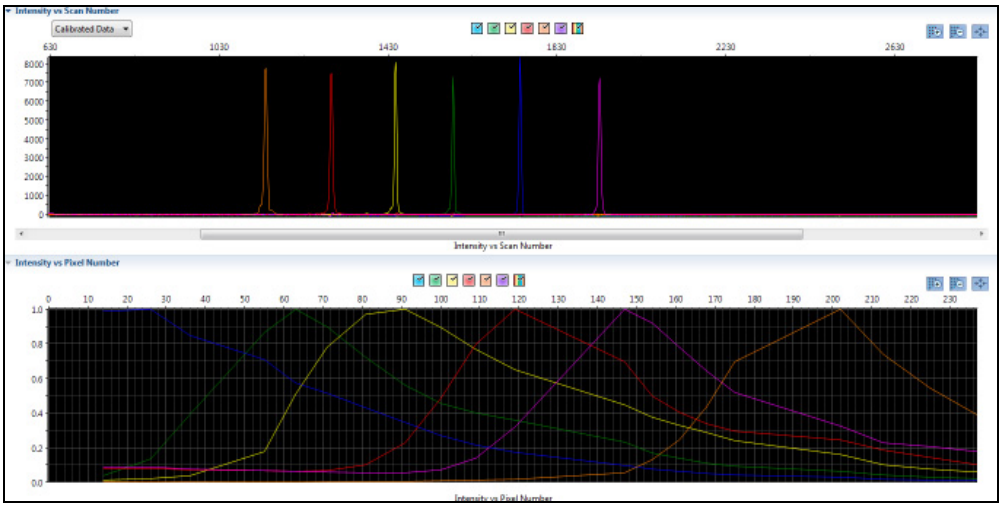
Parameter	Setting
Size Standard	GS600_LIZ_(60-460)
Size Range	Partial
Sizing Start Size	60 bp
Sizing Stop Size	460 bp
Size Calling Method	Local Southern Method
After checking the "Use Baselining" box:	
Baseline Window Pts.	33
Peak Window Size	13

   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 (J6 Dye Set) (Part no. 4425042). The following figure is an example of a passing 6-dye spectral calibration.



Prepare samples for electrophoresis on the 3500/3500xL instruments

Prepare the samples for electrophoresis immediately before loading.

1. Calculate the volume of Hi-Di™ Formamide and GeneScan™ 600 LIZ® Size Standard v2.0 needed to prepare the samples:

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

Note: Include additional samples in your calculations to 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. Pipet the required volumes of components into an appropriately sized polypropylene tube.
3. Vortex the tube, then centrifuge briefly.
4. 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.

5. 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.
6. Heat the reaction plate in a thermal cycler for 3 minutes at 95°C.
7. Immediately place the plate on ice for 3 minutes.
8. Place the plate assembly on the autosampler.
9. Start the electrophoresis run.

Section 3.2 3130/3130*xl* instruments

Set up the 3130/3130*xl* instruments for electrophoresis

Reagents and parts

Appendix C, "Ordering information" on page 109 lists the required materials 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.

Do not refreeze kit components after thawing.

Electrophoresis software setup and reference documents

The following table lists data collection software and the run modules that can be used to analyze PCR products generated by this kit. For details on the procedures, refer to the documents listed in the table.

Genetic Analyzer	Operating System	Data Collection Software	Additional software	Run modules and conditions	References
3130 *-or-* 3130*xl*†	Windows® 7	Data Collection Software v4	3130/3730 DC v4 6-Dye Module v1 (contact Life Technologies)	• HIDFragmentAnalysis36_POP4_1 Injection conditions for 3130: 3 kV/5 sec Injection conditions for 3130*xl*: 3 kV/10 sec Alternate injection conditions for the 3130*xl*: 3 kV/13 sec‡ Run conditions: 15 kV/1500 sec • Dye Set J6	*Applied Biosystems 3130 Series Data Collection Software v4 Getting Started Guide* (Pub. no. 4477796)

† We conducted validation studies for the Yfiler® Plus Kit using the 3130*xl*, 3500, or 3500xL configurations.

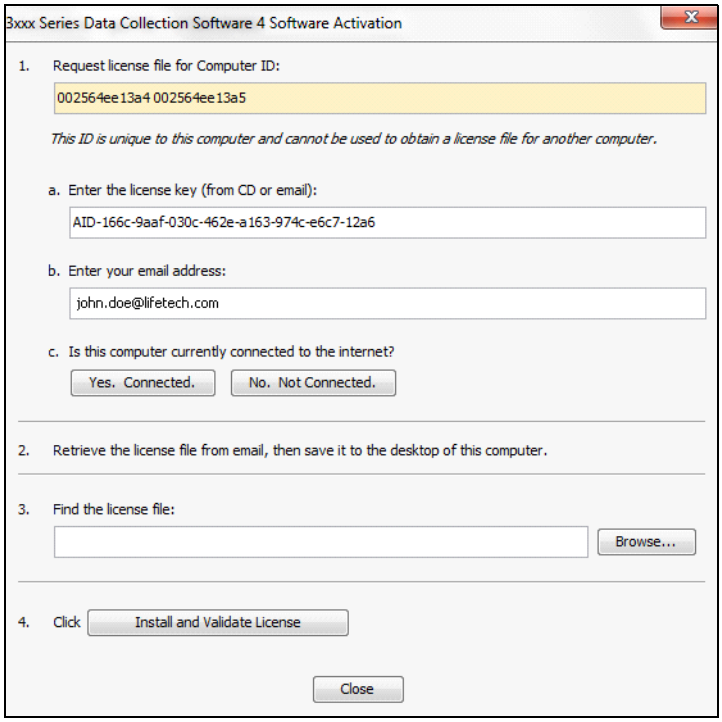
‡ This kit was developed using two injection voltage conditions for the 3130*xl*; 3 kV/10 sec and 3 kV/13 sec. You are encouraged to explore both options during validation to determine which protocol provides the best results on your instrumentation.

Obtain and activate the 6-dye license for the instrument

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 Life Technologies 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 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.

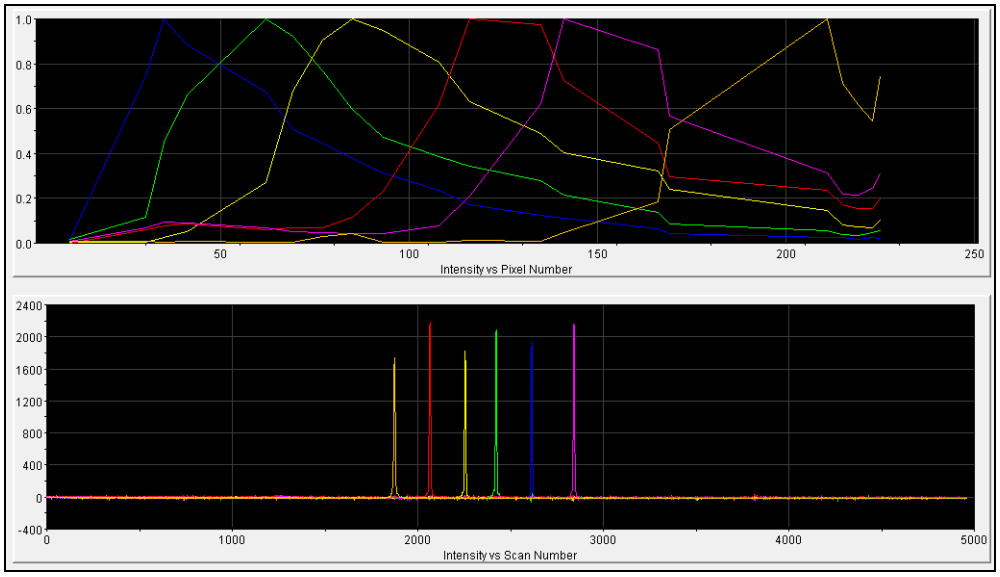
4. Select **Tools ▸ License Manager** to display the Software Activation dialog box.



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 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 (J6 Dye Set) (Part no. 4425042). The following figure is an example of a passing 6-dye spectral calibration.



Prepare samples for electrophoresis on the 3130/3130*xl* instruments

Prepare the samples for electrophoresis immediately before loading.

1. Calculate the volume of Hi-Di™ Formamide and size standard needed to prepare the samples:

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

Note: Include additional samples in your calculations to 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. Pipet the required volumes of components into an appropriately sized polypropylene tube.
3. Vortex the tube, then centrifuge briefly.

4. 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.
5. 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.
6. Heat the reaction plate in a thermal cycler for 3 minutes at 95°C.
7. Immediately place the plate on ice for 3 minutes.
8. Place the plate assembly on the autosampler.
9. Start the electrophoresis run.

4 Analyze Data

Overview of GeneMapper® *ID-X* Software v1.4

GeneMapper® *ID-X* Software v1.4 or higher analyzes 4-dye, 5-dye, and 6-dye data and is required to correctly analyze data generated using the Yfiler® Plus Kit. After electrophoresis, the data collection software stores information for each sample in a .fsa or .hid file. Using GeneMapper® *ID-X* Software v1.4 or higher enables you to analyze and interpret the data from the .fsa or .hid files.

Instruments

Refer to "Instrument and software overview" on page 15 for a list of compatible instruments.

Before you start

When using GeneMapper® *ID-X* Software v1.4 or higher to perform human identification (HID) analysis with Yfiler® Plus kits, be aware that:

- HID analysis requires at least one allelic ladder sample per run folder. Your laboratory can use multiple ladder samples in an analysis, provided individual laboratories conduct the appropriate validation studies.

  For multiple ladder samples, the GeneMapper® *ID-X* Software calculates allelic bin offsets by using an average of all ladders that use the same panel within 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 ladder(s) within 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. Failure to apply this setting for ladder samples results in failed analysis.

- 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 Yfiler® Plus Allelic Ladders do exist. Off-ladder (OL) alleles may contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the ±0.5-nt bin window of any known allelic ladder allele or virtual bin.

  Note: If a sample allele peak is called as an off-ladder allele, the sample result needs to be verified according to the laboratory's protocol.

Set up GeneMapper® *ID-X* Software for data analysis

Panel, bin, and stutter file version

The file names shown in this section may differ from the file names you see when you download or import files. If you need help determining the correct files to use, contact your local Life Technologies Human Identification representative, or go to **www.lifetechnologies.com/support ▸ Software, Patches & Updates ▸** GeneMapper® *ID-X* Software.

The instructions and examples in this section refer to the latest version of panel, bin, and stutter file available at the time of publication.

Before using the software for the first time

Before you use GeneMapper® *ID-X* Software v1.4 to analyze data for the first time, you must do the following:

1. Check **www.lifetechnologies.com/support ▸ Software, Patches & Updates ▸** GeneMapper® *ID-X* Software to obtain the latest Yfiler® Plus Kit panel, bin, and stutter files.
2. Download and import the files into the GeneMapper® *ID-X* Software, as explained in "Import panels, bins, and marker stutter" on page 48.

   Note: When downloading new versions of analysis files, refer to the associated Read Me file for details of changes between software file versions. If you have validated previous file versions for data analysis, conduct the appropriate internal verification studies before using new file versions for operational analysis.
3. Create an analysis method, as explained in "Create an analysis method" on page 52.
4. Define custom views of analysis tables.

   Refer to Chapter 1 of the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub no. 4375574) for more information.
5. Define custom views of plots.

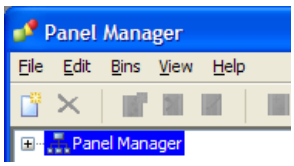
   Refer to Chapter 1 of the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub no. 4375574) for more information.

Check panel, bin, and stutter file version

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

   IMPORTANT! For logon instructions, refer to the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub no. 4375574).
2. Select **Tools ▸ Panel Manager**.
3. Check the version of files imported into the Panel Manager:

   a. Select **Panel Manager** in the navigation pane.



b. Expand the Panel Manager folder and any sub-folders to identify the analysis file version 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 and check the version of panel, bin, and stutter files installed.
5. If newer versions are available on the website, download and import as described below.

Import panels, bins, and marker stutter

To import the latest Yfiler® Plus Kit panel, bin set, and marker stutter files from our web site into the GeneMapper® *ID-X* Software database:

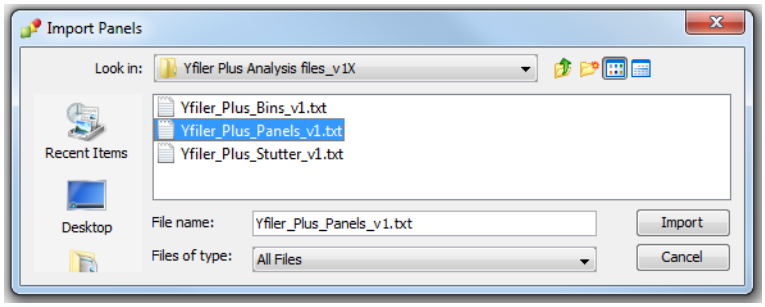
1. Download and open the file containing panels, bins, and marker stutter:
   a. Go to **www.lifetechnologies.com/support ▸ Software, Patches & Updates ▸ GeneMapper® *ID-X* Software**. Download the file **Yfiler Plus Analysis files_v1X** or its latest version.
   b. Unzip the file.
2. Start the GeneMapper® *ID-X* Software, then log in with the appropriate user name and password.

IMPORTANT! For logon instructions, refer to the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574).

3. Select **Tools ▸ Panel Manager**.
4. Find, then open the folder containing the panels, bins, and marker stutter:
   a. Select **Panel Manager** in the navigation pane.
   b. Select **File ▸ Import Panels** to open the Import Panels dialog box.
   c. Navigate to, then open the Yfiler Plus Analysis files_v1X folder that you unzipped in step 1 on page 48.

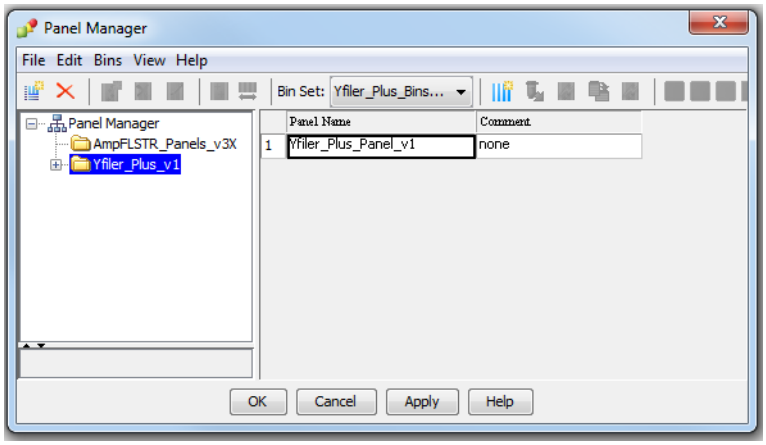
5. Select Yfiler_Plus_Panels_v1.txt (or the version you installed), then click **Import**.

   Note: Importing this file creates a new folder in the navigation pane of the Panel Manager "Yfiler_Plus_Panels_v1". This folder contains the panel for the Yfiler® Plus Kit and associated markers.



6. Import Yfiler_Plus_Bins_v1.txt:

   a. Select the **Yfiler_Plus_Panels_v1** folder in the navigation pane.

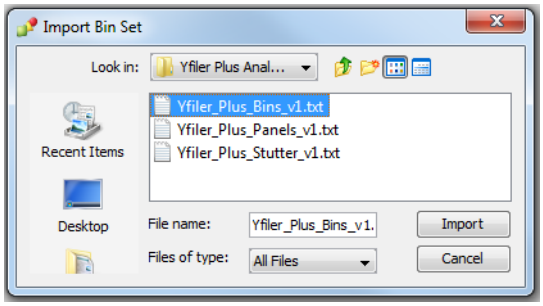


   b. Select **File** ▸ **Import Bin Set** to open the Import Bin Set dialog box.

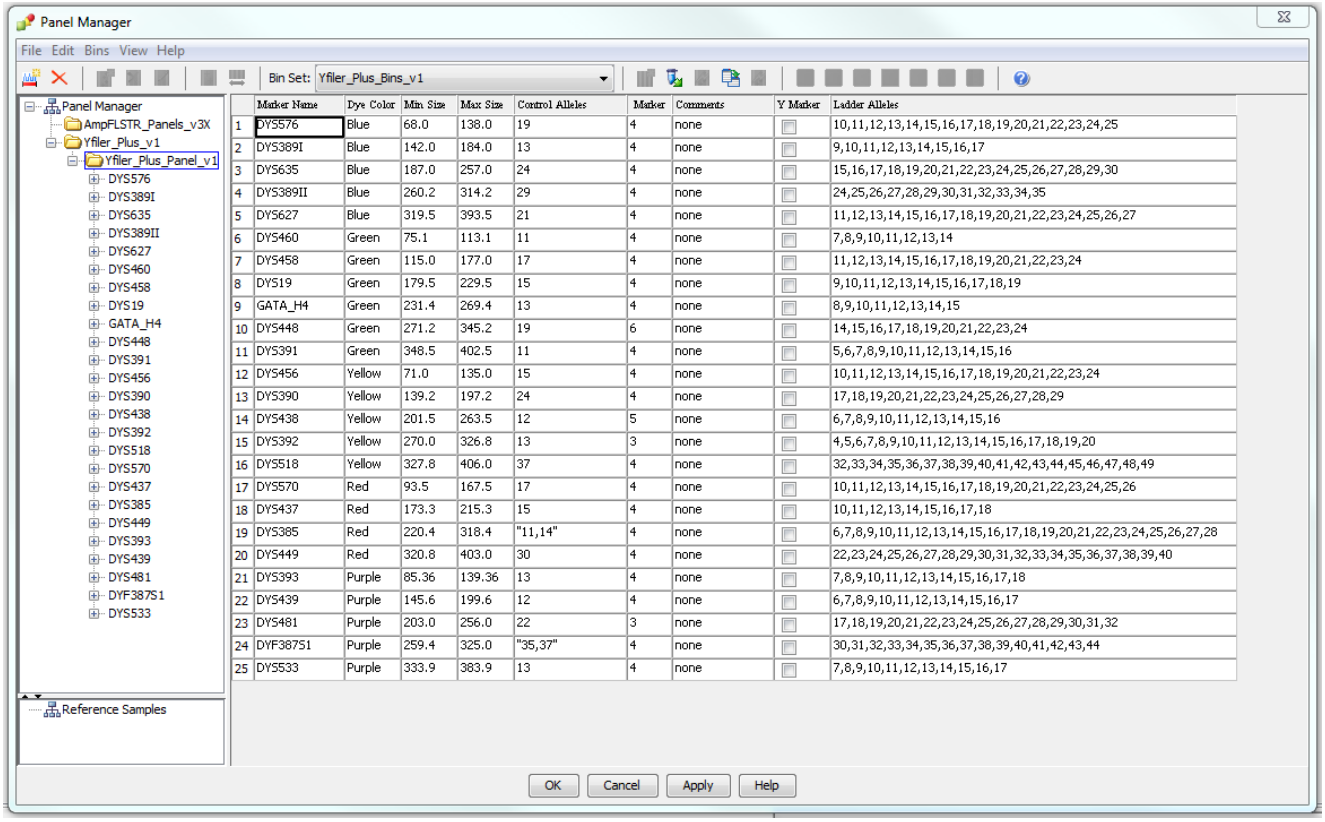
   c. Navigate to, then open the **Yfiler_Plus_Panels_v1** folder.

   d. Select **Yfiler_Plus_Bins_v1.txt**, then click **Import**.

      Note: Importing this file associates the bin set with the panels in the **Yfiler_Plus_Panels_v1** folder.



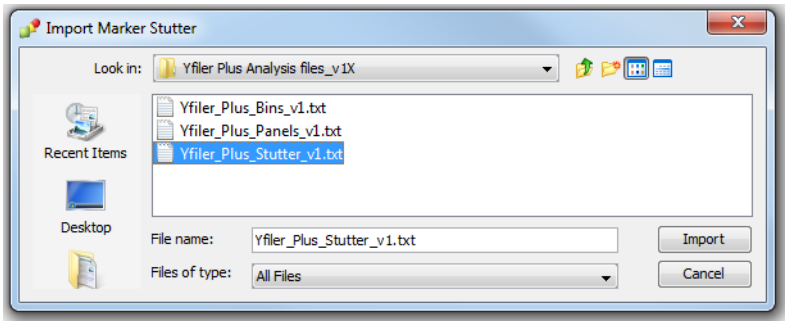
7. To view the imported panels in the navigation pane, double-click the **Yfiler_Plus_Panels_v1** folder to display the panel information in the right pane.



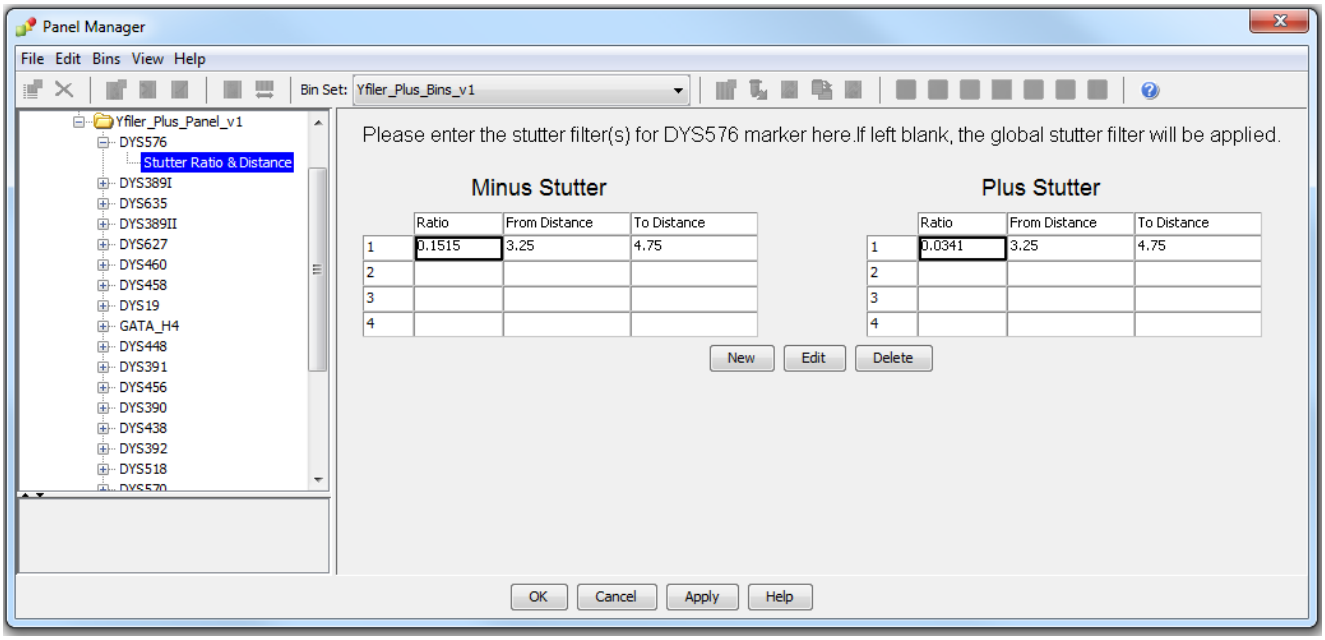
	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker	Comments	Y Marker	Ladder Alleles
1	DYS576	Blue	68.0	138.0	19	4	none		10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25
2	DYS389I	Blue	142.0	184.0	13	4	none		9,10,11,12,13,14,15,16,17
3	DYS635	Blue	187.0	257.0	24	4	none		15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30
4	DYS389II	Blue	260.2	314.2	29	4	none		24,25,26,27,28,29,30,31,32,33,34,35
5	DYS627	Blue	319.5	393.5	21	4	none		11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27
6	DYS460	Green	75.1	113.1	11	4	none		7,8,9,10,11,12,13,14
7	DYS458	Green	115.0	177.0	17	4	none		11,12,13,14,15,16,17,18,19,20,21,22,23,24
8	DYS19	Green	179.5	229.5	15	4	none		9,10,11,12,13,14,15,16,17,18,19
9	GATA_H4	Green	231.4	269.4	13	4	none		8,9,10,11,12,13,14,15
10	DYS448	Green	271.2	345.2	19	6	none		14,15,16,17,18,19,20,21,22,23,24
11	DYS391	Green	348.5	402.5	11	4	none		5,6,7,8,9,10,11,12,13,14,15,16
12	DYS456	Yellow	71.0	135.0	15	4	none		10,11,12,13,14,15,16,17,18,19,20,21,22,23,24
13	DYS390	Yellow	139.2	197.2	24	4	none		17,18,19,20,21,22,23,24,25,26,27,28,29
14	DYS438	Yellow	201.5	263.5	12	5	none		6,7,8,9,10,11,12,13,14,15,16
15	DYS392	Yellow	270.0	326.8	13	3	none		4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20
16	DYS518	Yellow	327.8	406.0	37	4	none		32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49
17	DYS570	Red	93.5	167.5	17	4	none		10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26
18	DYS437	Red	173.3	215.3	15	4	none		10,11,12,13,14,15,16,17,18
19	DYS385	Red	220.4	318.4	"11,14"	4	none		6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28
20	DYS449	Red	320.8	403.0	30	4	none		22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40
21	DYS393	Purple	85.36	139.36	13	4	none		7,8,9,10,11,12,13,14,15,16,17,18
22	DYS439	Purple	145.6	199.6	12	4	none		6,7,8,9,10,11,12,13,14,15,16,17
23	DYS481	Purple	203.0	256.0	22	3	none		17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32
24	DYF387S1	Purple	259.4	325.0	"35,37"	4	none		30,31,32,33,34,35,36,37,38,39,40,41,42,43,44
25	DYS533	Purple	333.9	383.9	13	4	none		7,8,9,10,11,12,13,14,15,16,17

8. Import Yfiler_Plus_Stutter_v1:
   a. Select the **Yfiler_Plus_Panels_v1** 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_v1X folder.
   d. Select **Yfiler_Plus_Stutter_v1**, then click **Import**.

   Note: Importing this file associates the marker stutter ratio with the bin set in the Yfiler_Plus_Panels_v1 folder and overwrites any existing stutter ratios associated with the panels and bins in that folder.



9. View the imported marker stutters in the navigation pane:
   a. Double-click the **Yfiler_Plus_Panels_v1** folder to display its list of kits in the right pane.
   b. Double-click the **Yfiler_Plus_Panels_v1** folder to display its list of markers below it.
   c. Double-click **DYS576**, then click **Stutter Ratio & Distance** to display the Stutter Ratio & Distance view for the marker in the right pane.



10. Click **Apply**, then **OK** to add the Yfiler® Plus Kit panel, bin set, and marker stutter to the GeneMapper® *ID-X* Software database.

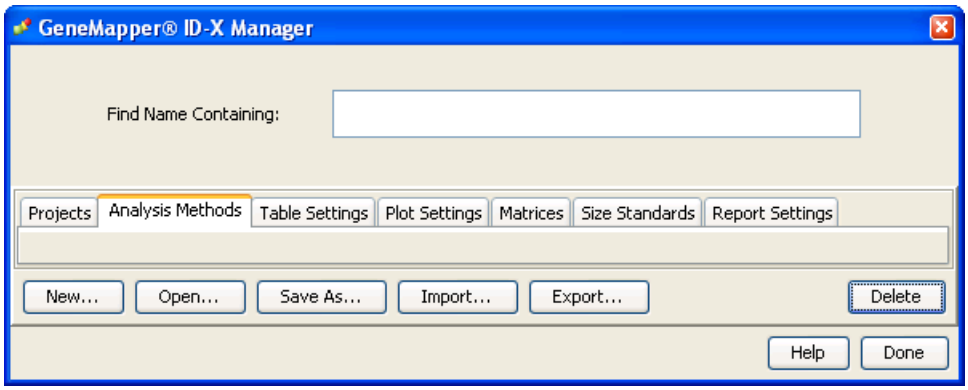
IMPORTANT! If you close the Panel Manager without clicking **Apply**, the panels, bin sets, and marker stutter will not be imported into the GeneMapper® *ID-X* Software database.

Create an analysis method

Use the following procedure to create an analysis method for the Yfiler® Plus Kit.

IMPORTANT! Analysis methods are version-specific, so you must create an analysis method for each version of the software. For example, an analysis method created for GeneMapper® *ID-X* version 1.2 is not compatible with earlier versions of GeneMapper® *ID-X* Software or with GeneMapper® *ID* Software version 3.2.1.

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



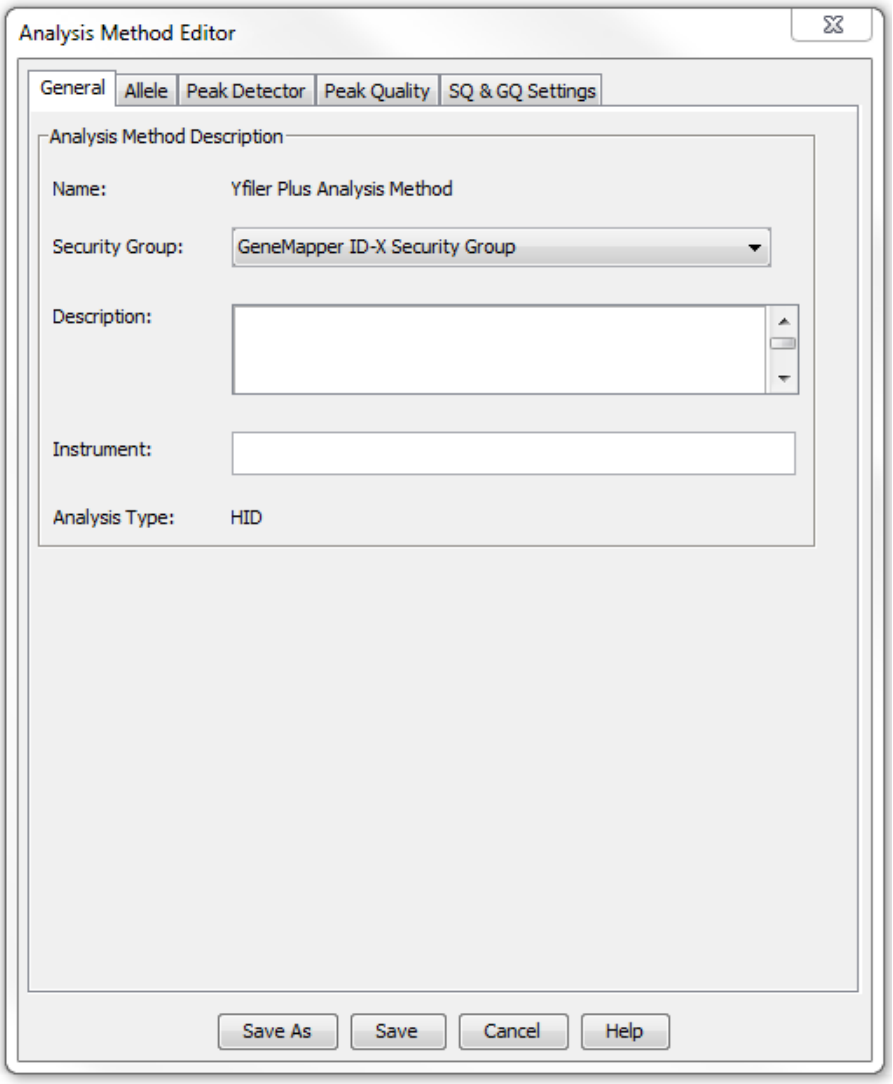
2. Select the **Analysis Methods** tab, then click **New** to open the Analysis Method Editor with the **General** tab selected.

   The figures below show the settings for each tab of the Analysis Method Editor. Configure the Analysis Method Editor tab settings as shown in the figures below, unless the instructions state otherwise.

   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.

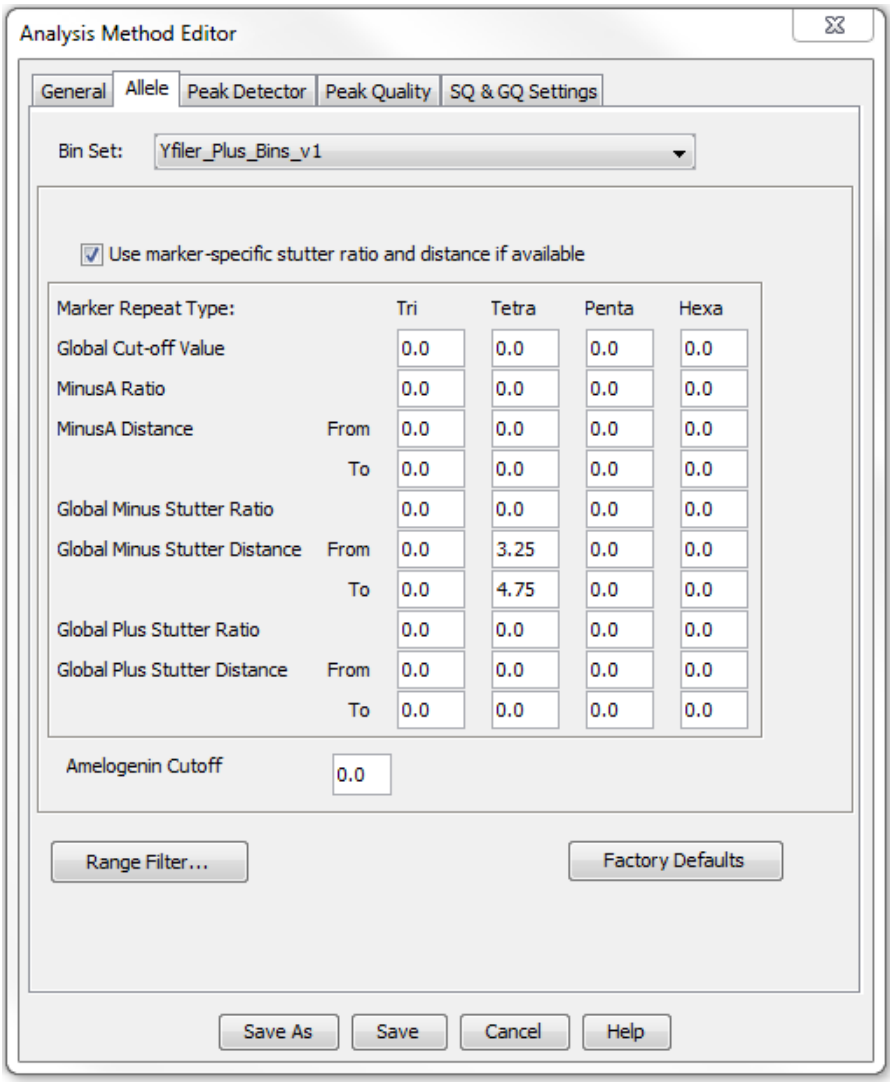
3. After you enter settings in all tabs, click **Save**.

General tab settings



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

Allele tab settings



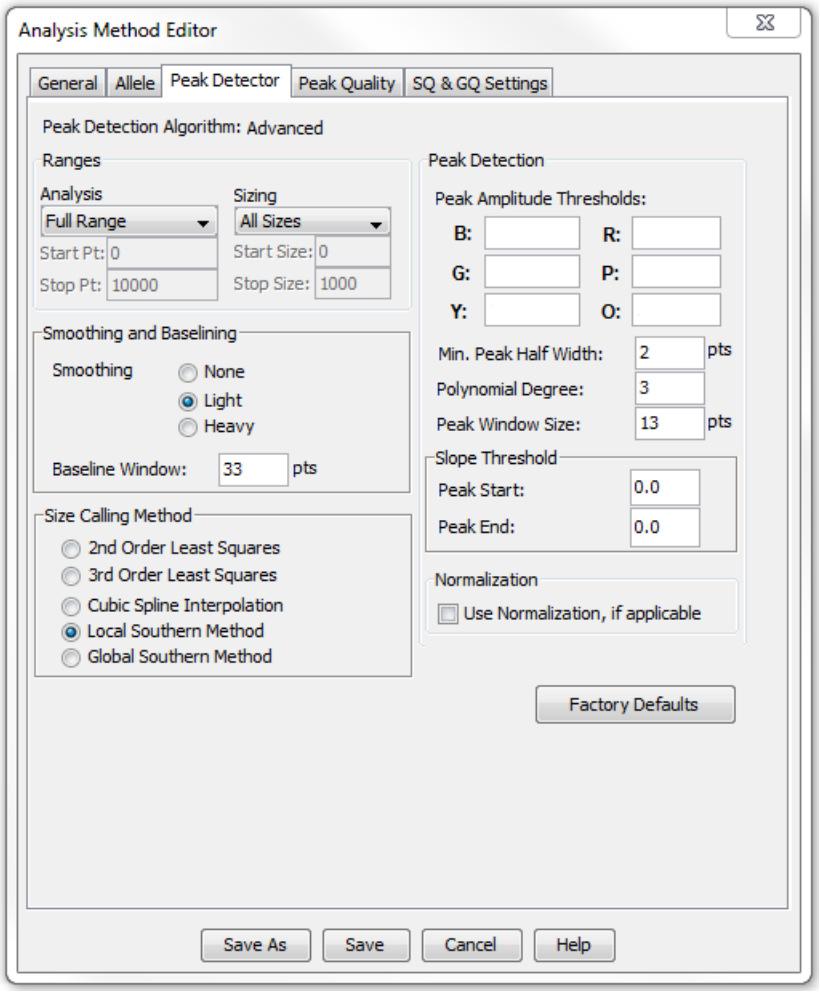
The settings shown in the screen shot above were used during the developmental validation of the Yfiler® Plus Kit. To specify settings:

- In the Bin Set field, select the **Yfiler_Plus_Bins_v1** bin set.
- GeneMapper® *ID-X* Software v1.0.1 or higher allows you to specify 4 types of marker repeat motifs: tri, tetra, penta, and hexa. You can enter parameter values for each type of repeat in the appropriate column.
- Specify the appropriate filter settings. To apply the stutter ratios contained in the Yfiler_Plus_Stutter_v1 file, select the "Use marker-specific stutter ratio if available" check box (selected by default).

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

Peak Detector tab settings

IMPORTANT! The Local Southern Sizing algorithm has been validated for analysis of Yfiler® Plus Kit data on 3130- and 3500-series instruments.

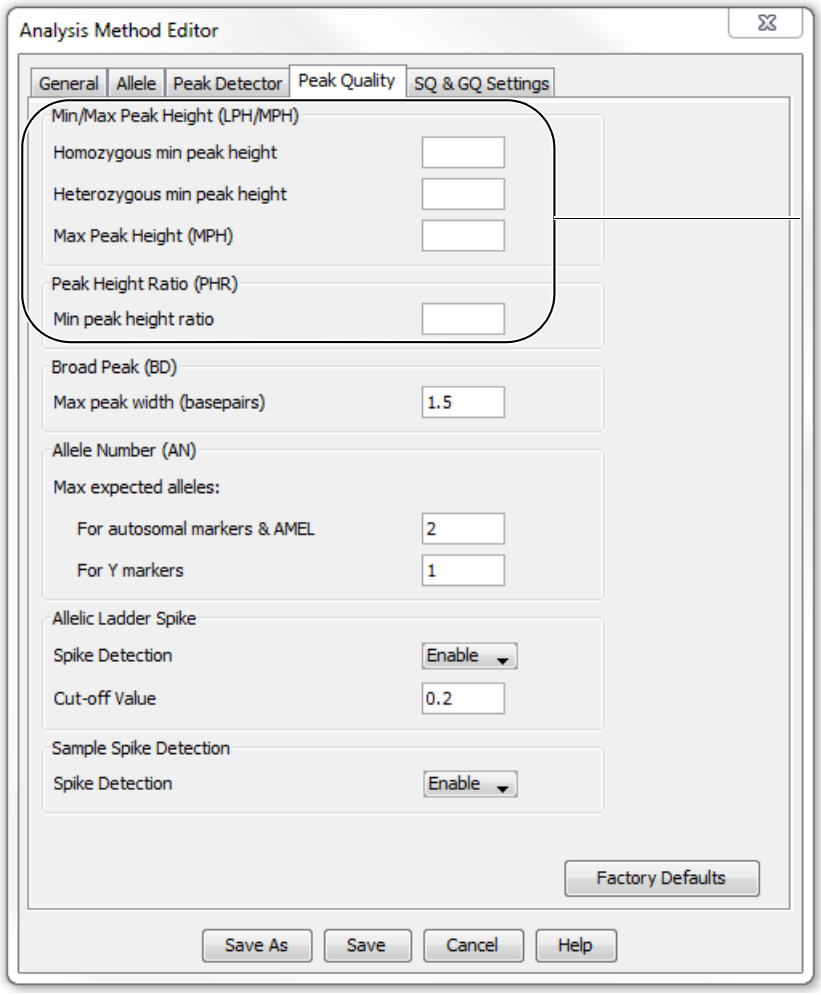


IMPORTANT! Perform the appropriate internal validation studies to determine the appropriate peak amplitude thresholds for interpretation of Yfiler® Plus Kit data.

Fields include:

- **Peak amplitude thresholds** – 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.
- **Size calling method** – The Yfiler® Plus Kit has been validated using the Local Southern sizing method (60–460 base pairs). Select alternative sizing methods only after performing the appropriate internal validation studies.
- **Normalization** – 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.

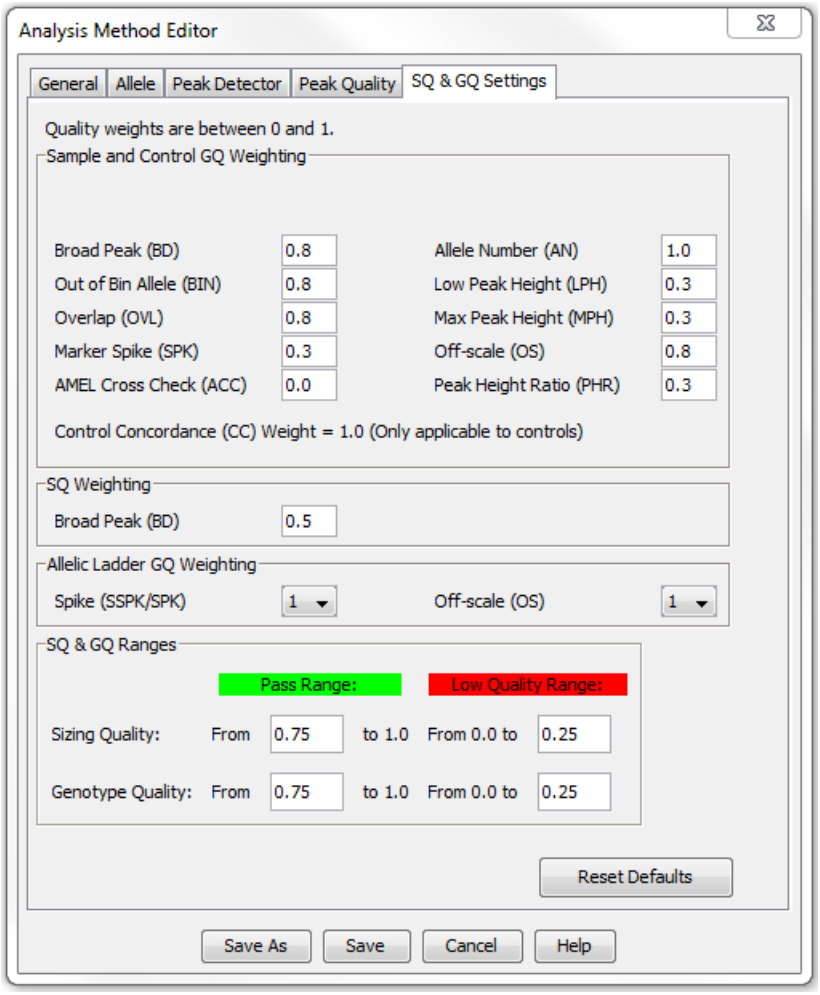
Peak Quality tab settings



Perform internal validation studies to determine 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 Yfiler® Plus Kit data.

SQ & GQ tab settings



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

Create a size standard

The GS600_LIZ_(60-460) size standard definition provided with GeneMapper® *ID-X* Software v1.4 and used with the Local Southern size-calling method contains the following peaks:

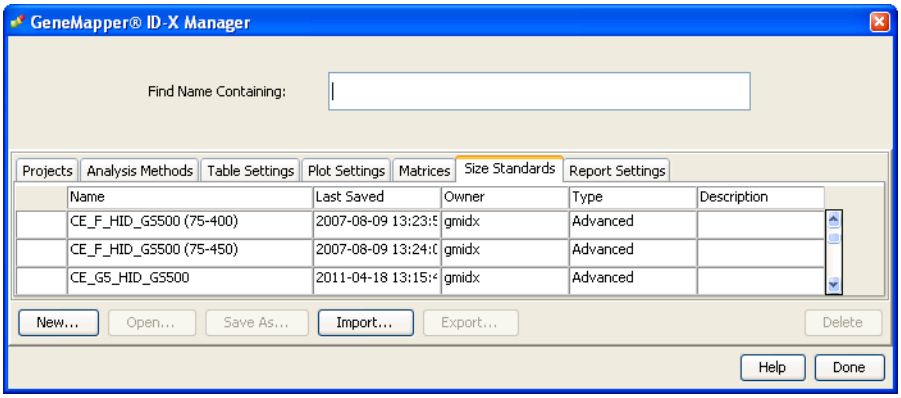
GeneScan™ 600 LIZ® Size Standard v2.0
60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, and 460

Note: This size standard definition has been validated for use with the Yfiler® Plus Kit on the Applied Biosystems® 3130/3130*xl* and 3500/3500xL instruments.

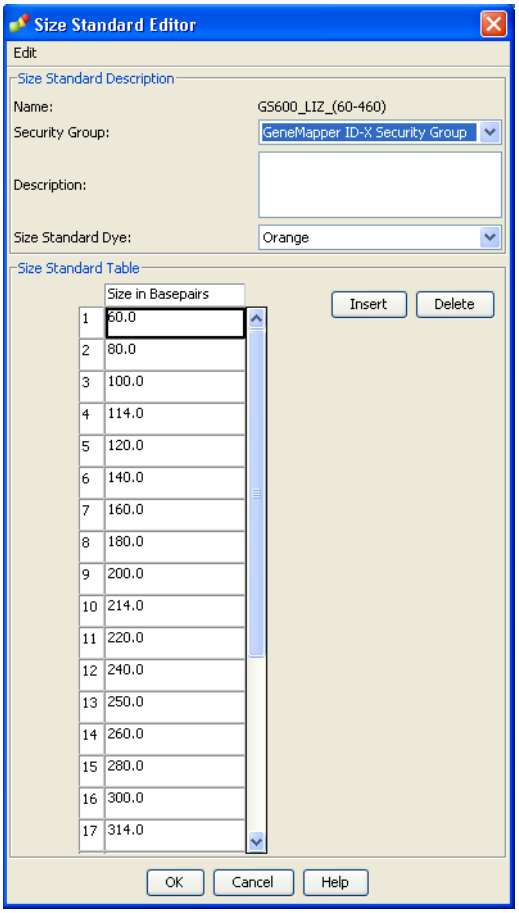
If you need to create your own size standard definition, use the following procedure to create the size standard definition file:

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

2. Select the **Size Standards** tab, then click **New**.



3. Complete the Name field as shown below or with a name of your choosing. In the Security Group field, select the Security Group appropriate to your software configuration from the drop-down list. In the Size Standard Dye field, select **Orange**. In the Size Standard Table, enter the sizes specified on page 57.



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 containing the sample files.
2. Apply analysis settings to the samples in the project.

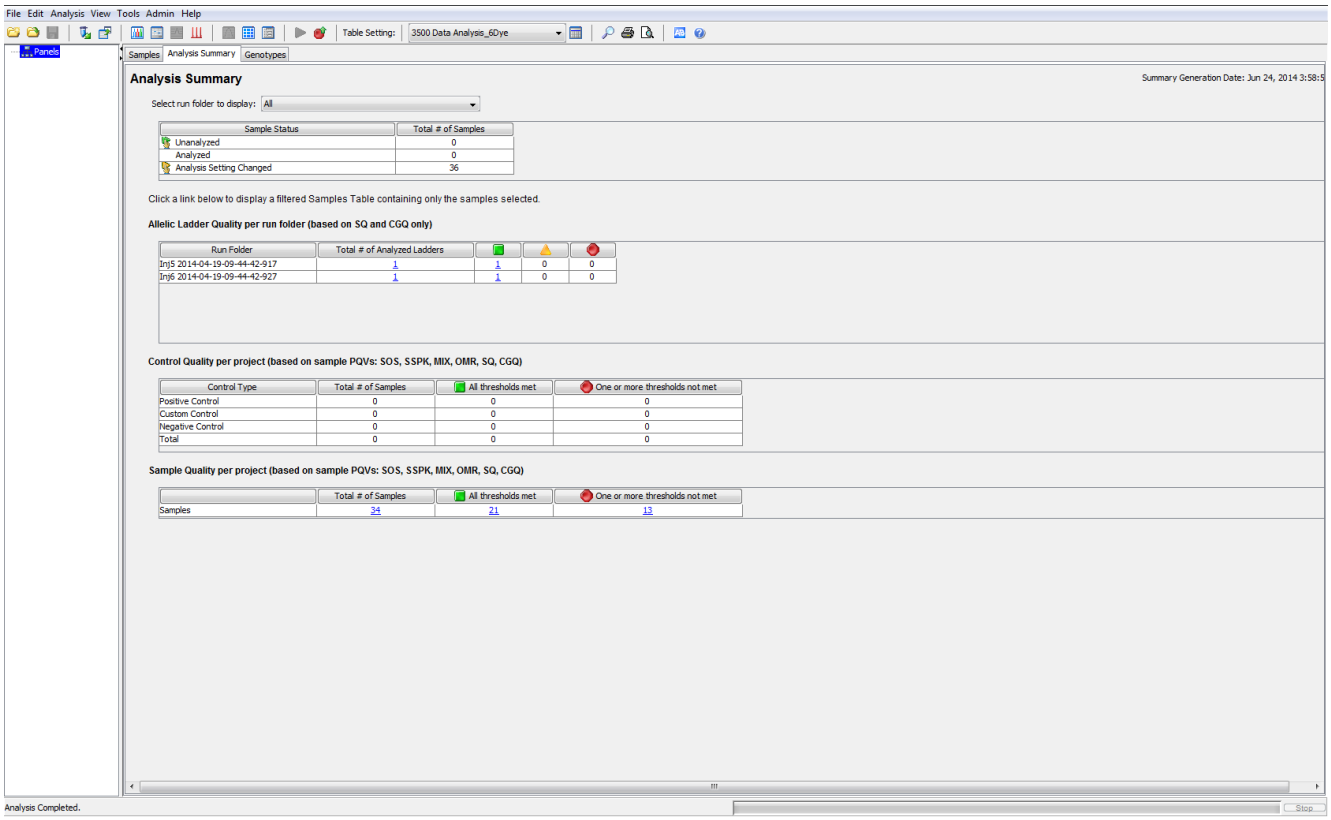
Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	Yfiler_Plus_AnalysisMethod_v1 (or the name of the analysis method you created)
Panel	Yfiler_Plus_Panels_v1 (or the version installed)
Size Standard	Use a size range of 60-460 bp for Local Southern size-calling method.†

† The Yfiler® Plus Kit was originally validated using 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 Kit.

Note: For more information about how the Size Caller works, refer to the *GeneScan™ Analysis Software for the Windows® NT Operating System Overview of the Analysis Parameters and Size Caller User Bulletin* (Pub. no. 4335617).

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 extending to the right with the percentage indicated.
   - 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 becomes available upon completion of the analysis.

   Analysis summary window after analysis



Examine and edit a project

You can display electropherogram plots from the Samples and Genotypes tabs of the Project window to examine the data. These procedures start with the Analysis Summary tab of the Project window (assuming the analysis is complete).

For more information

For more information, refer to:

- *GeneMapper® ID-X Software v1.4 New Features and Installation Procedures User Bulletin* (Pub. no. 4477684)
- *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574)
- *GeneMapper® ID-X Software Version 1.0 Quick Reference Guide* (Pub. no. 4375670)
- *GeneMapper® ID-X Software Version 1.0 Reference Guide* (Pub. no. 4375671)
- *GeneMapper® ID-X Software Version 1.1(Mixture Analysis) Getting Started Guide* (Pub. no. 4396773)
- *GeneMapper® ID-X Software Version 1.2 Reference Guide* (Pub. no. 4426481)
- *GeneMapper® ID-X Software Version 1.2 Quick Reference Guide* (Pub. no. 4426482)

5 Experiments and Results

Overview

This chapter provides results of the developmental validation experiments we performed using the Yfiler® Plus PCR Amplification Kit.

Importance of validation

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

Experiment conditions

We performed experiments to evaluate the performance of the Yfiler® Plus Kit 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).

This chapter will discuss many of the experiments we performed and examples of the results we obtained. We chose conditions that produced optimum PCR product yield and that met reproducible performance standards when using a peak amplitude threshold of 175 RFUs. 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.

Developmental validation

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 Yfiler® Plus Kit and established that the concentration of each component was within the range where data indicated that the amplification met the required performance criteria for specificity, sensitivity, and reproducibility. For example, 1 ng of DNA Control 007 was amplified in the presence of varying concentrations of magnesium chloride, and the results were analyzed on an Applied Biosystems® 3500xL Genetic Analyzer (Figure 4) The performance of the multiplex is most robust within ±20% of the optimal magnesium chloride concentration.

Figure 4 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 the 3500xL Genetic Analyzer. Y-axis scale is 0 to 13,000 RFU.

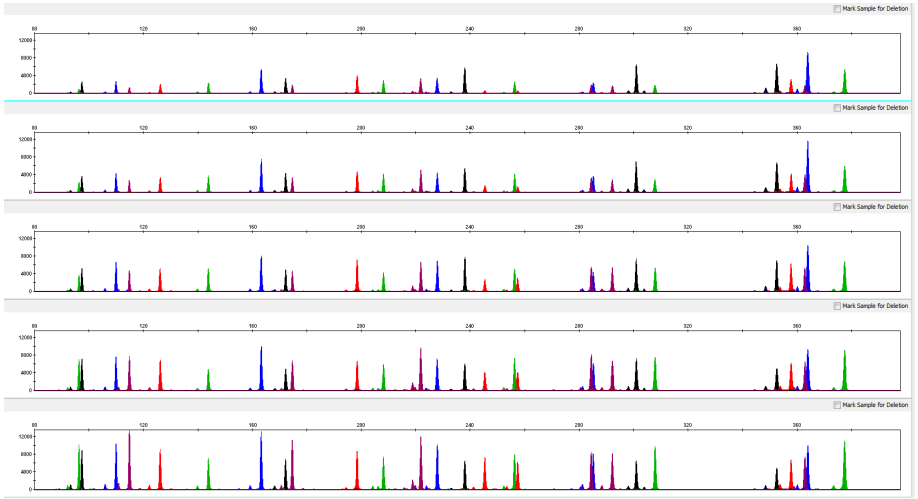


Figure 5 Electropherograms obtained from amplification of a blood sample on FTA® card amplified with the Yfiler® Plus Kit in the presence of varying concentrations of magnesium chloride and analyzed on an Applied Biosystems® 3500xL Genetic Analyzer (Y-axis scale 0 to 10,000 RFU).

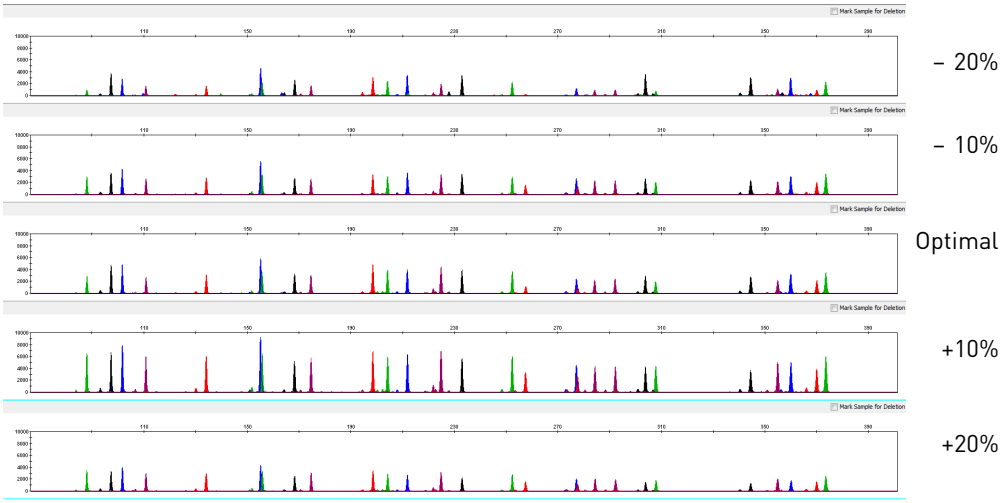
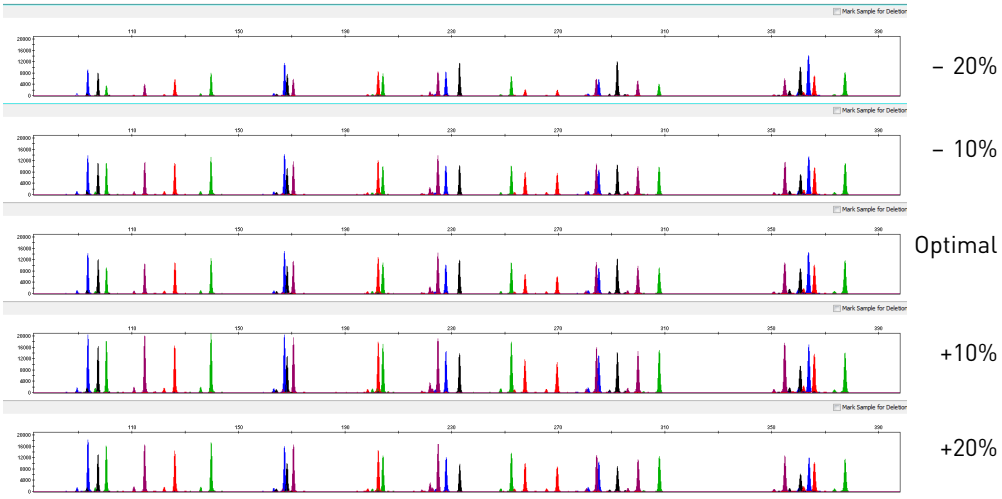


Figure 6 Electropherograms obtained from amplification of a buccal sample on FTA® card amplified with the Yfiler® Plus Kit in the presence of varying concentrations of magnesium chloride and analyzed on an Applied Biosystems® 3500xL Genetic Analyzer (Y-axis scale 0 to 28,000 RFU).



Thermal cycler parameters

Thermal cycling parameters were established for amplification of the Yfiler® Plus Kit in the Veriti® 96-Well Thermal Cycler. Thermal cycling times and temperatures of GeneAmp PCR systems were verified. Annealing and denaturation temperature windows were tested around each stipend to verify that a ±1.0°C window produced a specific PCR product with the desired specificity for male DNA.

The effects of denaturation and annealing temperatures on the amplification of Yfiler® Plus Kit loci were examined using the control DNA 007, male-female DNA mixtures, blood-FTA®, and buccal-FTA® samples.

The denaturation temperatures tested were 93, 94, and 95°C, all for 4-second hold times on the Veriti® 96-Well Thermal Cycler. The annealing temperatures tested were 60.5, 61, 61.5, 62, and 62.5°C (Figures 7, 8, and 9), for 1-minute hold times in the Veriti® 96-Well 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, 61.5, and 62°C produced robust profiles with no significant cross reactivity to 1 µg of female DNA. At 62.5°C, the yield of the majority of loci was significantly reduced. This should pose 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 7 Amplification of a mixture of 1 ng of male 007 DNA and 1 µg of female 9947 DNA at annealing temperatures of 60.5, 61, 61.5, 62, 62.5, and 63°C, analyzed on an Applied Biosystems® 3500xL Genetic Analyzer (Y-axis scale is 0 to 8,000 RFU).

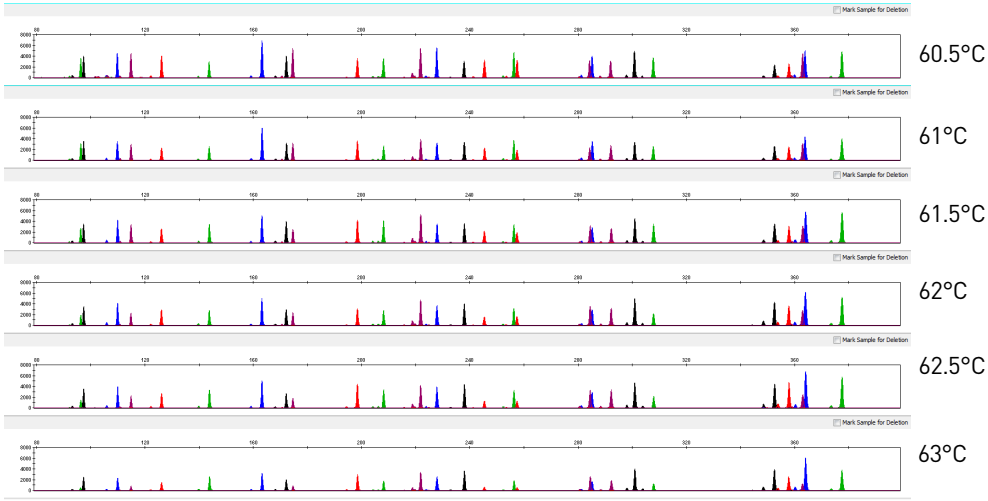


Figure 8 Electropherograms obtained from amplification of a blood sample on an FTA® card at annealing temperatures of 60.5, 61, 61.5, 62, and 62.5°C, analyzed on an Applied Biosystems® 3500xL Genetic Analyzer (Y-axis scale is 0 to 12,000 RFU).

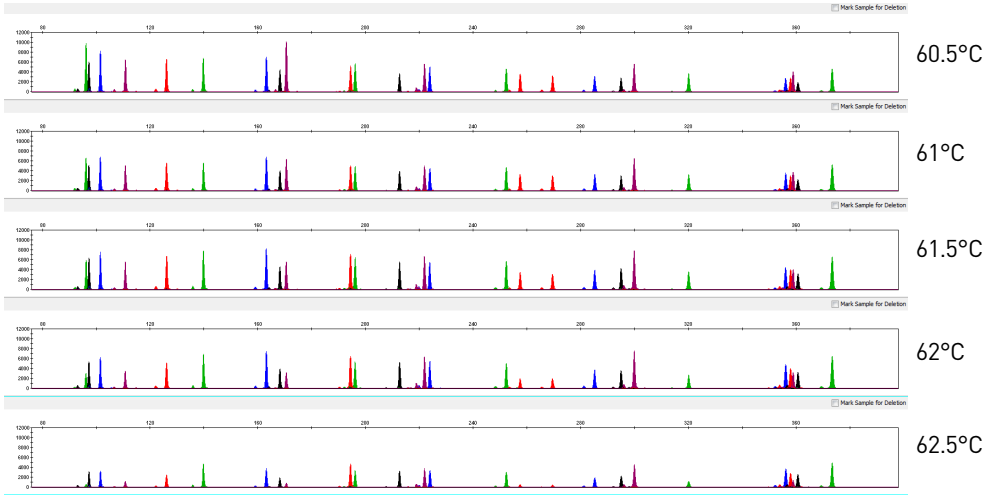
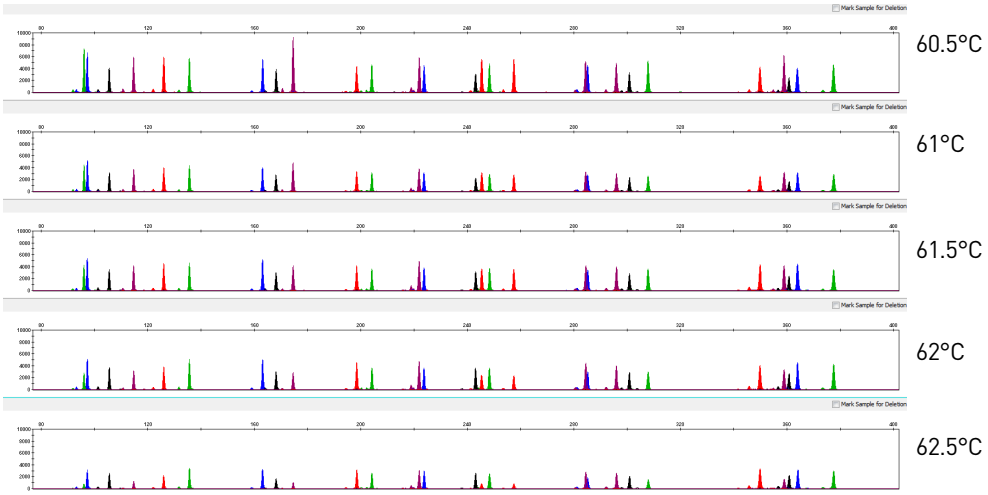


Figure 9 Electropherograms obtained from amplification of a buccal sample on an FTA® card at annealing temperatures of 60.5, 61, 61.5, 62, and 62.5°C, analyzed on an Applied Biosystems® 3500xL Genetic Analyzer (Y-axis scale is 0 to 10,000 RFU).



Accuracy, precision, and reproducibility

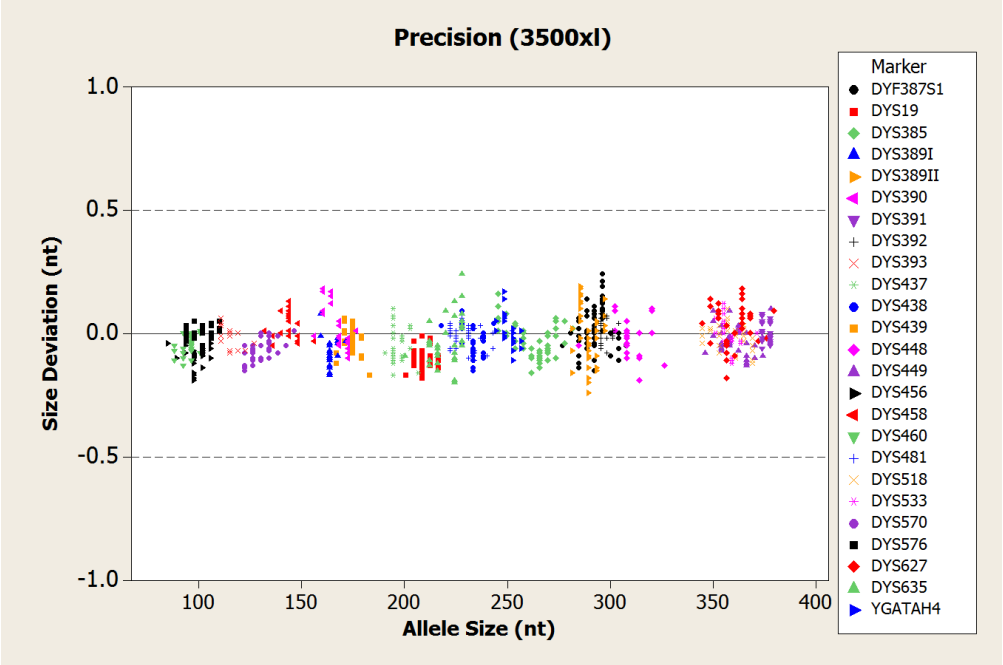
SWGDAM Guideline 3.5

"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

Laser-induced fluorescence detection of length polymorphism at short tandem repeat loci is not a novel methodology (Holt *et al.*, 2000; and Wallin *et al.*, 2002). However, accuracy and reproducibility of Yfiler® Plus Kit profiles have been determined from various sample types. Figure 10 shows the size differences that are typically observed between sample alleles and allelic ladder alleles on the Applied Biosystems® 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 10 Size deviation of 78 samples analyzed on the 3500xL Genetic Analyzer



Precision and size windows

Sizing precision allows for determination of accurate and reliable genotypes. Sizing precision was measured on the 3130*xl*, 3500, and 3500xL Genetic Analyzers. The recommended method for genotyping is to use a ±0.5-nt "window" around the size obtained for each allele in the Yfiler® Plus Allelic Ladder. A ±0.5-nt window allows for the detection and correct assignment of alleles. Any sample allele that sizes outside a window could be either of the following:

- An "off-ladder" allele, for example, an allele of a size that is not represented in the Yfiler® Plus Allelic Ladder
- An allele that does correspond to an allelic ladder allele, 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. Table 8 on page 93 indicates typical precision results obtained from the seven injections of the Yfiler® Plus Allelic Ladder analyzed on the 3130*xl*, 3500, and 3500xL Genetic Analyzers (36-cm capillary and POP-4® polymer). The size standard used was GeneScan™ 600 LIZ® Size Standard v2.0. The results were obtained within a set of injections on a single capillary array.

As indicated above, sample alleles may occasionally size outside of the ±0.5-nt window for a respective allelic ladder allele because of measurement error. The frequency of such an occurrence is lowest in detection systems having the smallest standard deviations in sizing. Table 8 on page 93 illustrates the tight clustering of allele sizes obtained on the 3500xL Genetic Analyzer, where the standard deviation in sizing is typically less than 0.15 nt. The instance of a sample allele sizing outside of the ±0.5-nt window because of measurement error is relatively rare when the standard deviation in sizing is approximately 0.15 nt or less (Smith, 1995).

For sample alleles that do not size within a ±0.5-nt window, the PCR product must be rerun to distinguish between a true off-ladder allele vs. measurement error of a sample allele that corresponds with an allele in the allelic ladder. Repeat analysis, when necessary, provides an added level of confidence to the final allele assignment. GeneMapper® *ID-X* Software automatically flags sample alleles that do not size within the prescribed window around an allelic ladder allele.

It is important to note that while the precision within a set of capillary injections is very good, the determined allele sizes vary between platforms. Cross-platform sizing differences arise from a number of parameters, including type and concentration of polymer mixture, run temperature, and electrophoresis conditions. Variations in sizing can also be found between runs on the same instrument and between runs on different instruments because of these parameters. We strongly recommend that the allele sizes obtained be compared to the sizes obtained for known alleles in the Yfiler® Plus Allelic Ladder from the same run and then converted to genotypes. For more information on precision and genotyping, see Lazaruk *et al.*, 1998 and Mansfield *et al.*,1998.

Extra Peaks in the electropherogram

Causes of extra peaks

Peaks other than the target alleles may be detected on the electropherogram displays. Several causes for the appearance of extra peaks, including the stutter product (at the n–4 position), incomplete 3′ A nucleotide addition (at the n–1 position), artifacts, and mixed DNA samples (see "SWGDAM Guideline 3.8" on page 87).

Stutter products

Stutter is a well-characterized PCR artifact that refers to the appearance of a minor peak one repeat unit smaller (or less frequently, one repeat larger) than the major STR product (Butler, 2005; Mulero *et al.*, 2006). Sequence analysis of stutter products at tetranucleotide STR loci has revealed that the stutter product is missing a single tetranucleotide core repeat unit relative to the main allele (Walsh *et al.*, 1996).

Most STR loci produce minus-stutter peaks as a by-product of PCR amplification. A process of "slippage" has been proposed as a molecular mechanism for stutter, where the Taq DNA polymerase enzyme "slips" on the template DNA during replication and produces a minority PCR product that is shorter than the template strand, usually by one repeat unit. The stutter process may also occur in the opposite direction to produce amplicon DNA that is usually one repeat unit longer than the template strand, termed plus-stutter. While plus-stutter is normally much less significant (<5%) than minus-stutter in STR loci with tetranucleotide repeats, the incidence of plus-stutter is more significant in the trinucleotide repeat-containing loci DYS481 and DYS392 as shown in Table 3 on page 71.

GeneMapper® *ID-X* analysis files supplied for use with theYfiler® Plus Kit contain plus-stutter filters for several markers to prevent these peaks from being called in normal profiles. To obtain a Technical Note regarding plus stutter in STR chemistries, see your local Life Technologies HID support representative.

A non-standard (minus 2-nt) stutter has been observed in certain Y-STR loci that include more complex nucleotide sequences including regions of dinucleotide repeats as shown in Figure 18 on page 77. In cases where these stutter peaks exceed the peak amplitude threshold (e.g., 175 RFU), they may be detected by analysis software as additional alleles in the profile. GeneMapper® *ID-X* analysis files supplied for use with the Yfiler® Plus Kit contain a minus 2-nt stutter filter for DYS19, DYS481, DYS533 and DYS627 to prevent these peaks from being called in normal profiles.

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. Such measurements have been made at Life Technologies for amplified samples at the loci used in the Yfiler® Plus Kit. All data were generated on the 3500xL Genetic Analyzer.

The stutter measurements were derived from DNA extracted from blood samples acquired from the Interstate Blood Bank (Memphis, Tennessee) and Boca Biolistics (Coconut Creek, Florida). The samples were collected in the United States (with no geographical preference) from randomly-selected individuals of known ethnicities.

Some of the general conclusions from these measurements and observations are as follows:

- For each Yfiler® Plus Kit locus, the stutter percent generally increases with allele length, as shown in Figure 11 through Figure 17 on the following pages. Smaller alleles display a lower level of stutter relative to the longer alleles within each locus. Alleles at the low/high extreme end of the range have minimal data points due to poor representation in the Life Technologies data set. Some of these alleles were not represented at all.
- Each allele within a locus displays percent stutter that is reproducible.
- Stutter filter sets in GeneMapper® *ID-X* Software, calculated as the mean stutter for the locus plus three standard deviations, are shown in Table 3. Peaks in the stutter position that are above the stutter filter percentage specified inthe software are not filtered. Peaks in the stutter position that have not been filtered and remain labeled can be further evaluated. For evaluation of mixed samples, see "Mixture studies" on page 87.
- The measurement of percent stutter for alleles that are off-scale may be unusually high due to artificial truncation of the main allele peak.

Table 3 Marker-specific stutter filter percentages for Yfiler® Plus Kit loci

Locus designation	% Minus stutter†	% Plus stutter†	%Minus-2 /%Plus-2 nt stutter†
DYS576	15.15	3.38	‡
DYS389I	9.16	3.45	‡
DYS635	13.38	3.3	‡
DYS389II	18.79	3.73	‡
DYS627	15.18	2.62	2.71
DYS460	11.65	4.27	‡
DYS458	15.31	2.52	‡
DYS19	12.68	3.72	10.1/3.42
YGATAH4	11.53	2.27	‡
DYS448	4.68	2.29	‡
DYS391	9.99	3.41	‡
DYS456	15.36	3.74	‡
DYS390	13.58	3.51	‡
DYS438	5.86	2.76	‡
DYS392	16.94	11	‡
DYS518	25.5	4.85	‡
DYS570	15.65	2.88	‡
DYS437	8.13	1.65	‡
DYS385	18.32	3.7	‡
DYS449	23.24	4.2	‡
DYS393	14.07	4.95	‡
DYS439	9.89	3.39	‡
DYS481	28.55	5.59	9.55
DYF387S1	15.71	NA	‡
DYS533	12	4.6	1.88

† The stutter filters are displayed as percentages in the GeneMapper ID-X Yfiler_Plus_Stutter.txt file.
‡ Undetermined

IMPORTANT! The values in Table 3 were determined by Life Technologies during the developmental validation studies. We recommend that laboratories perform their own internal validation studies to determine the appropriate values to use.

Figure 11 Minus stutter percentages for the DYF387S1, DYS19, and DYS385 loci. (Blue, green, black, red, and purple colors indicate loci labeled with 6-FAM™, VIC™, NED™, TAZ™, and SID™ dyes, respectively.)

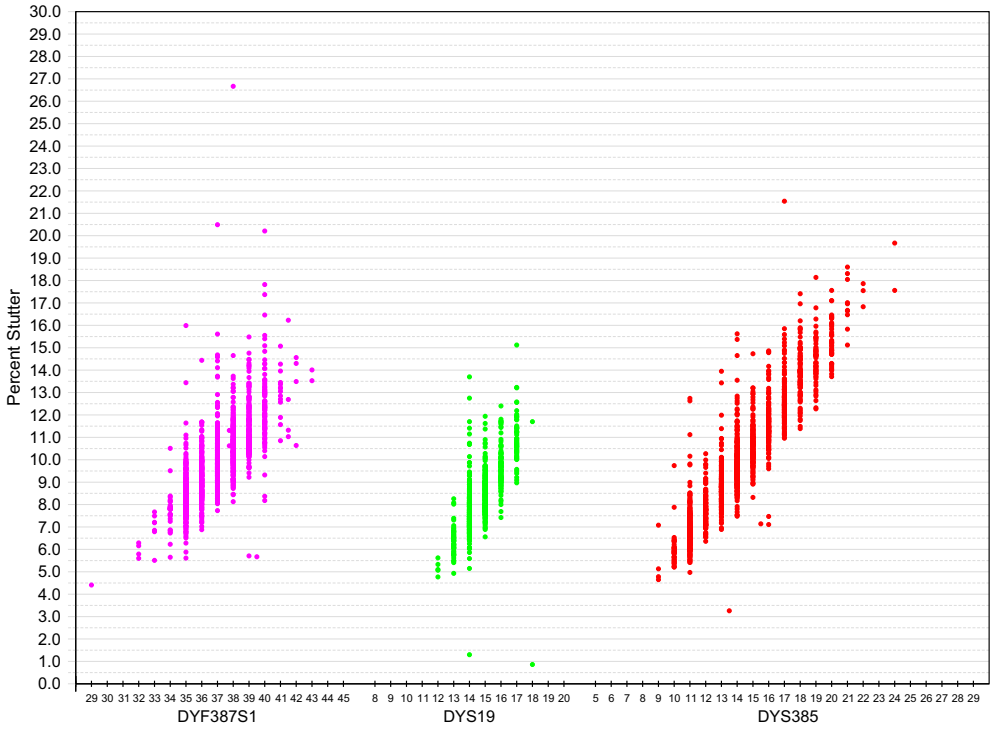


Figure 12 Minus stutter percentages for the DYS389I, DYS389II, DYS390, and DYS391 loci. (Blue, green, black, red, and purple colors indicate loci labeled with 6-FAM™, VIC™, NED™, TAZ™, and SID™ dyes, respectively.)

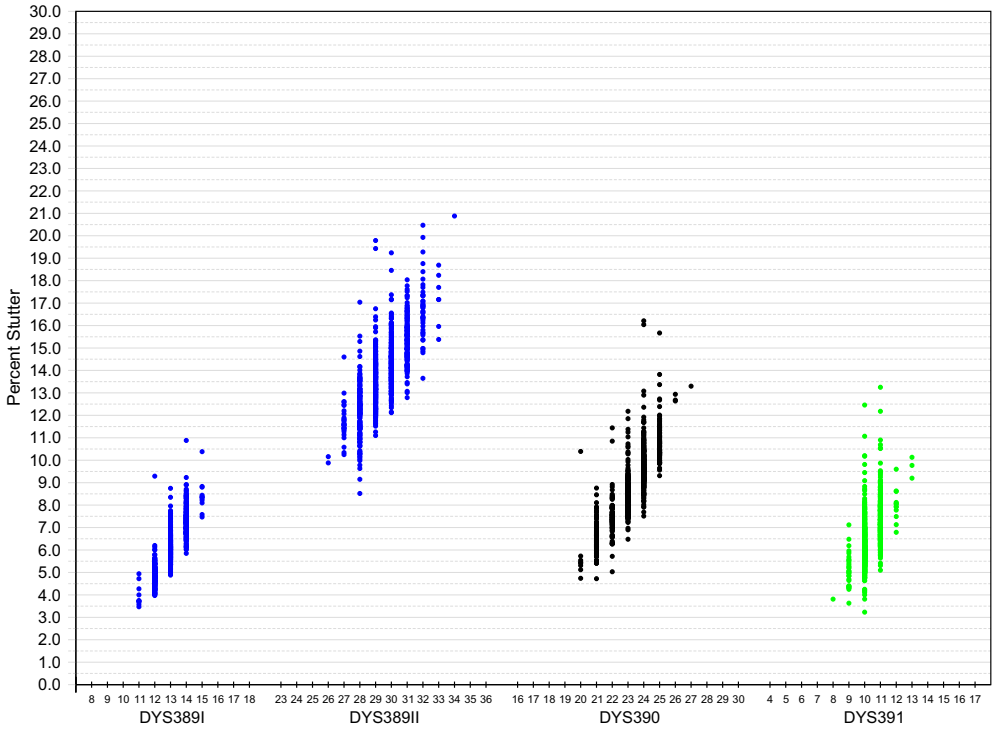


Figure 13 Minus stutter percentages for the DYS392, DYS393, DYS437, and DYS438 loci. (Blue, green, black, red, and purple colors indicate loci labeled with 6-FAM™, VIC™, NED™, TAZ™, and SID™ dyes, respectively.)

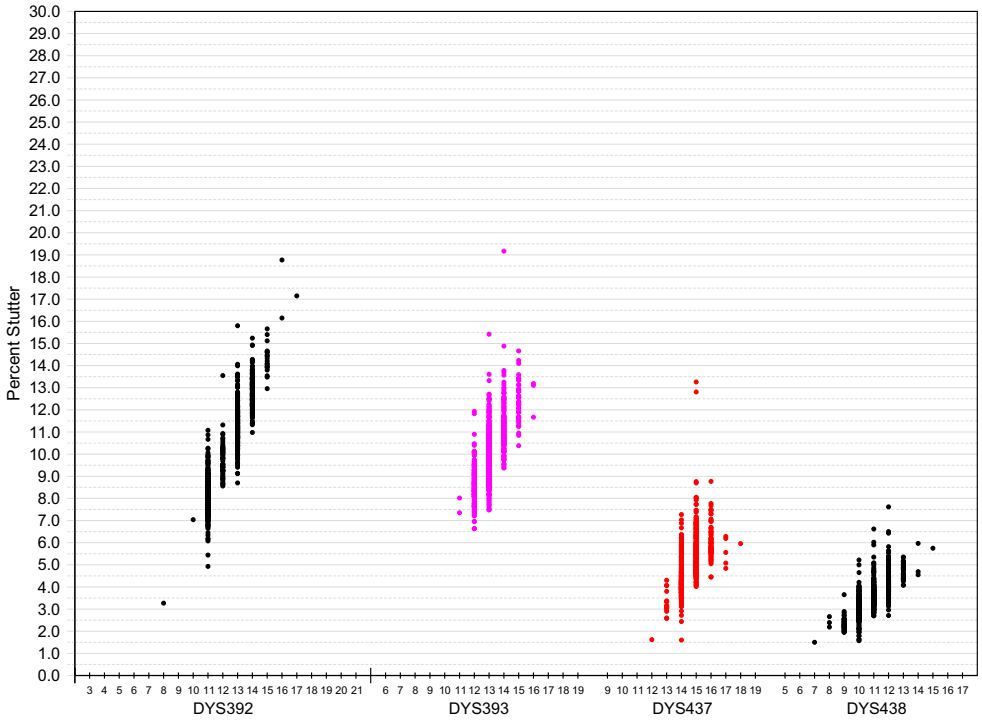


Figure 14 Minus stutter percentages for the DYS439, DYS448, DYS449, and DYS456 loci. (Blue, green, black, red, and purple colors indicate loci labeled with 6-FAM™, VIC™, NED™, TAZ™, and SID™ dyes, respectively.)

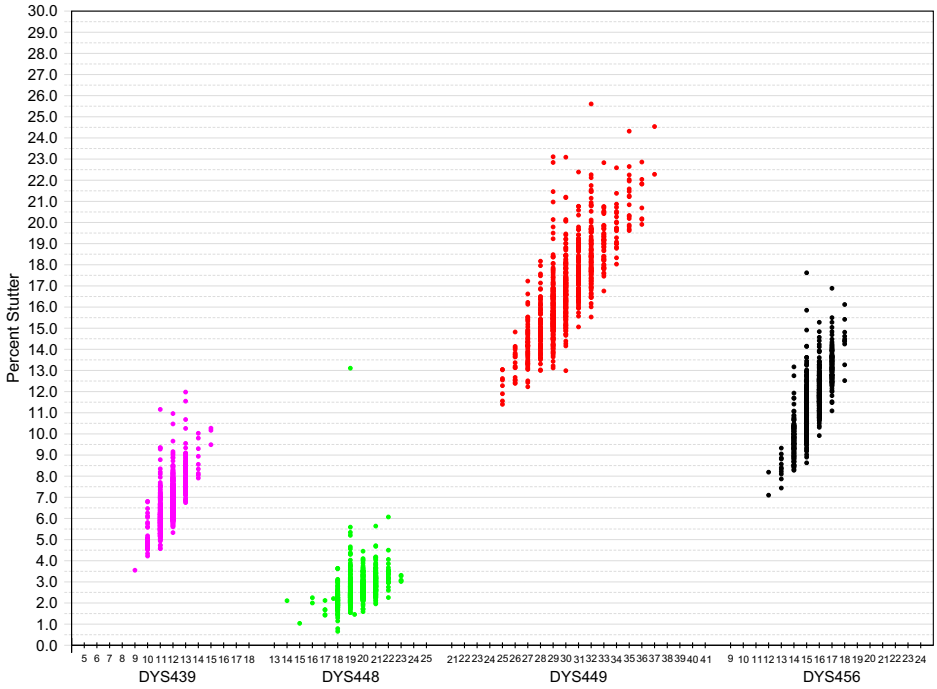


Figure 15 Minus stutter percentages for the DYS458, DYS460, DYS481, and DYS518 loci. (Blue, green, black, red, and purple colors indicate loci labeled with 6-FAM™, VIC™, NED™, TAZ™, and SID™ dyes, respectively.)

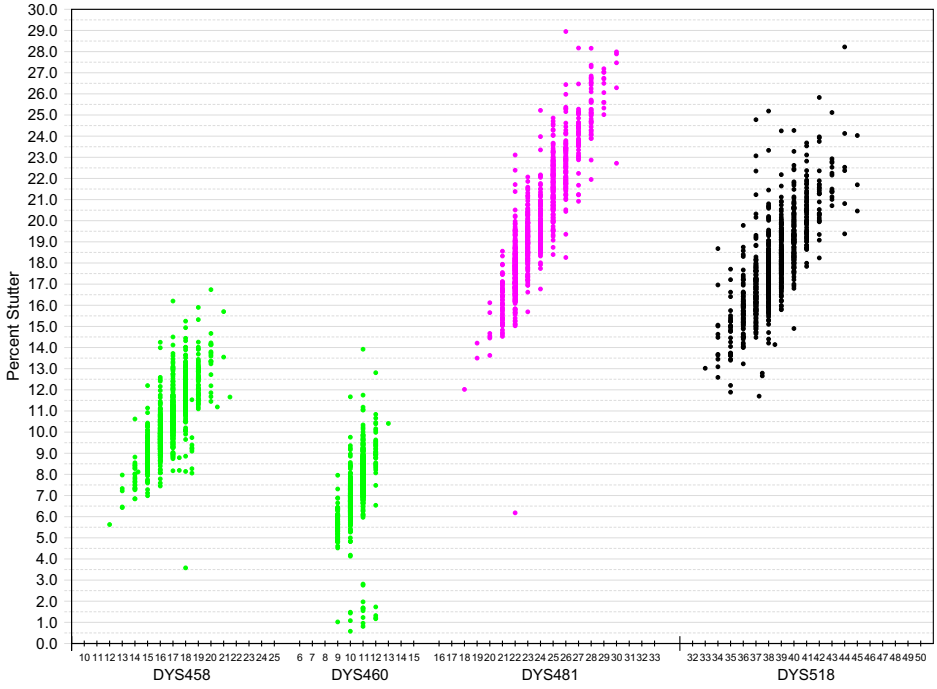


Figure 16 Minus stutter percentages for the DYS533, DYS570, and DYS576 loci. (Blue, green, black, red, and purple colors indicate loci labeled with 6-FAM™, VIC™, NED™, TAZ™, and SID™ dyes, respectively.)

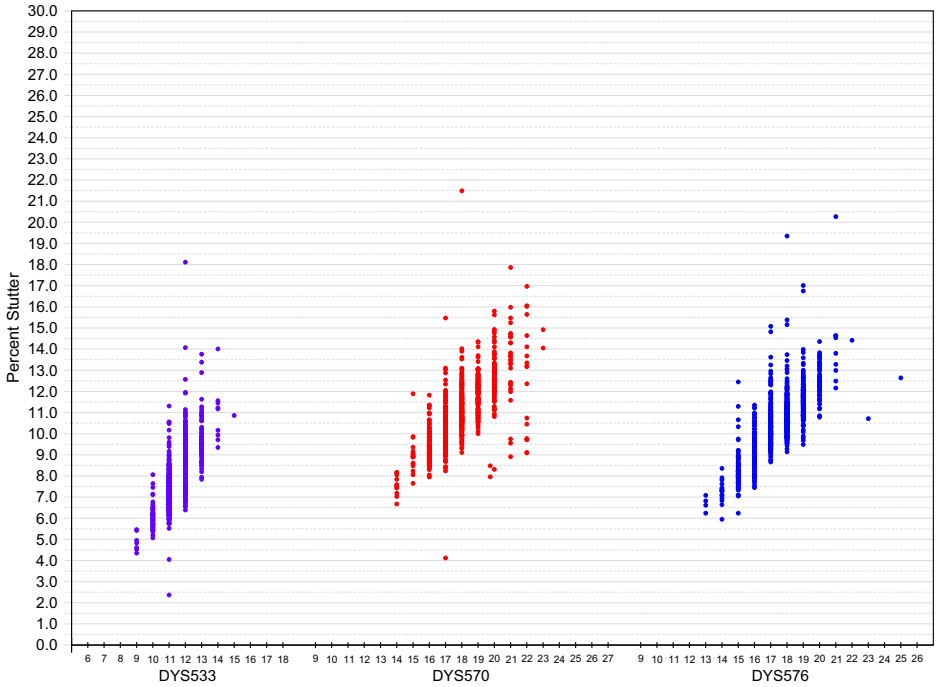


Figure 17 Minus stutter percentages for the DYS627, DYS635, and YGATAH4 loci. (Blue, green, black, red, and purple colors indicate loci labeled with 6-FAM™, VIC™, NED™, TAZ™, and SID™ dyes, respectively.)

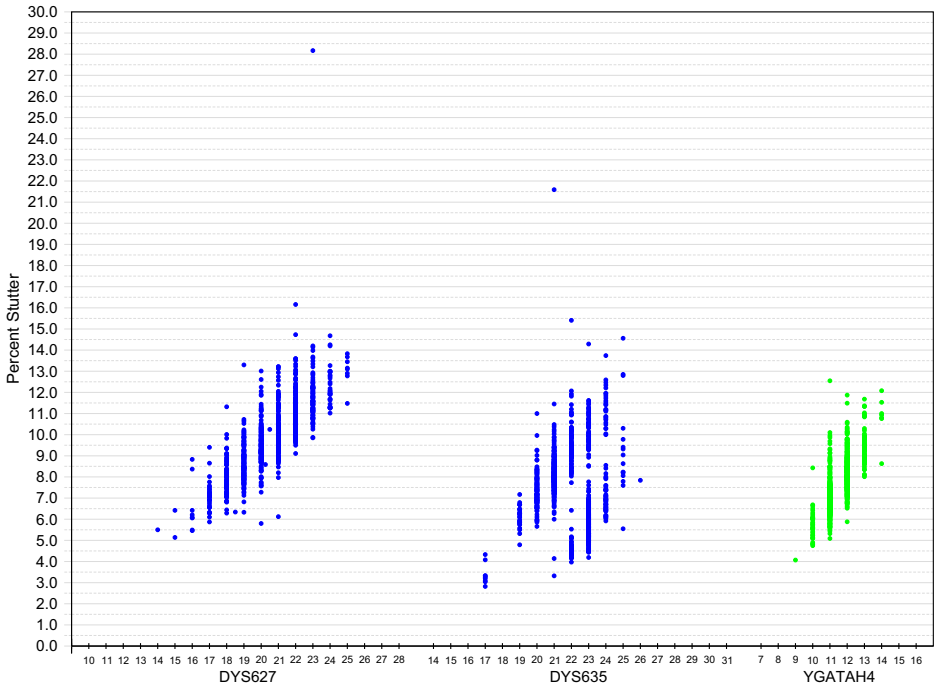
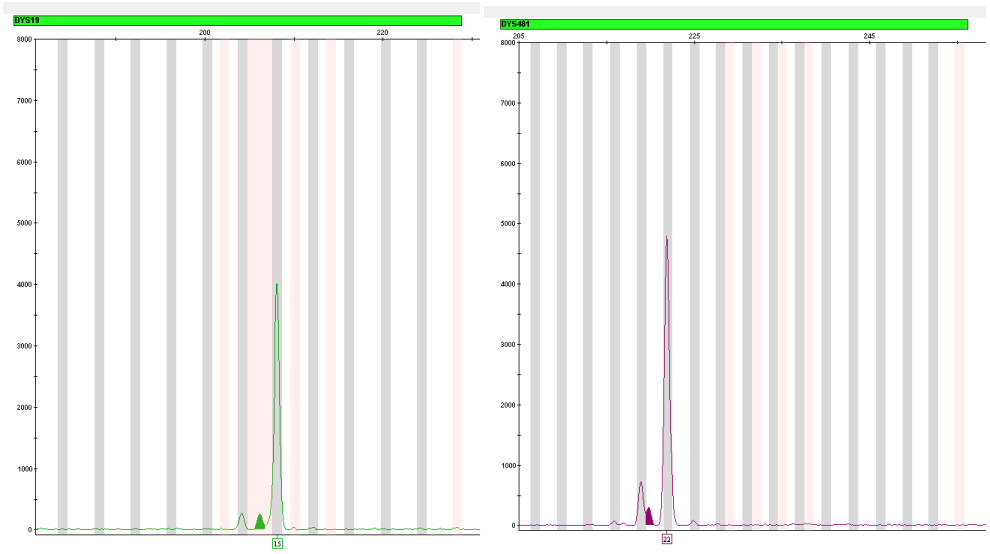


Figure 18 Example of reproducible 2-nt stutters in the DYS19 (left) and DYS481 (right) loci.



Addition of 3´A

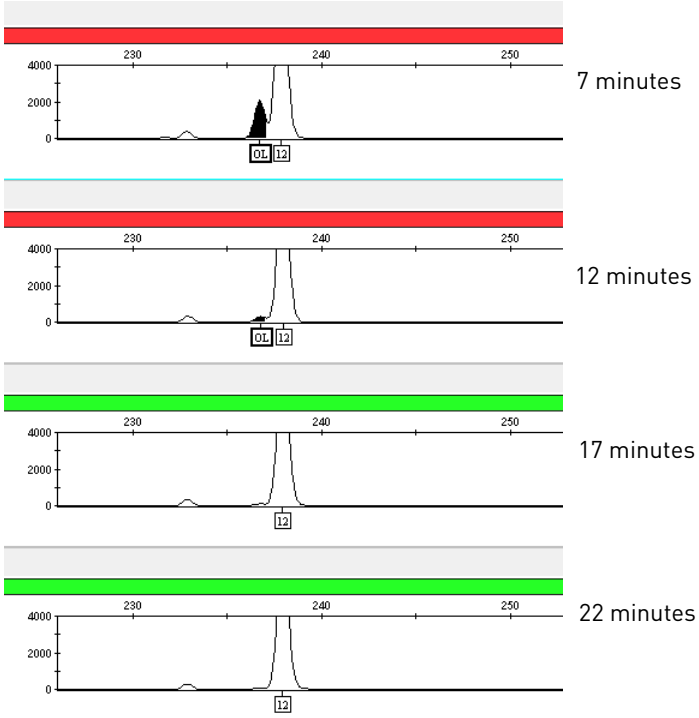
Many DNA polymerases can catalyze the addition of a single nucleotide (predominately adenosine) to the 3´ ends of double-stranded PCR products (Clark, 1988; Magnuson *et al.*,1996). This non-template addition results in a PCR product that is one base pair longer than the actual target sequence, and the PCR product with the extra nucleotide is referred to as the "+A" form (Figure 19).

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 Kit includes two main design features that promote maximum A addition:

- The primer sequences have been optimized to encourage A addition.
- The final extension step is 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. See Figure 19 for an example of incomplete and normal +A addition. Final extension incubation for longer than the recommended 22 minutes may result in double +A addition, in which two non-template 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 19 Time course of 7, 12, 17 and 22 minutes during the final extension step. A mixture of 1 ng of male DNA and 1 ug of female DNA was amplified with increasing final extension times resulting in complete +A addition at the DYS438 locus and the disappearance of the OL (-A) shoulder peak.



Lack of full A nucleotide addition may be observed in Yfiler® Plus Kit results when the amount of input DNA is greater than recommended protocols. This is because more time is needed for the DNA Polymerase to add the A nucleotide to all molecules as more PCR product is generated. Amplification of too much input DNA will also result in off-scale data.

About artifacts

Artifacts and anomalies are seen in all molecular biological systems. Artifacts are typically reproducible while anomalies are non-reproducible, intermittent occurrences that are not observed consistently in a system (for example, spikes and baseline noise). Due to improvements in PCR primer manufacturing processes, the incidence of artifacts has been greatly reduced in the Yfiler® Plus PCR Amplification Kit. Kit electropherograms are essentially free of reproducible dye artifacts within the kit's read region for commonly used analytical thresholds.

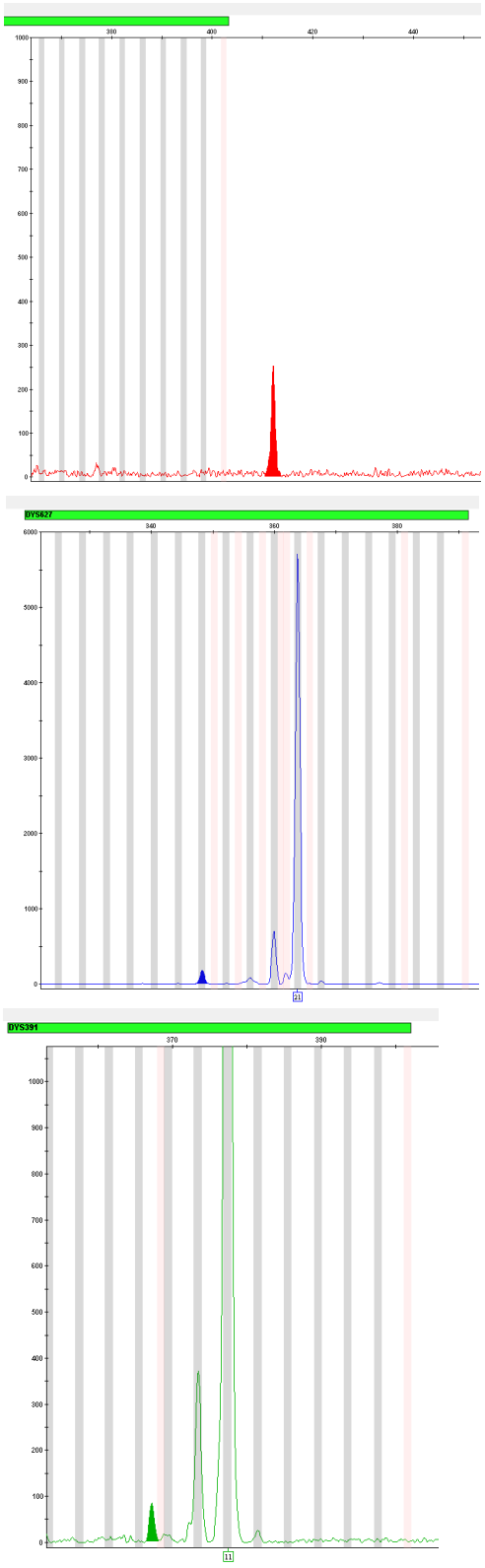
Other DNA-dependent artifacts

Additional reproducible DNA-dependent artifacts have been characterized and documented on Table 4. It is important to consider possible noise and artifacts when interpreting data from the Yfiler® Plus Kit on the Applied Biosystems® 3500/3500xL and 3130/3130*xl* Genetic Analyzers.

Table 4 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.
Y391 (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.

Figure 20 Examples of reproducible artifacts in data produced on the Applied Biosystems 3500/3500xL. The top panel is TAZ412, the middle panel is FAM348, and the bottom panel is Y391 (n-10)



Characterization of loci

SWGDAM Guideline 3.1

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

This section describes basic characteristics of the 27 loci that are amplified with the Yfiler® Plus Kit. These loci have been extensively characterized by other laboratories (Gusmao *et al*., 1999; Butler *et al*., 2002; Gonzalez-Neira *et al*., 2001; Hall and Ballantyne, 2003; Redd *et al*., 2002; Schoske *et al*., 2004; Ballantyne *et al*., 2012; Ballantyne *et al*., 2014).

Nature of the 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 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 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 Yfiler® Plus Kit 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 39 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).

Three CEPH family DNA sets were examined. 1 ng of DNA from each sample was amplified using the Yfiler® Plus Kit and the Identifiler® Kit, followed by analysis using a 3500xL Genetic analyzer. The families examined included #1333 (9 offspring, 7 males), #1340 (7 offspring, 5 males), and #1345 (7 offspring, 5 males), representing 23 meiotic divisions. The Identifiler® Kit results confirmed that the loci are inherited according to Mendelian rules, as reported in the literature (Nakahori *et al*.,1991; Edwards *et al*.,1992; Kimpton *et al*.,1992; Mills *et al*.,1992; Sharma and Litt, 1992; Li *et al*.,1993; Straub *et al*.,1993). The Yfiler® Plus Kit results confirmed that the loci were inherited according to a Y-linked (father to son) transmission. In no case was the maternal grandfather's Y-haplotype found in the offspring. Calculation of a mutation rate based on this small population size would be inaccurate due to the small sample size. The samples were reamplified and reinjected to confirm the allele call.

Mapping

The Yfiler® Plus Kit loci have been mapped and the chromosomal 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

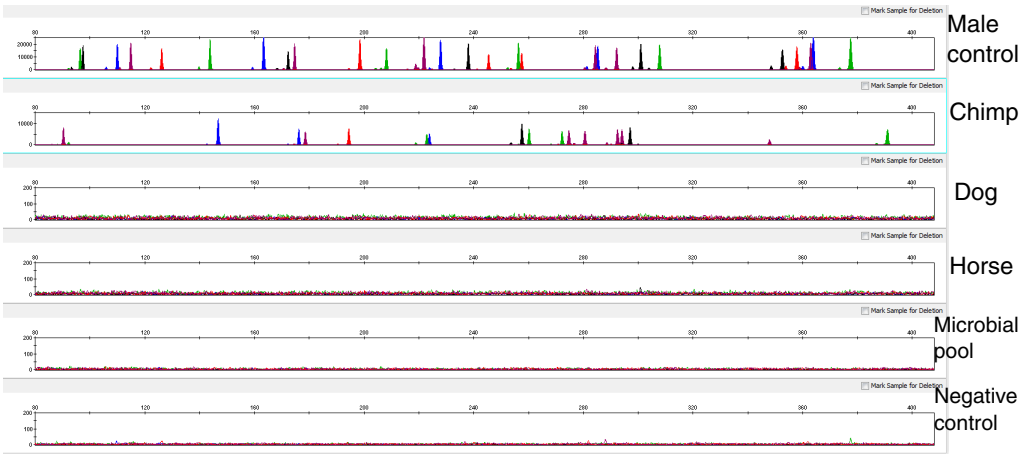
"The ability to detect genetic information from non-targeted species (e.g., detection of microbial DNA in a human assay) should be determined." (SWGDAM, December 2012).

The Yfiler® Plus Kit provides the required degree of specificity such that it is specific to primates. Other species do not amplify for the loci tested.

Nonhuman Studies

Nonhuman DNA may be present in forensic casework samples. The Yfiler® Plus Kit provides the required degree of specificity for the species tested (Figure 21 on page 82).

Figure 21 Representative electropherograms from a species specificity study including positive and negative control



The following experiments were conducted to investigate interpretation of Yfiler® Plus Kit results from nonhuman DNA sources.

The extracted DNA samples were amplified in Yfiler® Plus Kit reactions and analyzed using the 3100 Genetic Analyzer.

- **Primates** – Gorilla, chimpanzee, and macaque (1.0 ng each).
- **Non-primates** – Mouse, dog, pig, rat, sheep, horse, chicken and cow (10 ng each).
- **Microorganisms** – *Candida albicans*, *Neisseria gonorrhoeae*, *Escherichia coli* 0157:H7, *Bacillus subtilis*, *Staphylococcus aureus*, and *Lactobacillus rhamnosus* (5 ng each).

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

The remaining species tested did not yield reproducible detectable products.

Sensitivity

SWGDAM Guideline 3.3

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

Effect of DNA quantity on results and importance of quantitation

In a casework workflow, the optimal amount of input male DNA added to the Yfiler® Plus Kit should be between 0.5 and 1.0 ng for 30 cycles of amplification (Figure 22 on page 84). The DNA sample should be quantitated prior to amplification using a system such as the Quantifiler® HP (Human Plus) DNA Quantification Kit (Part no. 4482911) or the Quantifiler® Trio DNA Quantification Kit (Part no. 4482910). The final DNA concentration should be in the range of 0.05–0.10 ng/µL so that 0.5–1.0 ng of male DNA will be added to the PCR reaction in a volume of 10 µL. If the sample contains degraded DNA, amplification of additional DNA may be beneficial.

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

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

  Off-scale data is a problem for two reasons:

  – 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, which results in poor spectral separation ("pull-up").

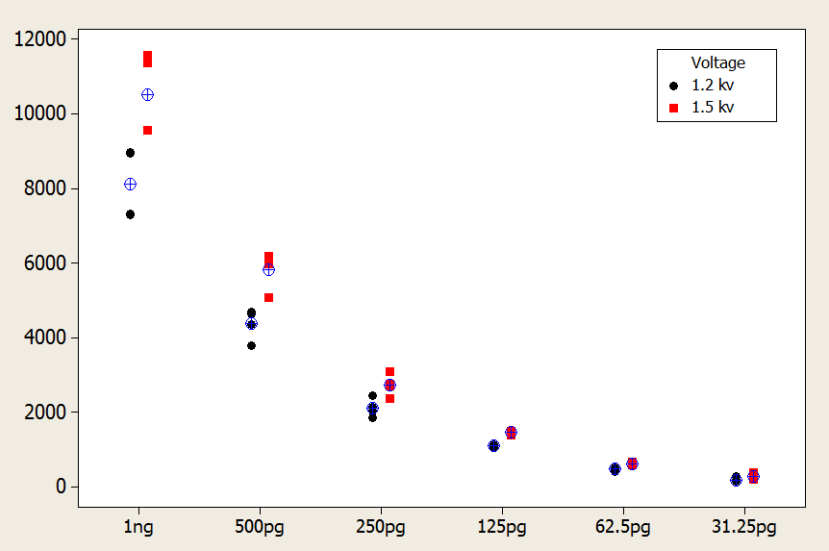
- Incomplete A nucleotide addition.

The sample can be re-amplified using less DNA.

Individual laboratories may find it useful to determine an appropriate minimum peak height threshold based on their own results and instruments using low amounts of input DNA. This kit was developed using two injection conditions (3130*xl*; 3 kV/10 sec and 3 kV/13 sec, 3500; 1.2 kV/16 sec and 1.5 kV/16 sec, 3500xL; 1.2 kV/24 sec and 1.5 kV/24 sec). You are encouraged to explore both options during validation to determine which protocol provides the best results on your instrumentation. The enhanced injection conditions resulted on average improvements in peak height of 25% (Figure 22 on page 84).

Note: Please refer to Section 2.2 on page 25 to optimize the PCR cycle number used for direct amplification from multiple sample types and substrate combinations.

Figure 22 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)

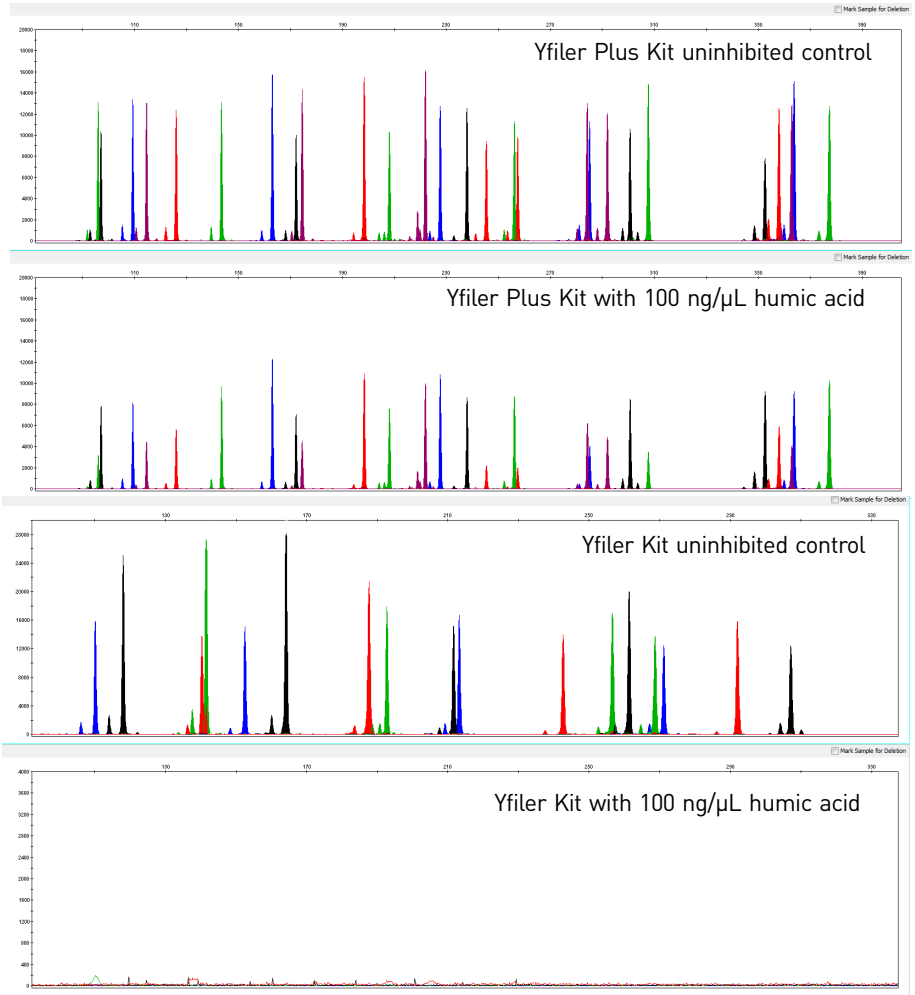
Lack of amplification of some loci

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

Effect of inhibitors

Traces of humic acid may inhibit the PCR amplification of DNA evidence collected from soil. Amplification of 1.0 ng of DNA Control 007 in the presence of increasing amounts of humic acid was performed using the Yfiler® Plus Kit (Figure 23 on page 85). The concentrations of humic acid tested were 0, 100, and 250 ng/µL. The same concentrations were tested with the Yfiler® Kit for comparison. At 250 ng/µL, neither kit yielded amplified products.

Figure 23 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 4000 RFUs for the bottom panel.



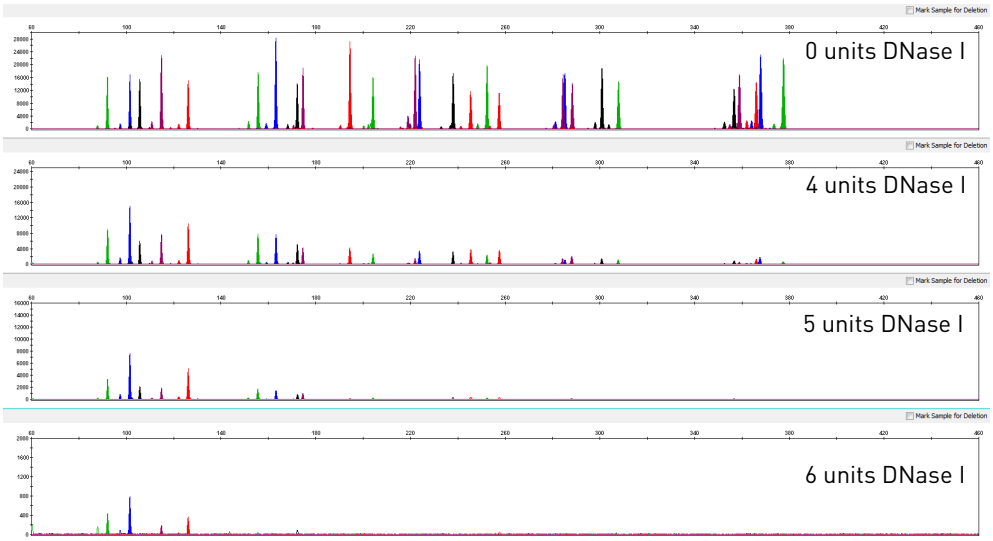
Degraded DNA

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 24).

Figure 24 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).

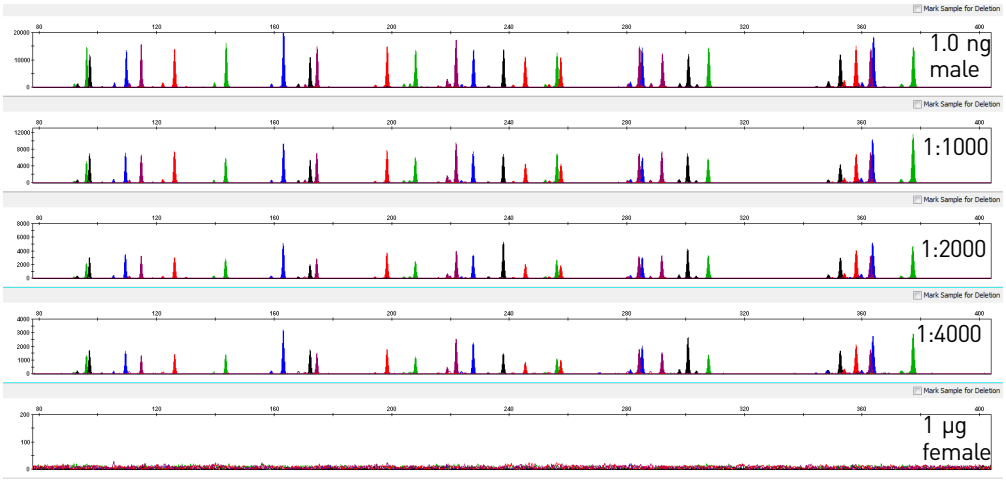
Evidence samples may contain DNA from more than one individual. The possibility of multiple contributors should be considered when interpreting the results. We recommend that individual laboratories assign a minimum peak height threshold based on validation experiments performed in each laboratory to avoid typing when stochastic effects are likely to interfere with accurate interpretation of mixtures.

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 1:4000 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 25.

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 25 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. Note that 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.

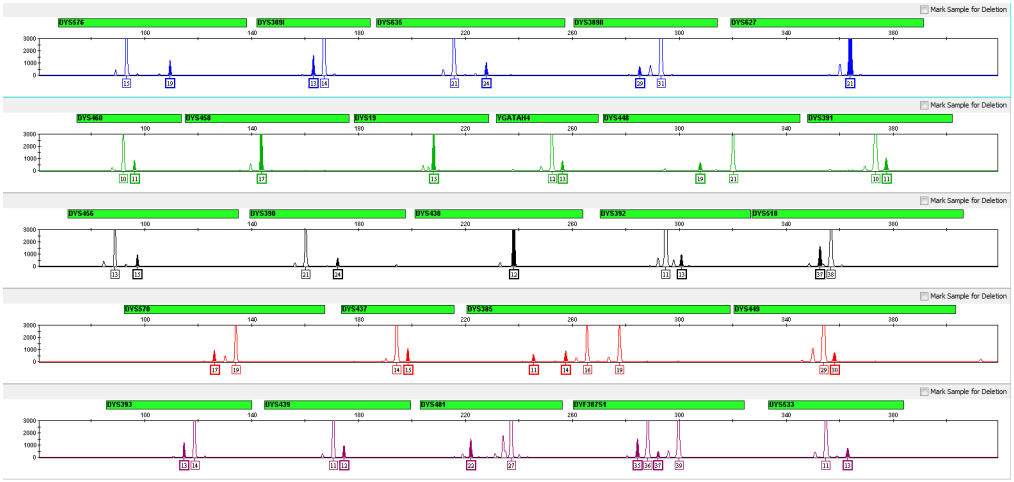
Mixtures of two male DNA samples were examined at various ratios (1:1 to 1:15). The total amount of genomic input DNA mixed at each ratio was 1 ng.

Table 5 Haplotypes of samples in Figure 26

Allele	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
DYS456	13	15
DYS390	21	24
DYS438	12	12
DYS392	11	13
DYS518	38	37
DYS570	19	17
DYS437	14	15
DYS385	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 26. 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 26 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)

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 Kit was used to generate the population data provided in this section. Samples were collected from individuals throughout the United States with no geographical preference.

Population	Number of samples
African-American	557
U.S. Caucasian	533
U.S. Hispanic	391
U.S. Asian	340

Gene diversity values

Table 6 shows the Yfiler® Plus Kit gene diversity in three populations, listed as percentages.

Table 6 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
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	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, p_i = allele frequency (Johnson *et al.*, 2003).

Analyzing the population data

In addition to the alleles that were observed and recorded in the Life Technologies 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 7 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 7 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. Hispanics (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 2000 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 8 Example of precision results of seven injections of the Yfiler® Plus Allelic Ladder run on the 3130*xl*, 3500, and 3500xL Genetic Analyzers

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

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

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

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

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

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

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

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

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

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

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

Follow the actions recommended in this appendix to troubleshoot problems that occur during analysis.

Table 9 Troubleshooting

Observation	Possible causes	Recommended actions
Faint or no signal from both the DNA Control 007 and the DNA test samples at all loci	Incorrect volume or absence of Master Mix or Primer Set	Repeat amplification.
	No activation of DNA Polymerase	Repeat amplification, making sure to hold reactions initially at 95°C for 1 minute.
	Master Mix not vortexed thoroughly before aliquoting	Vortex the Master Mix thoroughly.
	Primer Set exposed to too much light	Store the Primer Set protected from light.
	Evaporation.	Ensure that the plate is properly sealed with film and that you used a compression pad with the 9700 thermal cycler (a compression pad is not needed with the Veriti® thermal cycler).
	PCR System malfunction	Refer to the thermal cycler user's manual and check instrument calibration.
	Use of incorrect thermal cycling parameters	Check the protocol for correct thermal cycling parameters.
	MicroAmp® Base used with tray/ retainer set and tubes in GeneAmp® 9700	Remove MicroAmp® Base from tray/retainer set and repeat test.
	Insufficient PCR product electrokinetically injected	Prepare PCR product as described in "Prepare samples for electrophoresis on the 3500/3500xL instruments" on page 38, or "Prepare samples for electrophoresis on the 3130/3130xl instruments" on page 43.
	Degraded formamide	Check the storage of formamide; do not thaw and refreeze multiple times. Try Hi-Di™ Formamide.

Observation	Possible causes	Recommended actions
Positive signal from Control DNA 007 but partial or no signal from DNA test samples	Quantity of DNA test sample is below assay sensitivity	Quantify DNA and (when possible) add 1.0 ng of DNA. For low concentration samples, add up to 10 µL of the DNA sample to the reaction mix (see "Prepare the amplification kit reactions" on page 22).
	Test sample contains high concentration of PCR inhibitor (for example, heme compounds, certain dyes	Quantify DNA and add minimum necessary volume. Repeat test.
		Wash the sample in Centricon®-100 centrifugal filter unit. Repeat test.
	Test sample DNA is severely degraded	The Quantifiler® HP and Trio Kits can help evaluate sample integrity during the quantification step. If DNA is degraded, reamplify with an increased amount of DNA or use the AmpFℓSTR MiniFiler™ Kit.
	Dilution of test DNA in water or wrong buffer (for example, TE formula with incorrect EDTA concentration)	Redilute DNA using low TE buffer.
More than expected number of alleles present at a locus	Secondary gene duplication at DYS385 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.
	Presence of exogenous DNA	Use appropriate techniques to avoid introducing foreign DNA during laboratory handling.
	Amplification of stutter product (–1 repeat unit position)	See "Stutter products" on page 69.
	Mixed sample	
	Incomplete 3´ A base addition (n-1 nt position)	Be sure to include the final extension step of 60°C for 22 minutes in the PCR.
	Signal exceeds dynamic range of instrument (off-scale data)	Check that you are using the recommended number of PCR cycles (see "Perform PCR" on page 31). Repeat PCR amplification using reduced input DNA amount or use your laboratory's SOP to analyze off-scale data.
	Gene duplication	To confirm duplication, re-amplify with a different sample from the same individual.
	Poor spectral separation (bad matrix)	Follow the steps for creating a spectral file.
		Confirm that Filter Set J6 modules are installed and used for analysis.
	Too much DNA in reaction	Use recommended amount of template DNA (1.0 ng).
	Incomplete denaturation of double stranded DNA	Use recommended amount of Hi-Di™ Formamide and perform heat denaturation step according to the instructions in "Perform electrophoresis" on page 33.
Some but not all loci visible on electropherogram of DNA Test Samples	Less than 25 µL of PCR volume was used	Repeat amplification using the recommended PCR volume of 25 µL.

Observation	Possible causes	Recommended actions
STR profiles contain many off-scale alleles	DNA quantitation was not performed or not accurate	Verify the accuracy of the DNA quantitation protocol.
Poor peak height balance	Incorrect thermal cycling parameters	Check the protocol for correct thermal cycling parameters.

C

Ordering information

Equipment and materials not included

Table 10 Equipment

Equipment	Source
3500/3500xL Genetic Analyzer	Contact your local Life Technologies sales representative
Applied Biosystems® 3500/3500xL Genetic Analyzer for Human Identification	
Applied Biosystems® 3130/3130*xl* Genetic Analyzer	
Veriti® 96-Well Thermal Cycler	4479071
GeneAmp® PCR System 9700 with the Silver 96-Well Block	N8050001
GeneAmp® PCR System 9700 with the Gold-plated Silver 96-Well Block	4314878
Silver 96-Well Sample Block	N8050251
Gold-plated Silver 96-Well Sample Block	4314443
Tabletop centrifuge with 96-Well Plate Adapters (optional)	MLS (major laboratory supplier)
Harris Micro-Punch® tool, 1.2 mm	MLS
Copan® NUCLEIC-CARD™ collection device	4473980
Copan® NUCLEIC-CARD™ collection device, 1 Spot	4474001
Copan® NUCLEIC-CARD™ Color, 1 spot	4473974
CPA200 Semi-Automated Punch Instrument with a 1.2 mm punch head	Contact your local Life Technologies support representative for information.
CPA300 Fully-Automated Punch Instrument with a 1.2 mm punch head	
96 well, deep well plate	4392904

Table 11 Software

Software	Source
3500/3500xL Data Collection Software v2 (RUO)	4475183†
HID Updater 3500 Data Collection Software v2	4480670†
3130 Data Collection Software v4	4475105†
3130*xl* Data Collection Software v4	4475126†
3130/3730 Data Collection Software v4 6-Dye Module v1	†
GeneMapper® *ID-X* Software v1.4 Full Installation	4479707
GeneMapper® *ID-X* Software v1.4 Client Installation	4479711

† Contact your Life Technologies HID representative.

Table 12 Other items

Item†	Source
Yfiler® Plus PCR Amplification Kit, 100 reaction	4484678
Yfiler® Plus PCR Amplification Kit, 500 reaction	4482730
Prep-n-Go™ Buffer, (5 tubes) 1000 tests	4467079
Prep-n-Go™ Buffer for buccal swabs, 200 reactions	4471406
3130 Analyzer materials	
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3100/3130*xl* Genetic Analyzer Capillary Array, 36-cm	4315931
POP-4® Polymer for 3100/3100-*Avant* Genetic Analyzers	4316355
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
Running Buffer, 10X	402824
Hi-Di™ Formamide	4311320
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
MicroAmp® Optical 96-Well Reaction Plate	N8010560
3130*xl* Analyzer materials	
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
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
Running Buffer, 10X	402824
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
MicroAmp® Optical 96-Well Reaction Plate	N8010560
Hi-Di™ Formamide	4311320
For a complete list of parts and accessories for the 3130*xl* instrument, refer to Appendix A of the *3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* (Pub. no. 4352716).	
3500/3500xL Analyzer materials	
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
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
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

Item†	Source
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
For a complete list of parts and accessories for the 3500/3500xL instrument, refer to the *3500/3500xL Genetic Analyzer User Guide* (Pub. no. 4401661).	
PCR amplification	
MicroAmp® 96-Well Tray	N8010541
MicroAmp® Reaction Tube with Cap, 0.2-mL	N8010540
MicroAmp® 8-Tube Strip, 0.2-mL	N8010580
MicroAmp® 8-Cap Strip	N8010535
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
Other user-supplied materials	
Hi-Di™ Formamide, 25-mL	4311320
Aerosol resistant pipette tips	MLS
Microcentrifuge tubes	MLS
Pipettors	MLS
Tape, labeling	MLS
Tube, 50-mL Falcon	MLS
Tube decapper, autoclavable	MLS
Deionized water, PCR grade	MLS
Vortex	MLS

† For the Safety Data Sheet (SDS) of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

D

PCR Work Areas

Work area setup and lab design

Many resources are available for the appropriate design of a PCR laboratory. If you are using the Yfiler® Plus PCR Amplification Kit for:

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

The sensitivity of the Yfiler® Plus 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).

Also take care while handling and processing samples 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

IMPORTANT! These items should never leave the PCR Setup Work Area.

- Calculator
- Gloves, disposable
- Marker pen, permanent
- Microcentrifuge
- Microcentrifuge tubes, 1.5-mL, or 2.0-mL, or other appropriate clean 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.

You can use the following systems:

- GeneAmp® PCR System 9700 with the Silver 96-Well Block
- GeneAmp® PCR System 9700 with the Gold-plated Silver 96-Well Block

  IMPORTANT! The Yfiler® Plus Kit is not validated for use with the GeneAmp® PCR System 9700 with the Aluminium 96-Well Block. Use of this thermal cycling platform may adversely affect performance of the Yfiler® Plus Kit.

- Veriti® 96-Well Thermal Cycler

E

Safety

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

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). 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, and 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! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

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Documentation and Support

Related documentation

Document title	Part number
Applied Biosystems® 3130/3130xl Genetic Analyzers Getting Started Guide	4352715
Applied Biosystems® 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide	4352716
Applied Biosystems® 3130/3130xl Genetic Analyzers Quick Reference Card	4362825
Applied Biosystems® 3130/3130xl Genetic Analyzers AB Navigator Software Administrator Guide	4359472
Applied Biosystems® 3130/3100xl DNA Analyzers User Guide	4331468
Applied Biosystems® 3500/3500xL Genetic Analyzer Quick Reference Card	4401662
Applied Biosystems® *3500/3500xL Genetic Analyzer User Guide, Data Collection v2.0*	4476988
Applied Biosystems® 3500/3500xL Genetic Analyzer User Bulletin: Solutions to issues related to software, data, hardware, and consumables **Note:** Additional user bulletins may be available at **www.lifetechnologies.com**	4445098
GeneAmp® PCR System 9700 Base Module User's Manual	N805-0200
Veriti® 96-Well Thermal Cycler AmpFℓSTR® Kit Validation User Bulletin	4440754
Quantifiler® *HP and Trio DNA Quantification Kits User Guide*	4485354
Quantifiler® Kits: Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit User's Manual	4344790
PrepFiler® Forensic DNA Extraction Kit User Guide	4390932
GeneMapper® ID-X Software Version 1.2 Reference Guide	4426481
GeneMapper® ID-X Software Version 1.2 Quick Reference Guide	4426482
GeneMapper® ID-X Software Version 1.4 User Bulletin	4477684

Portable document format (PDF) versions of this guide and the documents listed above are available at **www.lifetechnologies.com**.

Note: To open the user documentation available from the our web site, use the Adobe® Acrobat® Reader® software available from **www.adobe.com**.

Obtain SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/sds**.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtain support

For HID support:

- **In North America** – Send an email to **HIDTechSupport@lifetech.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:

www.lifetechnologies.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited Product Warranty

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