applied biosystems

GlobalFiler™ Express PCR Amplification Kit

USER GUIDE

Catalog Numbers 4476609 and 4474665

Publication Number 4477672

Revision G

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

ThermoFisher SCIENTIFIC

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

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

Revision history: Pub. No. 4477672

Revision	Date	Description
G	13 October 2020	Add the SeqStudio™ Genetic Analyzer. Consolidate sample preparation for electrophoresis; it is the same for all instruments.
F	09 June 2020	In kit overview, change amplification time from ~80 to ~45 minutes. Update copyright page to latest template. On cover, update regulatory statement and remove licensing statement link.
E	21 December 2016	Revised the Peak Detector tab settings for GeneMapper™ *ID-X* Software analysis.
D	06 October 2016	Updated 3730 Peak Detector settings in Chapter 4. Add references to 3500 Series Data Collection 3 and GeneMapper ID-X v1.5. Non-technical changes: Reorganized Chapter 1 and Chapter 5.
C	May 2014	Added data to Chapter 5 about the evaluation of Hardy-Weinberg equilibrium.
B	April 2014	Added Master Mix Additive instructions. Updated the HID Updater 3500 DC v2.0 instructions, including sizing method information. Added Chapter 5, Experiments and Results.
A	October 2012	New document

Contents

1 Product information

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

Product description

Kit overview

The Applied Biosystems™ GlobalFiler™ Express PCR Amplification Kit is a 6-dye, short tandem repeat (STR) multiplex assay for the amplification of human genomic DNA.

The kit amplifies:

- 21 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338)
- 1 Y-STR (DYS391)
- 1 insertion/deletion polymorphic marker on the Y chromosome (Y indel)
- Amelogenin (sex determining marker)

The GlobalFiler™ Express PCR Amplification Kit combines the 13 original CODIS loci with 7 from the expanded European Standard Set of Loci (ESSL) and the highly discriminating SE33 locus. The kit delivers a 24-locus multiplex with the highest discrimination power of any Thermo Fisher Scientific Human Identification Kit, along with high sensitivity and tolerance to inhibitors. The concentration of 10 mini-STR loci that are entirely below 220 bp maximizes performance on degraded samples. The highly optimized buffer formulation contains an enzyme that allows completion of amplification in ~45 minutes.

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

Single-source sample types supported

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

- Blood and buccal samples on treated paper substrates.
- Blood and buccal 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.

Substrate examples

- Treated paper: NUCLEIC-CARD™ system or Whatman FTA™ cards
- Untreated paper: Bode Buccal DNA Collector™ or 903 paper
- Swab: FLOQSwabs™ or cotton swabs

Note: Our testing does not include blood samples on swab substrates. This sample type is not typically used for the collection of reference samples.

About the primers

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

The primers used in the kit are:

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

The GlobalFiler™ Express PCR Amplification Kit also includes the following primer additions and modifications:

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

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

Dyes used in the kit

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

Loci amplified by the kit

Table 1 GlobalFiler™ Express PCR Amplification Kit loci and alleles

Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	DNA Control 007
D3S1358	3p21.31	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20	6-FAM™	15, 16
vWA	12p13.31	11,12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24		14, 16
D16S539	16q24.1	5, 8, 9, 10, 11, 12,13, 14, 15		9, 10
CSF1PO	5q33.3-34	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		11, 12
TPOX	2p23-2per	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15		8, 8
Y indel	Yq11.221	1, 2	VIC™	2
Amelogenin	X: p22.1-22.3 Y: p11.2	X, Y		X, Y
D8S1179	8q24.13	5, 6, 7, 8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 19		12, 13
D21S11	21q11.2-q21	24, 24.2, 25, 26, 27, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 37, 38		28, 31
D18S51	18q21.33	7, 9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27		12, 15
DYS391	Yq11.21	7, 8, 9, 10, 11, 12, 13		11
D2S441	2p14	8, 9, 10, 11, 11.3, 12, 13, 14, 15, 16, 17	NED™	14, 15
D19S433	19q12	6, 7, 8, 9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, 19.2		14, 15
TH01	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3		7, 9.3

Table 1 GlobalFiler Express PCR Amplification Kit loci and alleles *(continued)*

Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	DNA Control 007
FGA	4q28	13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2	NED™	24, 26
D22S1045	22q12.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	TAZ™	11, 16
D5S818	5q21-31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18		11, 11
D13S317	13q22-31	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16		11, 11
D7S820	7q11.21-22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		7, 12
SE33	6q14	4.2, 6.3, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20.2, 21, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 27.2, 28.2, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35, 35.2, 36, 37		17, 25.2
D10S1248	10q26.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	SID™	12, 15
D1S1656	1q42.2	9, 10, 11, 12, 13, 14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18.3, 19.3, 20.3		13, 16
D12S391	12p13.2	14, 15, 16, 17, 18, 19, 19.3, 20, 21, 22, 23, 24, 25, 26, 27		18, 19
D2S1338	2q35	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28		20, 23

Standards and controls that are required

For the GlobalFiler™ Express PCR Amplification Kit, the panel of standards needed for PCR amplification, PCR product sizing, and genotyping are:

- **DNA Control 007**—A positive control for evaluating the efficiency of the amplification step and STR genotyping using the GlobalFiler™ Express Allelic Ladder. DNA Control 007 is present in the kit. See “DNA Control 007 profile” on page 13.
- **GeneScan™ 600 LIZ™ Size Standard v2.0**—Used for obtaining sizing results. This standard, which has been evaluated as an internal size standard, yields precise sizing results for PCR products. Order the GeneScan™ 600 LIZ™ Size Standard v2.0 (Cat. No. 4408399) separately.
- **GlobalFiler™ Express Allelic Ladder**—Developed for accurate characterization of the alleles amplified by the kit. The Allelic Ladder is present in the kit and allows automatic genotyping of most of the reported alleles for the loci in the kit. See “Allelic ladder profile” on page 12.

Allelic ladder profile

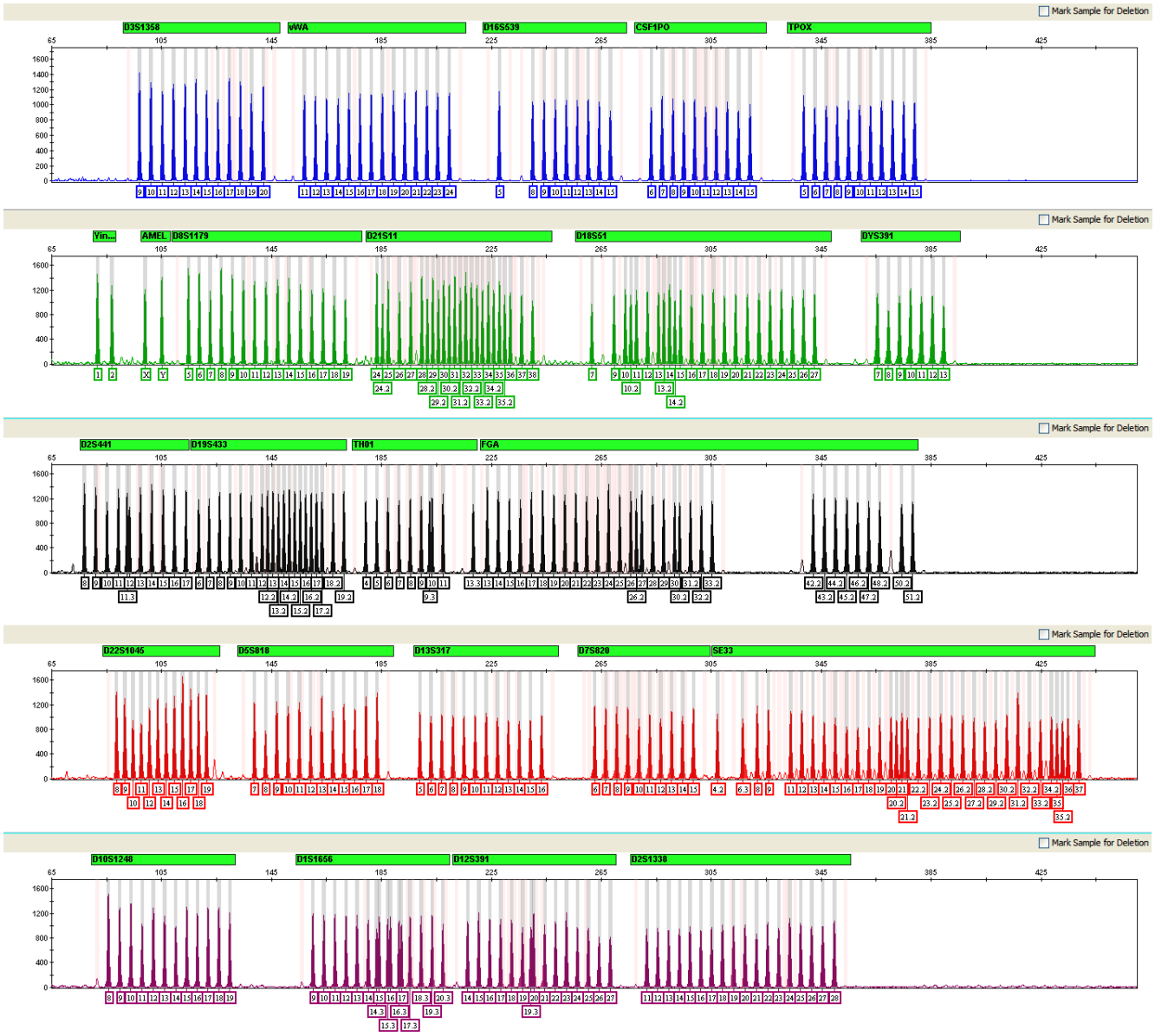


Figure 1 GeneMapper™ *ID-X* Software plot of the GlobalFiler™ Express Allelic Ladder

DNA Control 007 profile

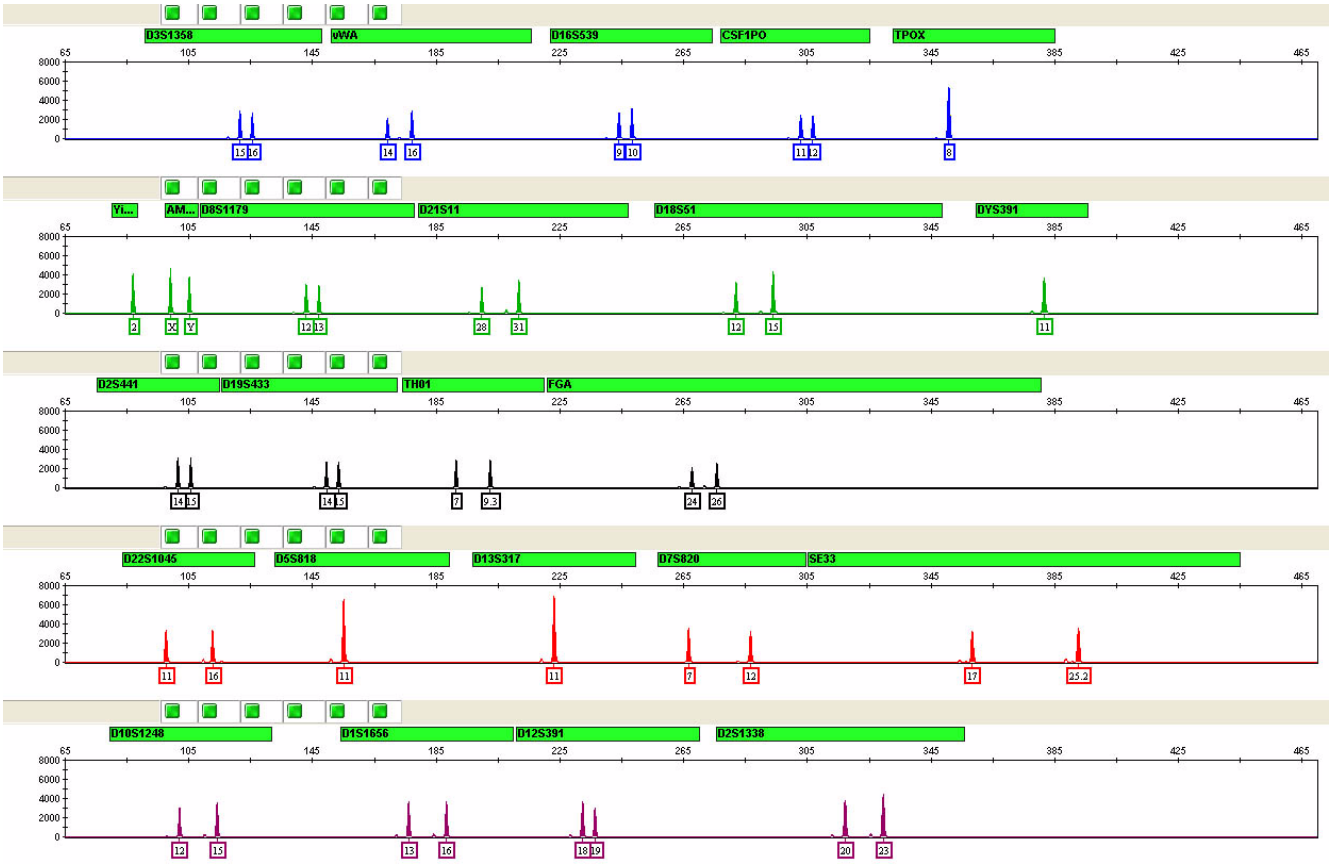


Figure 2 DNA Control 007 (1 ng) amplified with the GlobalFiler™ Express PCR Amplification Kit and analyzed on an Applied Biosystems™ 3500xL Genetic Analyzer (Y-axis scale 0 to 8,000 RFU).

Contents and storage

The GlobalFiler™ Express PCR Amplification Kit contains sufficient quantities of the following reagents to perform 200 (Cat. No. 4476609) or 1,000 (Cat. No. 4474665) amplifications at 15 µL/amplification.

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

IMPORTANT! Do not refreeze kit components after thawing.

Contents	Description	200 reactions (Cat. No. 4476609)	1,000 reactions (Cat. No. 4474665)	Storage
GlobalFiler™ Express Master Mix	Contains enzyme, salts, dNTPs, bovine serum albumin, enzyme, and 0.05% sodium azide in buffer and salt.	1 × 1.13 mL	1 × 5.64 mL	–25°C to –15°C on receipt. 2°C to 8°C after first use, for up to 6 months or up to the expiration date stated on the kit (whichever comes first).
Master Mix Additive	Reagent for one-time addition to the GlobalFiler™ Express Master Mix following first thaw.	1 × 0.1 mL	1 × 0.45 mL	–25°C to –15°C on receipt. Discard the tube after adding to the master mix.
GlobalFiler™ Express Primer Set	Contains forward and reverse primers to amplify human DNA targets.	1 × 1.2 mL	1 × 6 mL	–25°C to –15°C on receipt. 2°C to 8°C after first use, for up to 6 months or up to the expiration date stated on the kit (whichever comes first). Store protected from light.
GlobalFiler™ Express Allelic Ladder	Contains amplified alleles. See "Allelic ladder profile" on page 12 for information.	1 × 0.065 mL	1 × 0.15 mL	–25°C to –15°C on receipt. 2°C to 8°C after first use, up to the expiration date stated on the kit. Store protected from light.

(continued)

Contents	Description	200 reactions (Cat. No. 4476609)	1,000 reactions (Cat. No. 4474665)	Storage
DNA Control 007	Contains 2 ng/µL human male genomic DNA from cell line in 0.05% sodium azide and buffer[1] See "DNA Control 007 profile" on page 13 for information.	1 × 0.05 mL	1 × 0.1 mL	–25°C to –15°C on receipt. 2°C to 8°C after first use, up to the expiration date stated on the kit.

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

Required materials not supplied

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

Instrument and software compatibility

Instrument type	Validated models
Thermal cyclers	• ProFlex™ 96-well PCR System (Cat. No. 4484075) • ProFlex™ 2 × 96-well PCR System (Cat. No. 4484076) • Veriti™ 96-Well Thermal Cycler (Cat. No. 4479071) • GeneAmp™ PCR System 9700, 96-Well Silver (Cat. No. N8050001) • GeneAmp™ PCR System 9700, 96-Well Gold-Plated (Cat. No. 4314878) **IMPORTANT!** GlobalFiler™ Express PCR Amplification Kit is NOT validated for use with: • Veriti™ *Fast* 96-Well Thermal Cycler (Cat. No. 4375305) • GeneAmp™ PCR System 9700, 96-Well Aluminum (Cat. No. 4314879)

(continued)

Instrument type	Validated models
Genetic analyzers[1]	3500/3500xL Genetic Analyzer • 3500 Series Data Collection Software 1 (Windows™ Vista operating system) and HID Updater 3500 Data Collection Software v2 (Cat. No. 4480670) • 3500 Series Data Collection Software 2 (Windows™ 7 operating system) and HID Updater 3500 Data Collection Software v2 (Cat. No. 4480670) • 3500 Series Data Collection Software 3 (Windows™ 7 operating system) • 3500 Series Data Collection Software 3.1 (Windows™ 7 operating system) • 3500 Series Data Collection Software 4 (Windows™ 10 operating system) • 3500 Series HID Data Collection Software v4.0.1 (Windows™ 10 operating system)
	3130/3130*xl* Genetic Analyzer • 3130 Series Data Collection Software 4 (Windows™ 7 operating system) • 3130/3730 Data Collection 4 6-Dye Module v1
	3730/3730xl DNA Analyzer • 3730 Series Data Collection Software 4 (Windows™ 7 operating system) • 3730 Series Data Collection Software 4 6-Dye Module v1 • 3730xl Data Collection Software 5 (Windows™ 10 operating system) **Note:** For information on using the 3730xl DNA Analyzer, see the *3730xl Data Collection Software 5 for HID User Bulletin: New Features and Developmental Validation* (Pub. No. MAN0019461)
	SeqStudio™ Genetic Analyzer • SeqStudio™ Data Collection Software v1.2 • SeqStudio™ Data Collection Software v1.2.1
Analysis software	GeneMapper™ *ID-X* Software v1.4 or later Windows™ XP, Windows™ 7, or Windows™ 10 operating system

[1] We conducted validation studies using the 3130*xl*, 3500, 3500xL, and 3730xl instruments. For validation information on the 3730xl instrument, see the *3730xl Data Collection Software 5 for HID User Bulletin: New Features and Developmental Validation* (Pub. No. MAN0019461).

Workflow

Perform PCR on treated or untreated paper substrates

"Prepare the amplification kit reactions: treated paper substrate" on page 21 or

"Prepare the amplification kit reactions: untreated paper substrate" on page 23

▼

Obtain punch with Harris Manual Punch or BSD Semi-Automated Dried Sample Punch Instrument

▼

Untreated paper only: Process with Prep-n-Go™ Buffer

▼

Process with the GlobalFiler™ Express PCR Amplification Kit

▼

Perform PCR on swab substrates

"Prepare the reactions: swab substrate" on page 26

▼

Lyse in Prep-n-Go™ Buffer

▼

Process with the GlobalFiler™ Express PCR Amplification Kit

▼

Amplify with a recommended thermal cycler

▼

Perform electrophoresis

"Set up the SeqStudio™ instruments for electrophoresis (before first use of the kit)" on page 30 or

"Set up the 3500/3500xL instruments for electrophoresis (before first use of the kit)" on page 32 or

"Set up the 3130/3130xl instruments for electrophoresis (before first use of the kit)" on page 36 or

"Set up the 3730/3730xl instruments for electrophoresis (before first use of the kit)" on page 38

▼

"Prepare samples for electrophoresis" on page 41

▼

Analyze data

"Set up the GeneMapper™ ID-X Software for analysis (before first use of the kit)" on page 44

"Create an analysis method" on page 49

"Create a size standard definition file if needed" on page 57

"Analyze and edit sample files with GeneMapper™ ID-X Software" on page 60

"Examine or edit a project" on page 61

2 Perform PCR

Optimize PCR cycle number (before first use of the kit)

Before using the GlobalFiler™ Express PCR Amplification 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 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 buccal samples on swabs), perform separate sensitivity experiments for each sample type and substrate to be used for testing.

Procedural guidelines when optimizing PCR cycle number

- (Recommended) Use 26 samples so that you can complete electrophoresis using a single 96-well plate. This minimizes the impact of run-to-run variation on the results. Examples of PCR and electrophoresis plate layouts are provided on page 145.
- To maximize result quality, prepare and amplify Plate 1, then repeat for Plates 2 and 3. Do not prepare all 3 plates before amplification.
- To minimize the effect of instrument-to-instrument variation, use the same thermal cycler to amplify all 3 plates.

Select samples and prepare plates

1. Select 26 of each sample+substrate type. Ensure that the selected samples represent a "typical" range of samples analyzed in your laboratory.
2. Prepare the samples and the reactions as described in the appropriate protocols later in this chapter. Prepare sufficient PCR reagents to complete amplification of three replicate plates.
3. Create the first of 3 identical PCR plates (see page 145 for a suggested plate layout).

4. Amplify each plate using a different cycle number to determine the optimum conditions for use in your laboratory.

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

Sample type	Substrate		
	Treated paper	Untreated paper	Swab
Blood	25, 26, 27 cycles	25, 26, 27 cycles	N/A
Buccal	26, 27, 28 cycles	26, 27, 28 cycles	25, 26, 27 cycles

Determine optimum PCR conditions

1. Run the PCR products on the appropriate CE platform using the recommended protocol that is described in Chapter 3, “Perform electrophoresis”.

2. Based on the results of the sensitivity study, select the appropriate PCR cycle number for future experiments.

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

Instrument	Heterozygous peak height
3500 Series	3,000–12,000 RFU
3130 Series	1,000–3,000 RFU
3730 Series	3,000–12,000 RFU
SeqStudio™ Genetic Analyzer	3,000–12,000 RFU

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

Before you begin

Thaw reagents and prepare Master Mix (before first use of the kit)

1. Thaw the Master Mix, Master Mix Additive, and Primer Set, then vortex for 3 seconds.

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

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

2. Before opening the tubes or bottles, remove droplets from the caps by centrifuging the tubes briefly and tapping the bottles on the bench.

3. Add the following volumes of Master Mix Additive to the Master Mix:

Kit	Master Mix Additive volume
200 reactions	80 µL
1,000 reactions	390 µL

4. Gently invert the Master Mix tube 10 times, then centrifuge the tube briefly or tap the bottle on the bench.

5. Mark the cap of the Master Mix with a (+) to indicate that the Master Mix Additive has been added.

6. Discard the Master Mix Additive tube.

Treated paper substrates: prepare the amplification kit reactions

Sample preparation guidelines: treated paper substrate

- Do not add water to the wells on the reaction plate before adding the punches. If you observe static issues with the paper discs, you can prepare and dispense the 15-µL reaction mix into the wells of the reaction plate before adding the punches.

  Alternatively, dispense 3 µL of low-TE Buffer into each sample and negative amplification control well (NOT the positive amplification control wells) before adding the punches.
- Make the punch as close as possible to the center of the sample to ensure optimum peak intensity. Increasing the size of the punch may cause inhibition during PCR amplification.
- For manual punching: Place the tip of a 1.2 mm Harris Micro-Punch on the card, hold the barrel of the Harris Micro-Punch (do not touch the plunger), gently press and twist 1/4-turn, then eject the punch in to the appropriate well on the reaction plate.
- For automated punching: See the User Guide of your automated or semiautomated disc punch instrument for proper guidance.

Prepare low-TE buffer

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

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

   Note: Adjust the volumes accordingly for specific needs.

2. Aliquot, then autoclave the solutions.

3. Store the aliquots at room temperature.

Prepare the amplification kit reactions: treated paper substrate

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

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

1. Add samples to the MicroAmp™ Optical 96-well Reaction Plate:

To these wells of the plate ...	Add...	
Negative control	1.2 mm blank disc	
Test samples	1.2 mm sample disc	
Positive control **IMPORTANT!** Do not add a blank disc to the positive control well.	For 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 can be adjusted if peak heights are too high or too low for your optimized cycle number.

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

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

Reaction component	Volume per reaction
Master Mix	6.0 µL
Primer Set	6.0 µL
Low-TE buffer	3.0 µL

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

IMPORTANT! This kit is optimized for a 15-µL PCR volume to overcome the PCR inhibition that is expected when amplifying unpurified samples. Using a lower PCR reaction volume may reduce the ability of the kit chemistry to generate full STR profiles.

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

5. Dispense 15 µL of the reaction mix into each reaction well of a MicroAmp™ Optical 96-Well Reaction Plate.

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

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

IMPORTANT! If you are using the GeneAmp™ PCR System 9700 with silver or gold-plated silver block and adhesive clear film instead of caps to seal the plate wells, place a MicroAmp™ Optical Film Compression Pad (Cat. No. 4312639) on top of the plate to prevent evaporation during thermal cycling. Other validated thermal cyclers do not require a compression pad.

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

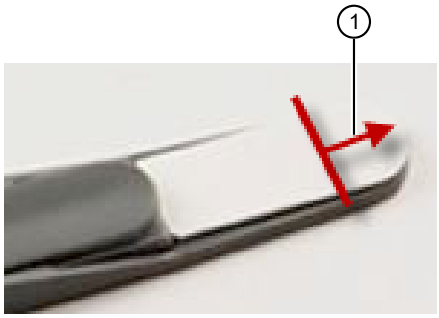
8. Amplify the samples as described in Chapter 2, “Perform PCR”.

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

Untreated paper substrates: prepare the amplification kit reactions

Sample preparation guidelines: untreated paper substrate

- Make a 1.2 mm punch as close as possible to the center of the sample to ensure optimum peak intensity. Increasing the size of the punch may cause inhibition during PCR amplification.
- If you are using a Bode Buccal DNA Collector™, make a 1.2 mm punch as close as possible to the tip of the DNA collector to ensure optimum peak intensity. A larger punch may cause inhibition during PCR amplification.
- For manual punching: Place the tip of a 1.2 mm Harris Micro-Punch on the card, hold the barrel of the Harris Micro-Punch (do not touch the plunger), gently press and twist 1/4-turn, then eject the punch in to the appropriate well on the reaction plate.
- For automated punching: See the User Guide of your automated or semiautomated disc punch instrument for proper guidance.



① Location of punch with a Bode Buccal DNA Collector™

Prepare the amplification kit reactions: untreated paper substrate

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

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

1. Add Prep-n-Go™ Buffer (Cat. No. 4467079) to the MicroAmp™ Optical 96-Well Reaction Plate:

To these wells ...	Add...	
Negative control	3 µL of Prep-n-Go™ Buffer	
Test samples	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. Add samples to the reaction plate:

To these wells ...	Add...	
Negative control	1.2 mm blank disc	
Test samples	1.2 mm sample disc	
Positive control **IMPORTANT!** Do not add a blank disc to the positive control well.	For 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.

3. Centrifuge the plate to ensure that the punches are immersed in the Prep-n-Go™ Buffer.

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

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

Reaction component	Volume per reaction
Master Mix	6.0 µL
Primer Set	6.0 µL

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

IMPORTANT! This kit is optimized for a 15-µL PCR volume to overcome the PCR inhibition that is expected when amplifying unpurified samples. Using a lower PCR reaction volume may reduce the ability of the kit chemistry to generate full STR profiles.

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

7. Dispense 12 µL of the reaction mix into each reaction well of a MicroAmp™ Optical 96-Well Reaction Plate.

   The final volume in each well is 15 µL (reaction mix plus Prep-n-Go™ Buffer and sample or positive control).

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

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

   IMPORTANT! If you are using the GeneAmp™ PCR System 9700 with silver or gold-plated silver block and adhesive clear film instead of caps to seal the plate wells, place a MicroAmp™ Optical Film Compression Pad (Cat. No. 4312639) on top of the plate to prevent evaporation during thermal cycling. Other validated thermal cyclers do not require a compression pad.

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

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

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

Swab substrates: prepare the amplification kit reactions

Sample preparation guidelines: swab substrate

- Detach each buccal swab head from the swab shaft before lysis.
- If you are using the heated lysis protocol, perform lysis in either of the following formats:
  - 1.5-mL tubes with a heat block (VWR™ Scientific Select dry heat block or similar)
  - PrepFiler™ 96-Well Processing Plates (Cat. No. A47010)
  - Robbins Scientific™ Model 400 Hybridization Incubator or similar

  - Agilent™ Benchtop Rack for 200 µL Tubes/V Bottom Plates (metal) or similar (Cat. No. 410094)

    IMPORTANT! Do not use a plastic plate adaptor.

- For optimum performance, lyse the entire swab. If you need to preserve the sample, use half of the lysate prepared from the entire swab.

Prepare the sample lysate: room temperature

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

1. Add 400 µL Prep-n-Go™ Buffer (Cat. No. 4471406) to 1.5-mL tubes or the appropriate wells of a PrepFiler™ 96-Well Processing Plate (Cat. No. A47010).
2. Into each tube or well, put the entire head of each swab, then let stand for 20 minutes at room temperature (20°C to 25°C) to lyse the sample.
3. After 20 minutes, transfer the sample lysate out of the sample plate into tubes or plates for storage, then discard the deep-well plate containing the swab heads.

   Note: To minimize the risk of contamination, do not remove the swab heads from the sample lysate plate before transferring the lysate.

4. Go to "Prepare the reactions: swab substrate" on page 26 or "Store the sample lysate" on page 27.

Prepare the sample lysate: heat protocol

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

1. Preheat the heat block to 90°C or the oven with metal plate adaptor to 99°C.
2. Add 400 µL Prep-n-Go™ Buffer (for buccal swabs, Cat. No. 4471406) to 1.5-mL tubes or the appropriate wells of a PrepFiler™ 96-Well Processing Plate (Cat. No. A47010).
3. Into each tube or well, put the entire head of each swab. If you are using tubes, cap the tubes. Let the tubes or plate stand for 20 minutes in the preheated heat block or oven to lyse the sample.
4. After 20 minutes, remove the tubes or the deep-well plate from the heat block or oven.
5. Let the lysate stand at room temperature for at least 15 minutes to cool the lysate (for accurate pipetting).
6. Transfer the sample lysate out of the 1.5-mL tubes or sample plate into tubes or plates for storage. Discard the 1.5-mL tubes or deep-well plate containing the swab heads.

   Note: To minimize the risk of contamination, do not remove the swab heads from the sample lysate plate before transferring the lysate.

7. Go to "Prepare the reactions: swab substrate" on page 26 or "Store the sample lysate" on page 27.

Prepare the reactions: swab substrate

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

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

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

To these wells ...	Add...	
Negative control	3 µL of Prep-n-Go™ Buffer	
Positive control	For 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. Vortex the Master Mix and Primer Set for 3 seconds. Before opening the tubes or bottles, remove droplets from the caps by centrifuging the tubes briefly or tapping the bottles on the bench.

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

Reaction component	Volume per reaction
Master Mix	6.0 µL
Primer Set	6.0 µL

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

IMPORTANT! This kit is optimized for a 15-µL PCR volume to overcome the PCR inhibition that is expected when amplifying unpurified samples. Using a lower PCR reaction volume may reduce the ability of the kit chemistry to generate full STR profiles.

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

5. Dispense 12 µL of the reaction mix into each reaction well of a MicroAmp™ Optical 96-Well Reaction Plate.

   The final volume in each well is 15 µL (reaction mix plus Prep-n-Go™ Buffer or sample lysate or positive control).

6. Add samples to the reaction plate:

To these well(s) of a MicroAmp™ Optical 96-Well Reaction Plate...	Add...	
Test samples	3 µL of sample lysate	
Positive control	For 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.

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

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

IMPORTANT! If you are using the GeneAmp™ PCR System 9700 with silver or gold-plated silver block and adhesive clear film instead of caps to seal the plate wells, place a MicroAmp™ Optical Film Compression Pad (Cat. No. 4312639) on top of the plate to prevent evaporation during thermal cycling. Other validated thermal cyclers do not require a compression pad.

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

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

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

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

Store the sample lysate

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

2. Store the sample lysate as needed:

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

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

Perform PCR

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

1. Program the thermal cycling conditions.

   IMPORTANT! If you are using the GeneAmp™ PCR System 9700, select the Max ramping mode. If you are using the ProFlex™ 96-well PCR System, select the GeneAmp™ PCR System 9700 simulation mode. If you are using the Veriti™ Thermal Cycler, select the 100% ramping rate. *Do not* use 9600 emulation mode.

Initial incubation step	Optimum cycle number[1]		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE		HOLD	HOLD
95°C, 1 minute	94°C, 3 seconds	60°C, 30 seconds	60°C, 8 minutes	4°C, up to 24 hours[2]

[1] See "Optimize PCR cycle number (before first use of the kit)" on page 18.
[2] The infinity (∞) setting allows an unlimited hold time.

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

   IMPORTANT! If you are using adhesive clear film instead of caps to seal the plate wells, be sure to place a MicroAmp™ Optical Film Compression Pad (Cat. No. 4312639) on top of the plate to prevent evaporation during thermal cycling. The Veriti™ Thermal Cycler, ProFlex™ 96-well PCR System, and ProFlex™ 2 × 96-well PCR System do not require a compression pad.

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

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

IMPORTANT! Protect the amplified DNA from light.

3 Perform electrophoresis

Allelic ladder requirements for electrophoresis

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

Instrument	Number of allelic ladders to run	One injection equals	Number of samples per allelic ladder(s)
3500	1 per 3 injections	8 samples	23 samples + 1 allelic ladder
3500*xl*	1 per injection	24 samples	23 samples + 1 allelic ladder
3130	1 per 4 injections	4 samples	15 samples + 1 allelic ladder
3130*xl*	1 per injection	16 samples	15 samples + 1 allelic ladder
3730/3730*xl*, 48-capillary	3 per injection	48 samples	15 samples + 1 allelic ladder
SeqStudio™	1 per 6 injections	4 samples	23 samples + 1 allelic ladder

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

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

Materials required for electrophoresis

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

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

Set up the SeqStudio™ instruments for electrophoresis (before first use of the kit)

Electrophoresis software setup

The following table lists the data collection software and the run modules that you can use to analyze PCR products generated by this kit. For details on the procedures, see the documents listed in “Documentation and support” on page 151.

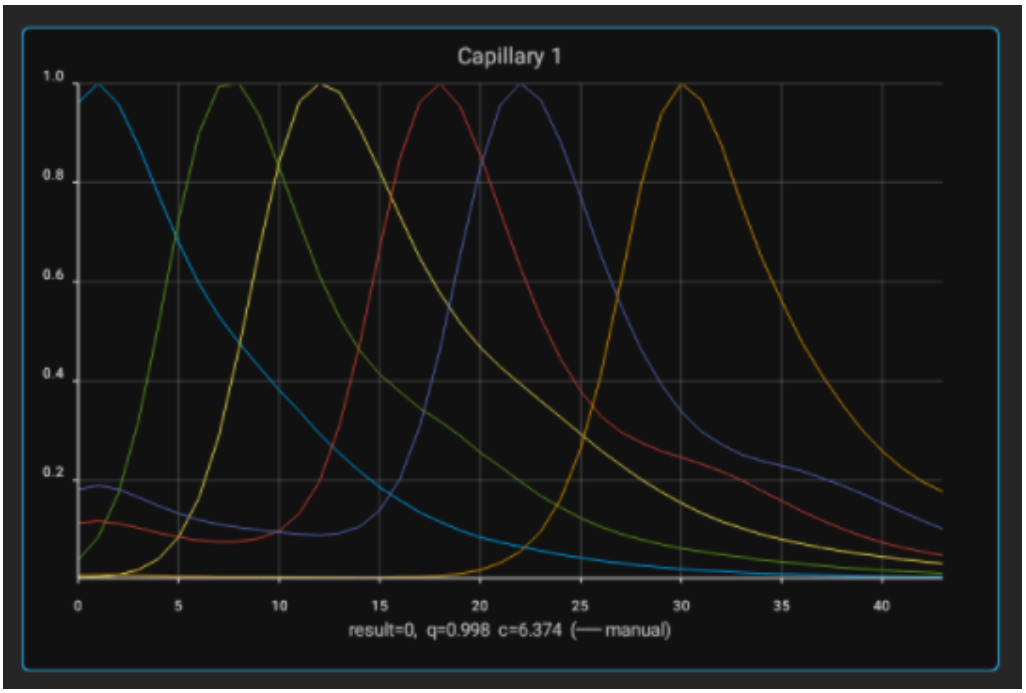
Genetic Analyzer	Data Collection Software	Additional software	Run modules and conditions
SeqStudio™	SeqStudio™ Data Collection Software v1.2.1	None	• Run Module: HIDAnalysis • Injection Conditions: 1.2 kV/10 sec • Run Conditions: 11 kV/1,120 sec • Dye Set J6 • Kit: GlobalFiler Express (to enable marker-to-marker pull-up reduction feature)

Perform spectral calibration

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

You need to perform manual calibration for each dye set only once before first use. To determine if a dye set requires manual calibration, review the calibration history for the dye set.

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



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

Electrophoresis software setup

The following table lists the data collection software and the run modules that you can use to analyze PCR products generated by this kit. For details on the procedures, see the documents listed in "Documentation and support" on page 151.

Note: We conducted original validation studies for the kit using the 3130*xl*, 3500, or 3500xL configurations. Subsequent validation studies for the kit were performed using the 3730*xl* 48-capillary configuration and the SeqStudio™ Genetic Analyzer.

Genetic analyzer	Operating system	3500 Data Collection Software	Additional software	Plate templates, assays, run modules, and conditions
3500 3500xL	Windows™ 10	v4.0.1	None	Assays: AB_J6_LS_POP4 (and _xl) and AB_J6OSR_LS_POP4 (and _xl), which contain instrument protocols AB_HID36_POP4_J6_NT3200 (and _xl) and AB_HID36_POP4_J6OSR_NT3200 (and _xl) All assays use the following conditions: • Run Module: HID36_POP4(xl) • Injection Conditions: 1.2kV/15 sec (24 sec for xl) • Run Conditions: 13kV/1,550 sec • Dye Set J6 or J6-OSR
3500 3500xL	Windows™ 7	v3, v3.1	None	Plate templates: 6dye_36_POP4 (and _xl) Assays (DCv3.1 and earlier): GF+Norm_POP4 (and _xl) and GF_POP4 (and _xl), which contain instrument protocol HID36_POP4 (and_xl)_J6_NT3200. All assays use the following conditions: • Run module: HID36_POP4(xl) • Injection conditions: 1.2 kV/15 sec (24 sec for xl) • Run conditions: 13 kV/1,550 sec • Dye Set J6
3500 3500xL	Windows™ 7	v2	HID Updater 3500 DC v2 (Cat. No. 4480670)	
3500 3500xL	Windows™ Vista	v1	HID Updater 3500 DC v2 (Cat. No. 4480670)	

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

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

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

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

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

Modify 3500 QC protocol

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

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

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

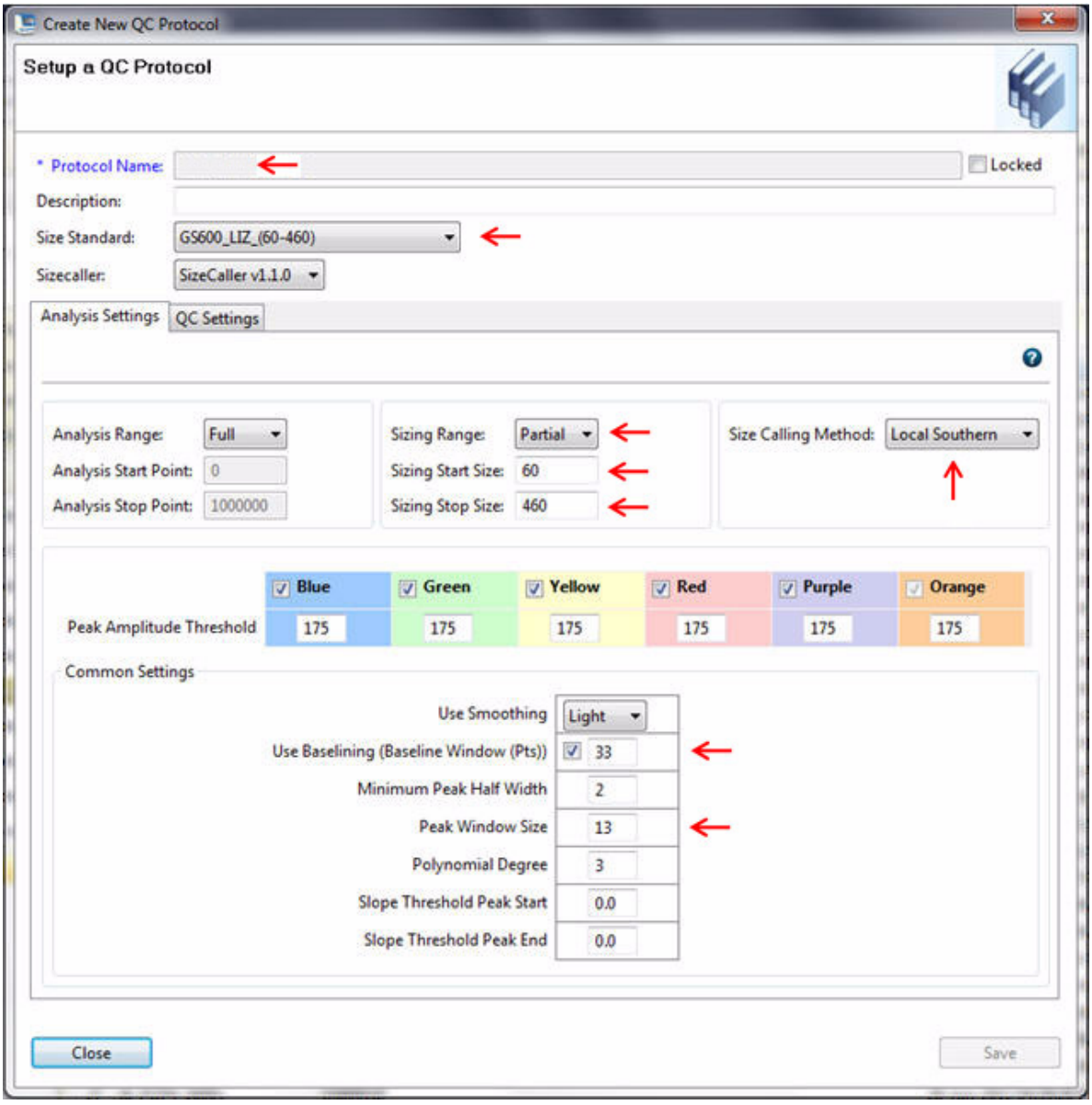
1. In the **Library** tab, open the **QC Protocol** window.
2. Create a new QC protocol:
   a. Name the new QC protocol according to your laboratory naming convention.
   b. Set the following parameters:

Parameter	Setting
Size Standard	GS600_LIZ_(60-460)
Size Range Sizing Start Size Sizing Stop Size	Partial 60 bp 460 bp

(continued)

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

c. Click **Save**.



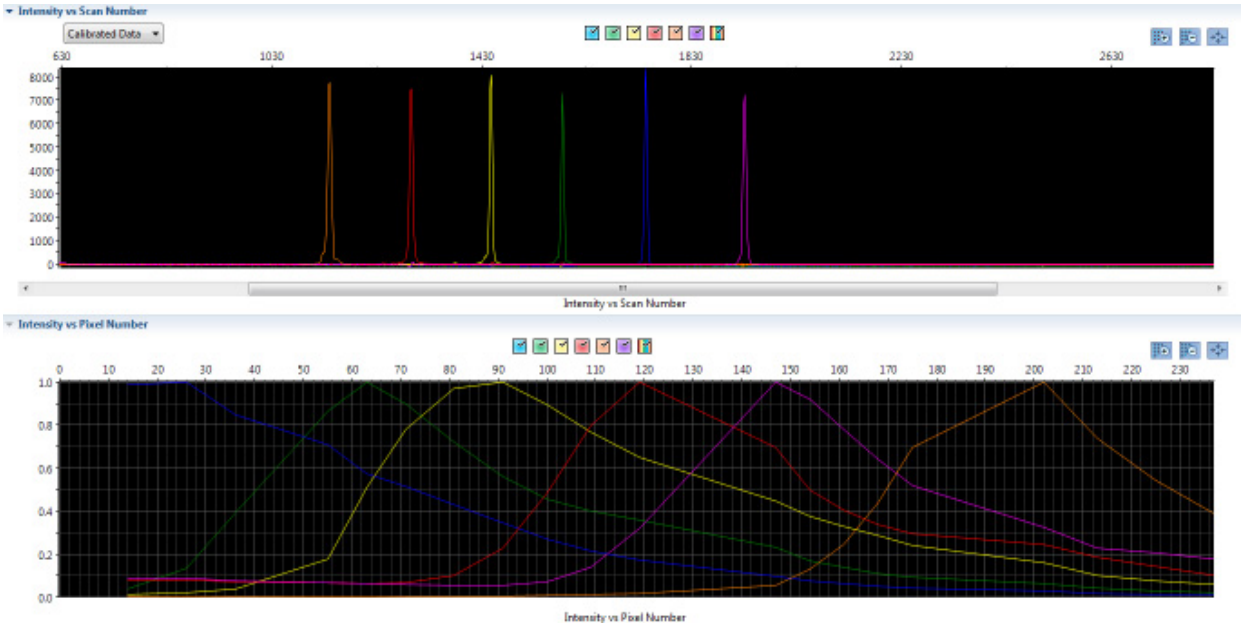
3. Add the QC protocol to the HID assay.

Perform spectral calibration

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

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

To enable off-scale recovery (OSR) with 3500 Series Data Collection Software 4.0.1, select the J6-OSR dye set in the spectral calibration and use the J6 OSR assay to run samples.



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

Electrophoresis software setup

The following table lists the data collection software and the run modules that you can use to analyze PCR products generated by this kit. For details on the procedures, see the documents listed in "Documentation and support" on page 151.

Note: We conducted original validation studies for the GlobalFiler™ Express PCR Amplification Kit using the 3130xl, 3500, or 3500xL configurations. Subsequent validation studies for the kit were performed using the 3730*xl* 48-capillary configuration and the SeqStudio™ Genetic Analyzer.

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

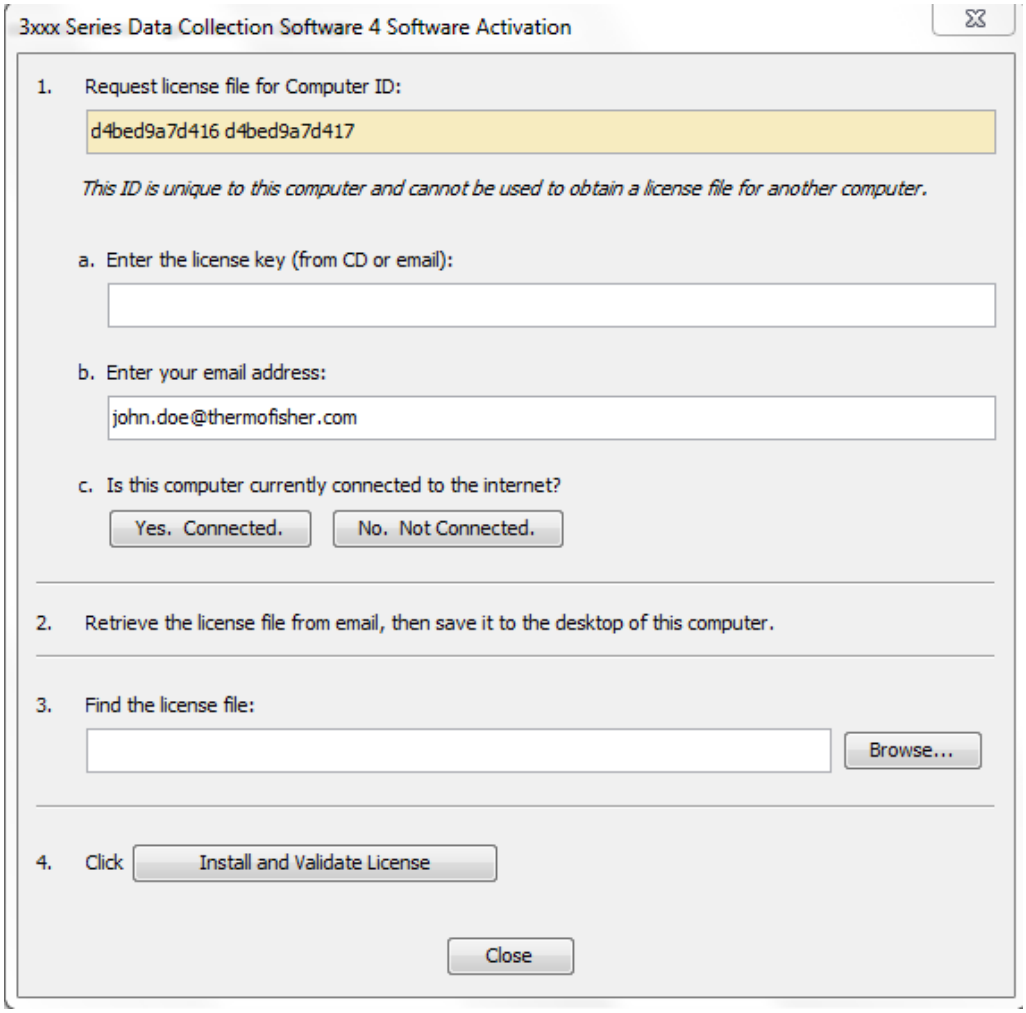
[1] Requires activation of 6-dye license.

Obtain and activate 6-dye license

1. Confirm that you are running Data Collection Software v4 (**Help ▸ About**).
2. Obtain a 3130 DC v4 6-Dye Module v1 License key. Contact your local Human Identification representative for information.
3. Ensure that all network cards in the computer are enabled.

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

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

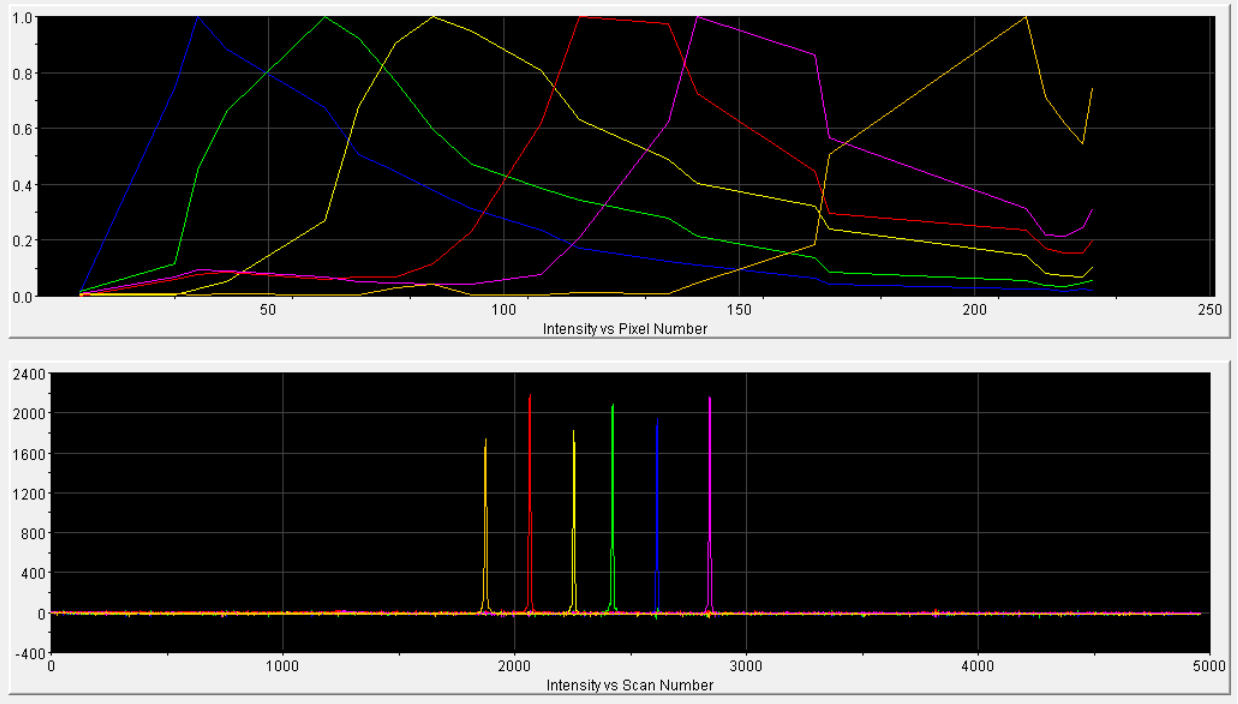


5. Request the software license file by performing steps 1a, 1b, and 1c as listed on the activation screen. The license file will be emailed to you.
6. Obtain the software license file from your email.
7. Make a copy of the software license file and keep it in a safe location.
8. Copy the software license file to the desktop of the Data Collection Software v4 computer.
9. If the Software Activation dialog box has closed, select **Tools ▸ License Manager**.
10. Click **Browse**, then navigate to the software license file saved on your computer.
11. Click **Install and Validate License**.
    A message is displayed when the license is installed and validated.
12. Click **Close**.

Perform spectral calibration

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

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



Set up the 3730/3730*xl* instruments for electrophoresis (before first use of the kit)

Electrophoresis software setup

The following table lists the data collection software and the run modules that you can use to analyze PCR products generated by this kit. For details on the procedures, see the documents listed in "Documentation and support" on page 151.

Genetic Analyzer	Operating System	Data Collection Software	Additional software	Run modules and conditions
3730*xl*	Windows™ 10	Data Collection Software v5	None	• GeneMapper36_POP7_1 Injection conditions: 2 kV/10 sec • Run conditions: 15 kV/1,200 sec • Dye Set J6
3730	Windows™ 7	Data Collection Software v4[1]	3130/3730 DC v4 6-Dye Module v1	

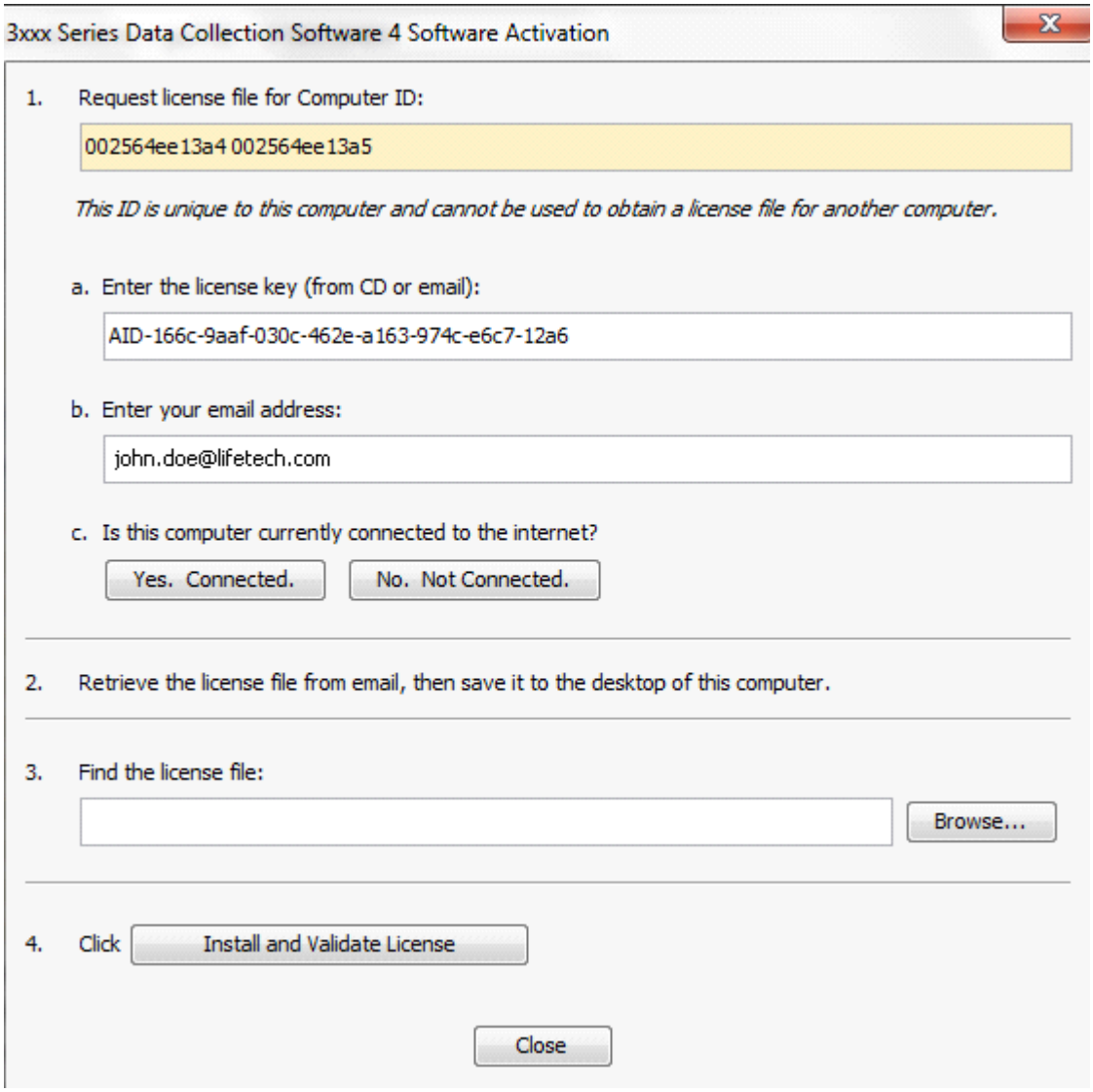
[1] Requires activation of 6-dye license.

Obtain and activate the 6-dye license

1. Confirm that you are running Data Collection Software v4 (**Help** ▸ **About**)
2. Obtain a 3730 DC v4 6-Dye Module v1 License key. Contact Thermo Fisher Scientific for information.
3. Ensure that all network cards in the computer are enabled.

   IMPORTANT! You can run the 3730 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.

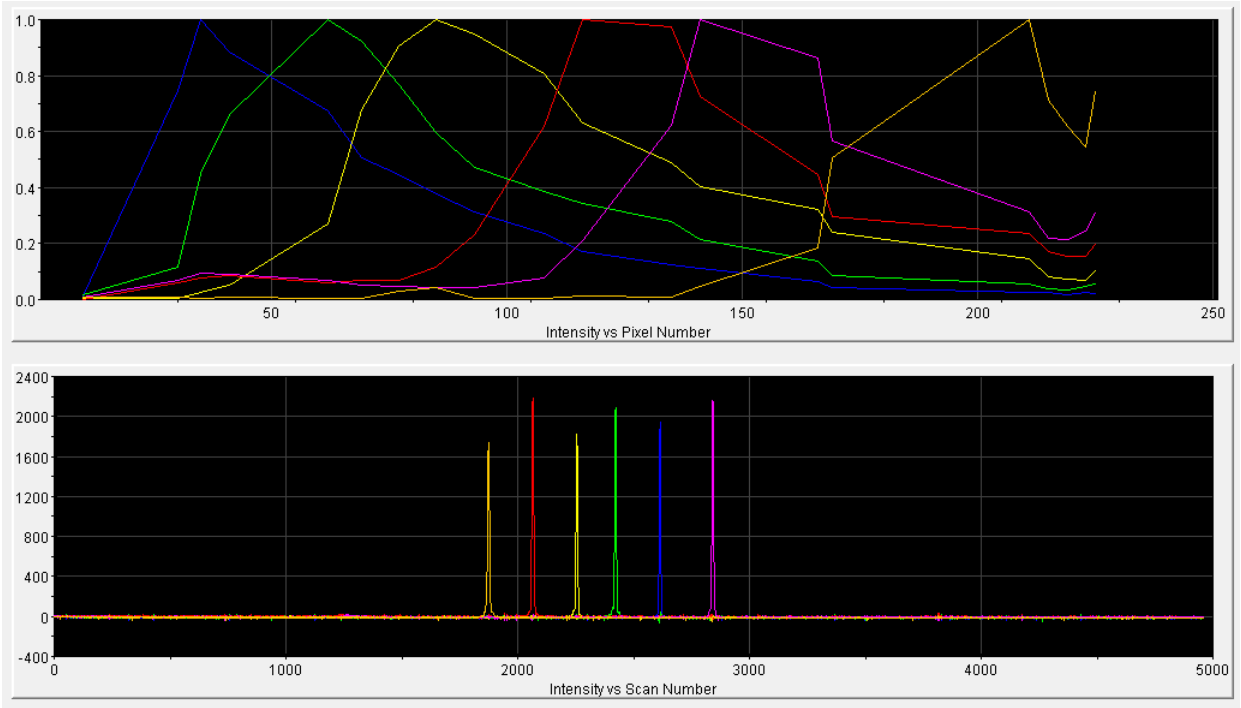


5. Request the software license file by performing steps 1a, 1b, and 1c as listed on the activation screen. The license file will be emailed to you.
6. Obtain the software license file from your email.
7. Make a copy of the software license file and keep in a safe location.
8. Copy the software license 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 Kit (Dye set J6, 6-dye) (Cat. No. 4425042).

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



Prepare samples for electrophoresis

Prepare the samples for electrophoresis immediately before loading.

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

Reagent	Volume per reaction
GeneScan™ 600 LIZ™ Size Standard v2.0	0.5 µL
Hi-Di™ Formamide	9.5 µL

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

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

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

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

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

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

Overview of GeneMapper™ *ID-X* Software

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

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

Allelic ladder requirements for data analysis

- HID analysis requires at least one allelic ladder sample per run folder. Perform the appropriate internal validation studies before you use multiple allelic ladder samples in an analysis.

  For multiple allelic ladder samples, the GeneMapper™ *ID-X* Software calculates allelic bin offsets by using an average of all allelic ladders that use the same panel in a run folder.
- Allelic ladder samples in an individual run folder are considered to be from a single run. When the software imports multiple run folders into a project, only the ladders in their respective run folders are used for calculating allelic bin offsets and subsequent genotyping.
- Allelic ladder samples must be labeled as "**Allelic Ladder**" in the **Sample Type** column in a project. Analysis will fail if the **Allelic Ladder Sample Type** is not specified.
- Injections containing the allelic ladder must be analyzed with the same analysis method and parameter values that are used for samples, to ensure proper allele calling.
- Alleles that are not in the allelic ladders do exist. Off-ladder (OL) alleles can contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the bin window of any known allelic ladder allele or virtual bin.

Note: If a sample allele peak is called as an off-ladder allele, verify the sample result according to your laboratory protocol.

File names and versions used in this section

The file names and version numbers of panel, bin, and stutter files that are shown in this section may differ from the file names that you see when you download or import files.

If you need help to determine the correct files to use, contact your local Human Identification representative, or go to **thermofisher.com/support**.

Set up the GeneMapper™ *ID-X* Software for analysis (before first use of the kit)

Workflow: Set up GeneMapper™ *ID-X* Software

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

"Check panel, bin, and stutter file versions on your computer" on page 44

▼

"(If needed) Download newer versions of panel, bin, and stutter files" on page 45

▼

"Import panels, bins, and marker stutter" on page 45

▼

"(Optional) Define custom table or plot settings" on page 48

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

1. Start the GeneMapper™ *ID-X* Software , then log in with the appropriate user name and password.
2. Select **Tools** ▸ **Panel Manager**.
3. Check the version of files that are currently available in the **Panel Manager**:
   a. Select **Panel Manager** in the navigation pane.
   b. Expand the **Panel Manager folder** and any subfolders to identify the analysis file version that is already installed for your kit choice.

4. Check the version of files available for import into the **Panel Manager**:
   a. Select **Panel Manager**, then select **File** ▸ **Import Panels** to open the **Import Panels** dialog box.
   b. Navigate to, then open the **Panels** folder, then check the version of panel, bin, and stutter files installed.
5. Check for newer versions of the files as described in the next procedure.

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

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

   Note: When downloading new versions of analysis files, see the associated **Read Me** file for details of changes between software file versions. Perform the appropriate internal validation studies before using new file versions for analysis.

3. Unzip the file.

Import panels, bins, and marker stutter

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

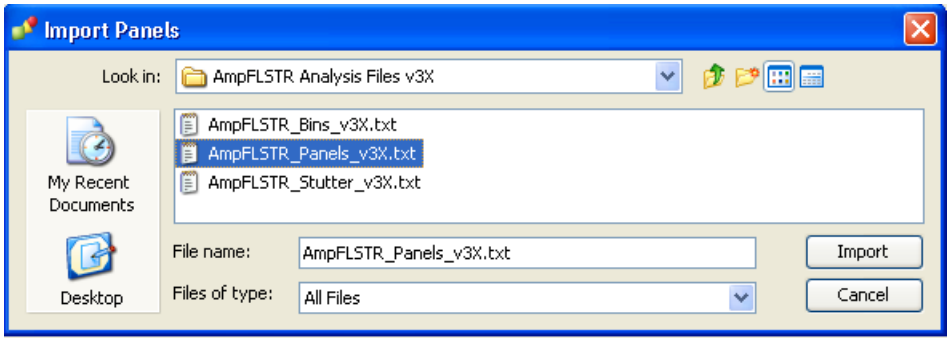
1. Start the GeneMapper™ *ID-X* Software, then log in with the appropriate user name and password.
2. Select **Tools** ▸ **Panel Manager**.
3. Find, then open the folder containing the panels, bins, and marker stutter:

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

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

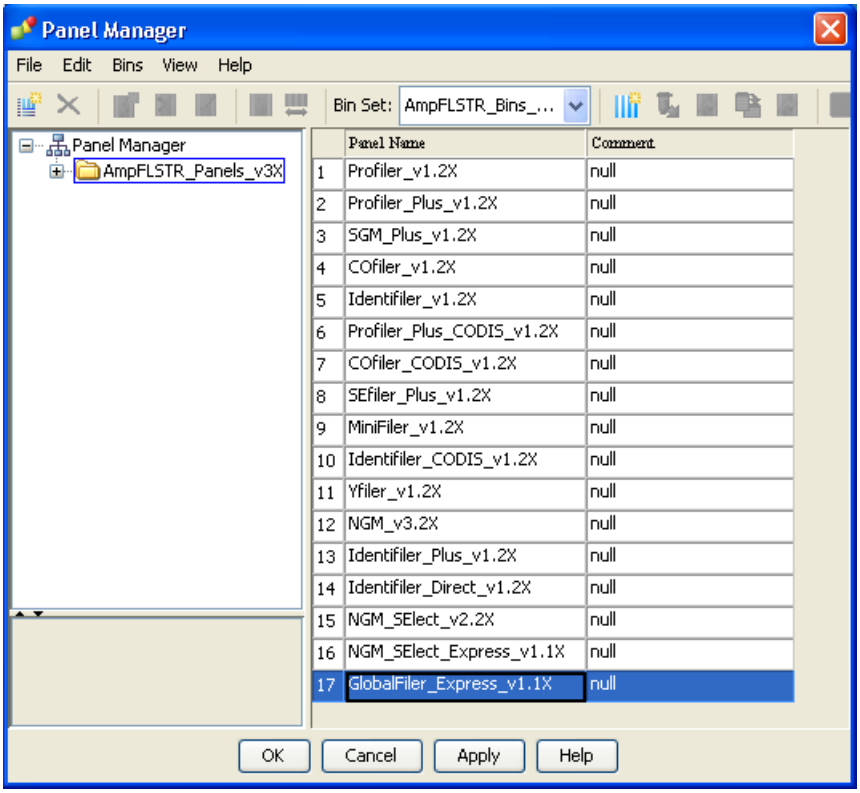
4. Select AmpFLSTR_Panels.txt, then click **Import**.

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



5. Import the bins file:

   a. Select the AmpFLSTR_Panels folder in the navigation pane.

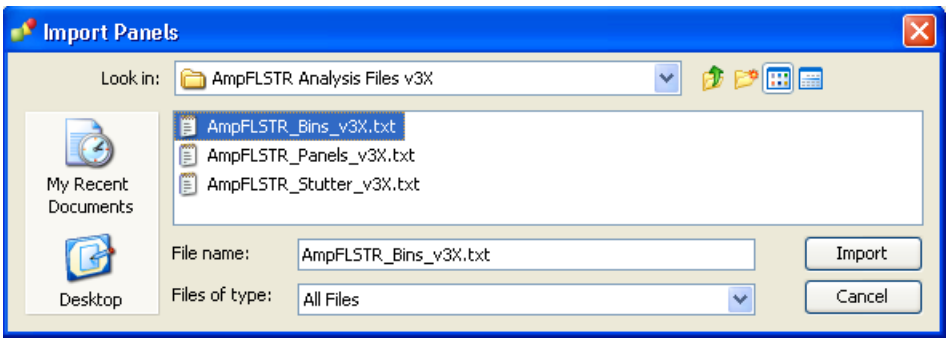


   b. Select **File** ▸ **Import Bin Set** to open the **Import Bin Set** dialog box.

   c. Navigate to, then open the AmpFLSTR Analysis Files folder.

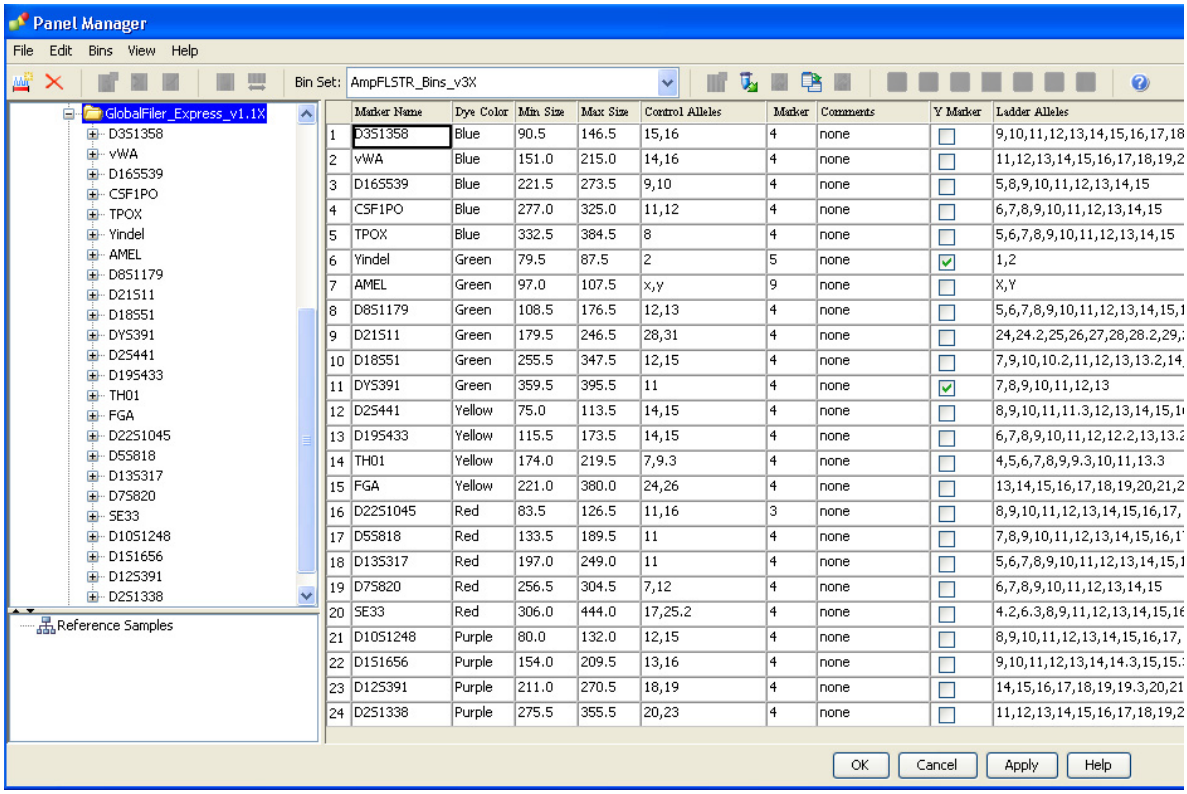
   d. Select AmpFLSTR_Bins.txt, then click **Import**.

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



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

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



	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker	Comments	Y Marker	Ladder Alleles
1	D3S1358	Blue	90.5	146.5	15,16	4	none	☐	9,10,11,12,13,14,15,16,17,18
2	vWA	Blue	151.0	215.0	14,16	4	none	☐	11,12,13,14,15,16,17,18,19,2
3	D16S539	Blue	221.5	273.5	9,10	4	none	☐	5,8,9,10,11,12,13,14,15
4	CSF1PO	Blue	277.0	325.0	11,12	4	none	☐	6,7,8,9,10,11,12,13,14,15
5	TPOX	Blue	332.5	384.5	8	4	none	☐	5,6,7,8,9,10,11,12,13,14,15
6	Yindel	Green	79.5	87.5	2	5	none	☑	1,2
7	AMEL	Green	97.0	107.5	x,y	9	none	☐	X,Y
8	D8S1179	Green	108.5	176.5	12,13	4	none	☐	5,6,7,8,9,10,11,12,13,14,15,1
9	D21S11	Green	179.5	246.5	28,31	4	none	☐	24,24.2,25,26,27,28,28.2,29,
10	D18S51	Green	255.5	347.5	12,15	4	none	☐	7,9,10,10.2,11,12,13,13.2,14
11	DYS391	Green	359.5	395.5	11	4	none	☑	7,8,9,10,11,12,13
12	D2S441	Yellow	75.0	113.5	14,15	4	none	☐	8,9,10,11,11.3,12,13,14,15,1
13	D19S433	Yellow	115.5	173.5	14,15	4	none	☐	6,7,8,9,10,11,12,12.2,13,13.2
14	TH01	Yellow	174.0	219.5	7,9.3	4	none	☐	4,5,6,7,8,9,9.3,10,11,13.3
15	FGA	Yellow	221.0	380.0	24,26	4	none	☐	13,14,15,16,17,18,19,20,21,2
16	D22S1045	Red	83.5	126.5	11,16	3	none	☐	8,9,10,11,12,13,14,15,16,17,
17	D5S818	Red	133.5	189.5	11	4	none	☐	7,8,9,10,11,12,13,14,15,16,1
18	D13S317	Red	197.0	249.0	11	4	none	☐	5,6,7,8,9,10,11,12,13,14,15,1
19	D7S820	Red	256.5	304.5	7,12	4	none	☐	6,7,8,9,10,11,12,13,14,15
20	SE33	Red	306.0	444.0	17,25.2	4	none	☐	4.2,6.3,8,9,11,12,13,14,15,16
21	D10S1248	Purple	80.0	132.0	12,15	4	none	☐	8,9,10,11,12,13,14,15,16,17,
22	D1S1656	Purple	154.0	209.5	13,16	4	none	☐	9,10,11,12,13,14,14.3,15,15.
23	D12S391	Purple	211.0	270.5	18,19	4	none	☐	14,15,16,17,18,19,19.3,20,21
24	D2S1338	Purple	275.5	355.5	20,23	4	none	☐	11,12,13,14,15,16,17,18,19,2

7. Import the stutter file:

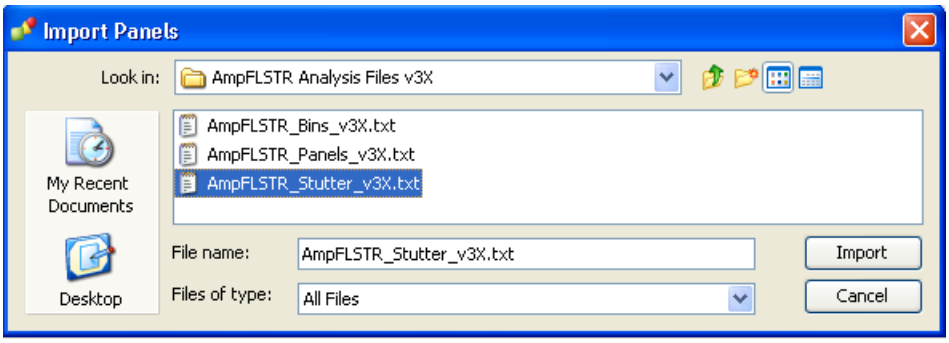
   a. Select the AmpFLSTR_Panels folder in the navigation panel.

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

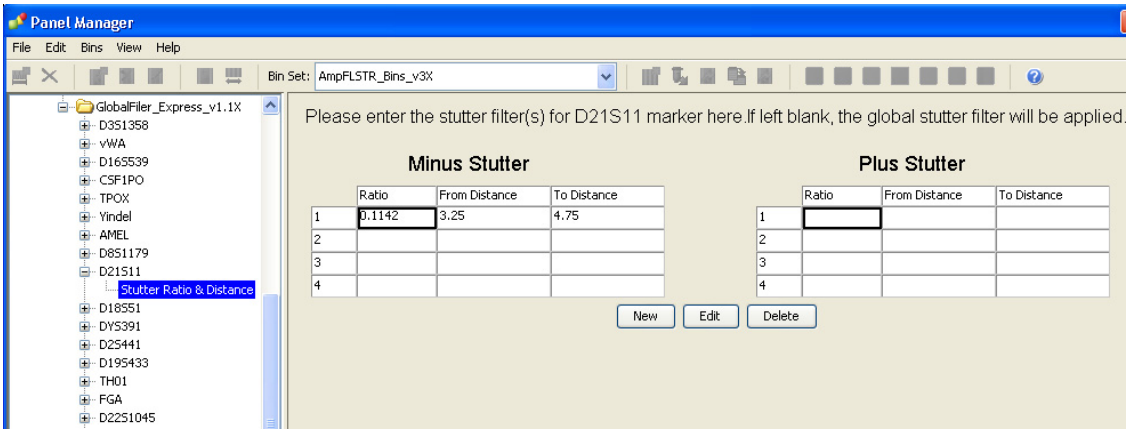
   c. Navigate to, then open the AmpFLSTR Analysis Files folder.

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

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



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



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

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

(Optional) Define custom table or plot settings

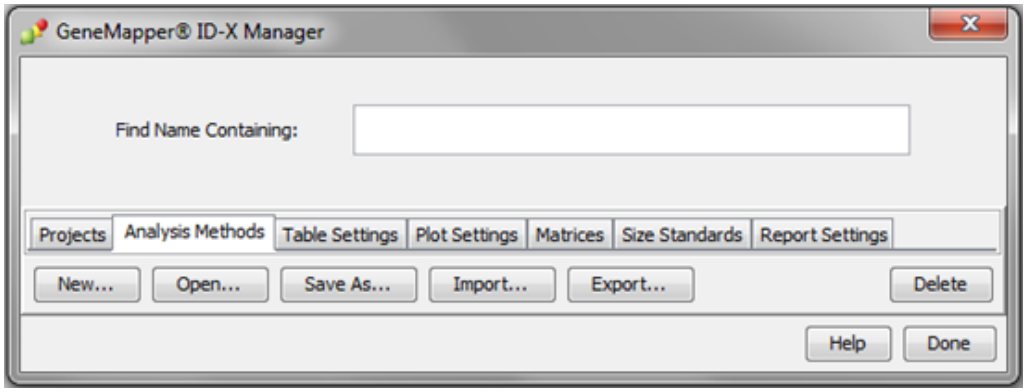
Default views for table and plot settings are provided with the software. For information on defining custom views, see *GeneMapper™ ID-X Software Getting Started Guide— Basic Features*.

Create an analysis method

Create an analysis method

IMPORTANT! Analysis methods are version-specific, so you must create an analysis method for each version of the software. For example, an analysis method that is created in GeneMapper™ *ID-X* Software version 1.2 is not compatible with analysis methods that are created in earlier versions of software, or with GeneMapper™ Software v3.2.1.

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



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

3. Enter the settings shown in the figures on the following pages.

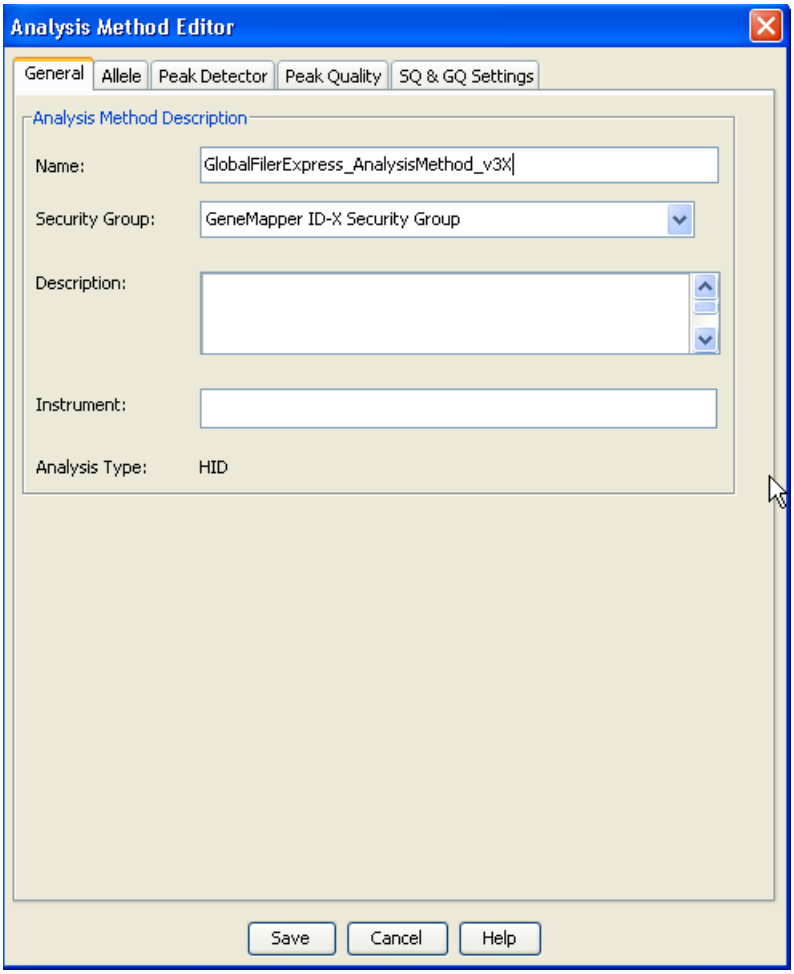
   Note: The **Analysis Method Editor** closes when you save your settings. To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.

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

Enter Analysis Method settings

Enter General tab settings

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



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

Enter Allele tab settings

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

1. Select the **AmpFlSTR_Bins_v3X** bin set.

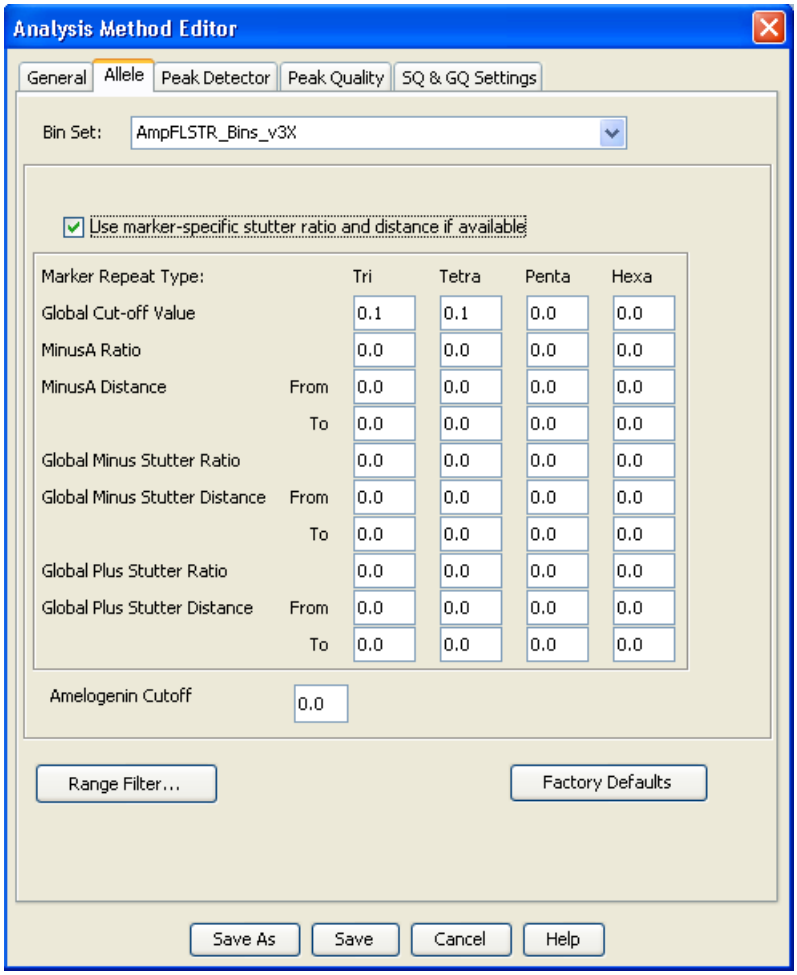


Figure 3 Settings used in developmental validation of the kit

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

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

4. Enter the appropriate filter settings.

Enter Peak Detector tab settings

Figure 4 shows the Peak Detector tab settings that are used in the developmental validation of the kit. Figure 5 shows the settings for use on the 3730/3730xl DNA Analyzer.

Enter the appropriate values for each field:

Field	Values to enter or select	Additional information
Ranges	Enter the values shown in Figure 4, or adjust as needed depending on the polymer and genetic analyzer that you are using.	—
Peak Detection	Enter the appropriate settings. **IMPORTANT!** Perform appropriate internal validation studies to determine the appropriate peak amplitude thresholds for interpretation of data.	The software uses these parameters to specify the minimum peak height, in order to limit the number of detected peaks. Although GeneMapper™ *ID-X* Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the genotype of these peaks.
Smoothing and Baseline	Enter the values shown in Figure 4, or adjust as needed dependent on the polymer you are using. 3730/3730xl DNA Analyzer with POP-7™ polymer only: With the **Smoothing** setting of **None**, the instances of spacing failures for the D2S441 and D1S1656 markers in some allelic ladder samples are significantly reduced. With the default **Smoothing** setting of **Light**, failures of base-pair spacing quality assessment are observed.	For more information, see the *GeneMapper™ ID-X Software v1.4 New Features and Installation Procedures User Bulletin* (Pub. No. 4477684 Rev. B), "Known issues: 3730 DNA Analyzer allelic ladder failures".
Size Calling Method	Select **3rd Order Least Squares**, or another method if validated by your internal validation. **IMPORTANT!** Because of the small amplicon sizes that are generated by the this kit, the **3rd Order Least Squares** sizing method is validated for use with this kit. Perform internal validation studies before using other sizing calling methods.	—
Normalization	*(Optional)* Select the **Normalization** checkbox.	A **Normalization** checkbox is available on this tab in GeneMapper™ *ID-X* Software for use in conjunction with data run on the 3500 Series Genetic Analyzers.

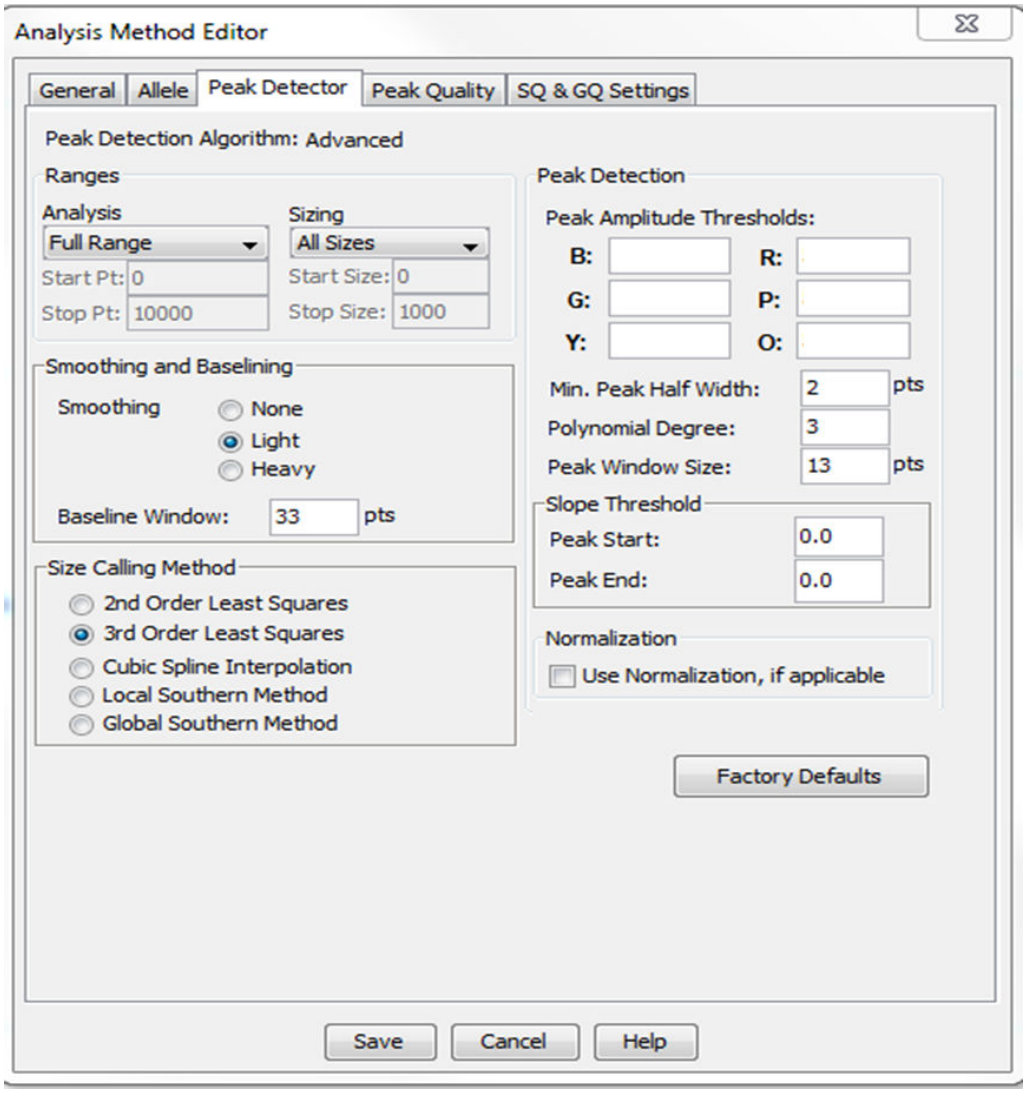


Figure 4 Settings used in developmental validation of the kit

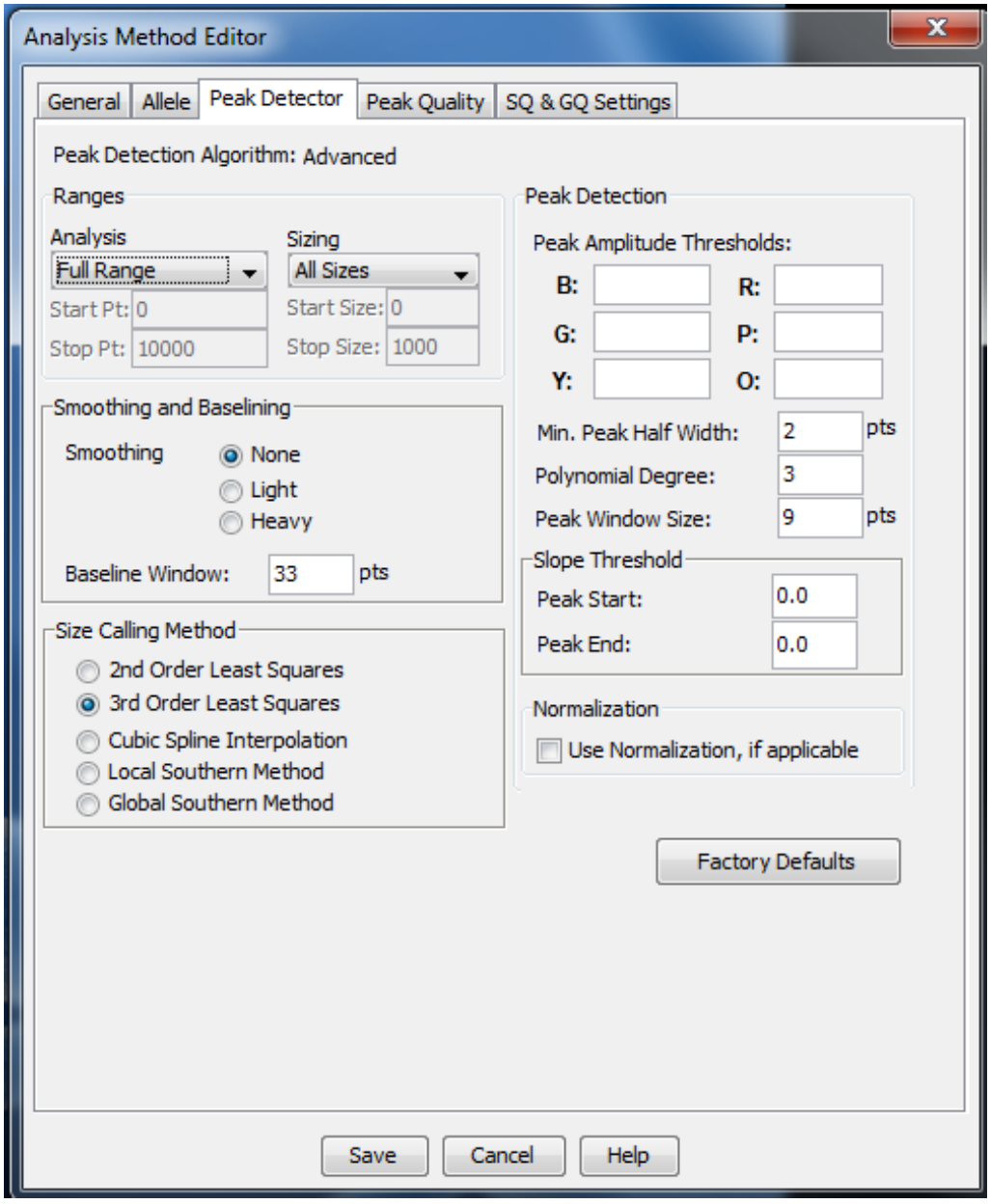
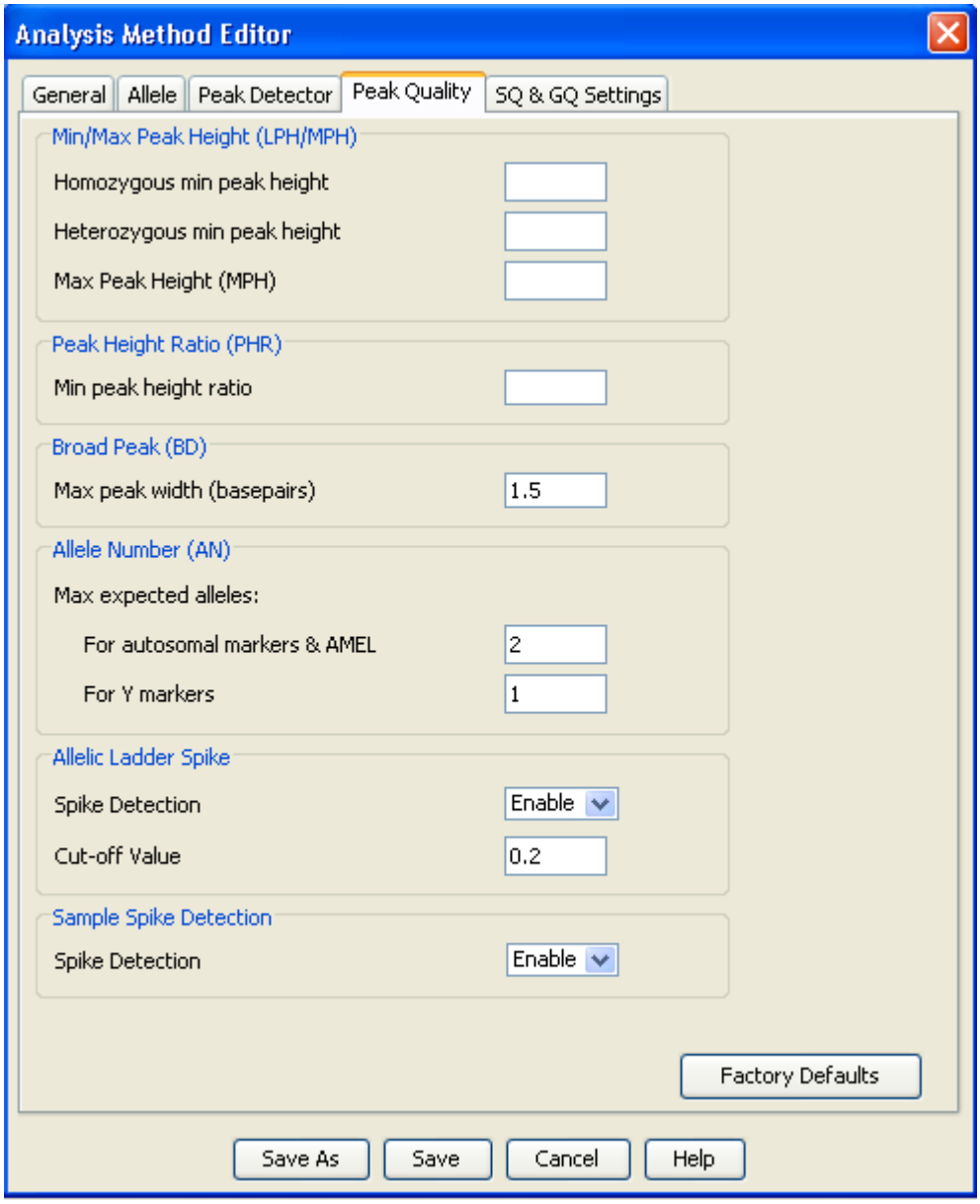


Figure 5 Settings for use on the 3730/3730xl DNA Analyzer.

Enter Peak Quality tab settings

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

Enter the following values:



Enter SQ and GQ tab settings

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

Enter the following values:

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

Create a size standard definition file if needed

If you cannot use the default settings that are provided, create a new size standard definition file.

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

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

This size standard definition has been validated for use with this kit on the genetic analyzers listed in “Instrument and software compatibility” on page 15. If you need to create your own size standard definition, see “Create a size standard definition file” on page 58.

If you use POP-7™ polymer on a 3730 instrument

The 60 bp size-standard peak may occasionally be obscured by the primer peak. The issue can be addressed by either of the following steps:

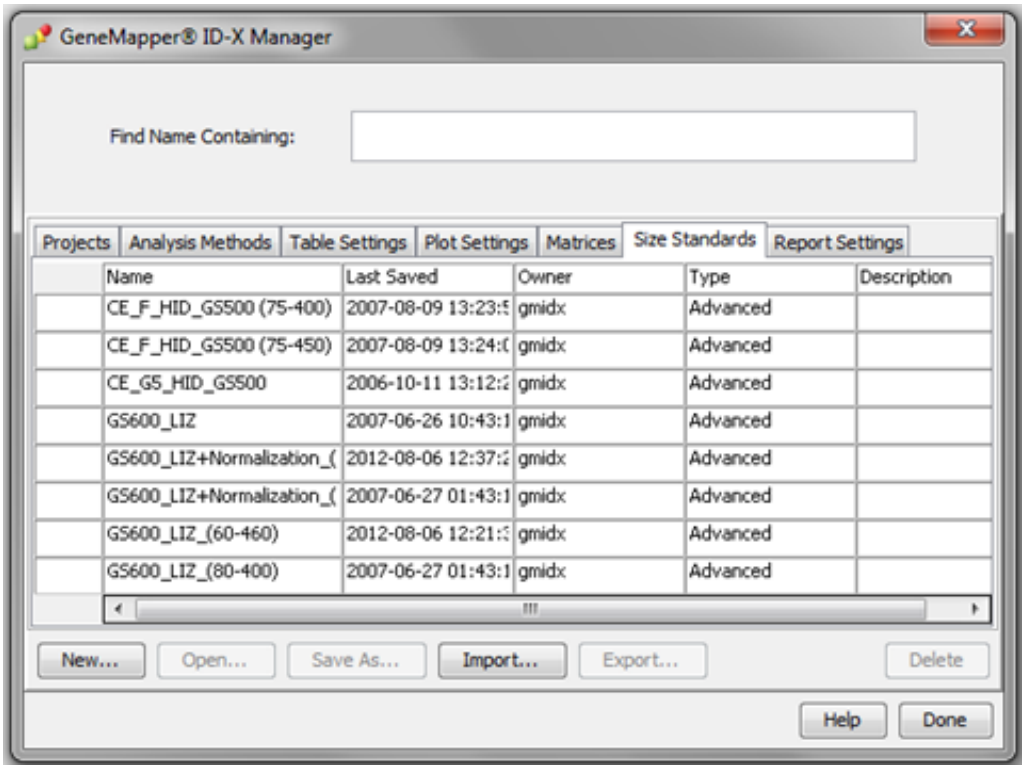
- Re-inject samples in which the 60 base-pair peak is not recognized.
- Use the 80–460 bp size-standard definition after performing appropriate validation studies (as a general rule, the 60 base-pair peak is not required for accurate fragment sizing with the 3rd Order Least Squares sizing method).

For more information, see the *GeneMapper™ ID-X Software v1.4 New Features and Installation Procedures User Bulletin* (Pub. No. 4477684 Rev. B), “Known issues: 3730 DNA Analyzer sizing failures”.

Create a size standard definition file

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

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

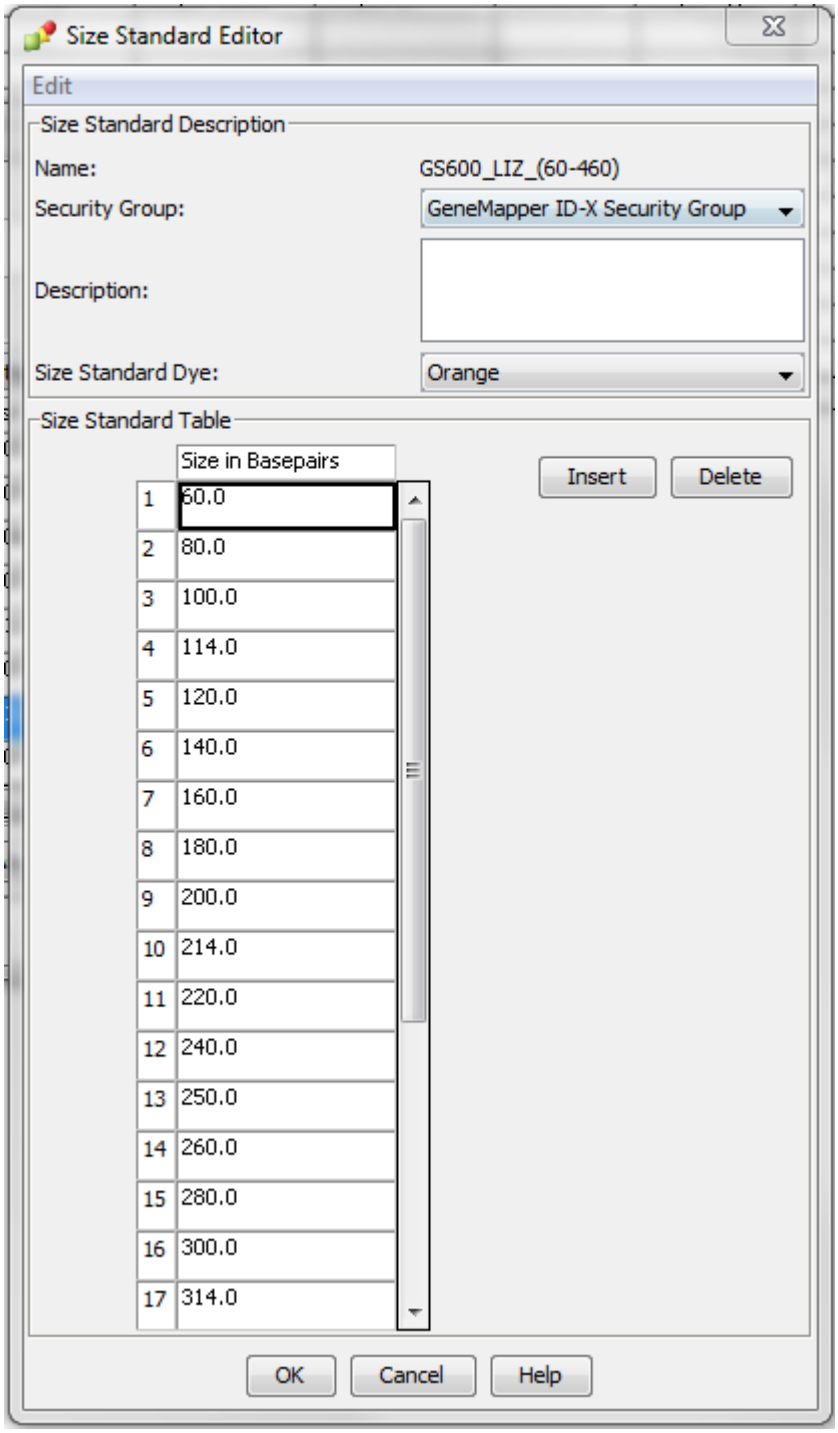


3. Specify settings in the **Size Standard Editor**:
   a. Enter a name as shown in the following figure or enter a new name.

   b. In the **Security Group** field, select the **Security Group** appropriate for your software configuration.

   c. In the **Size Standard Dye** field, select **Orange**.

d. In the **Size Standard Table**, enter the peak sizes that correspond to your size standard.



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

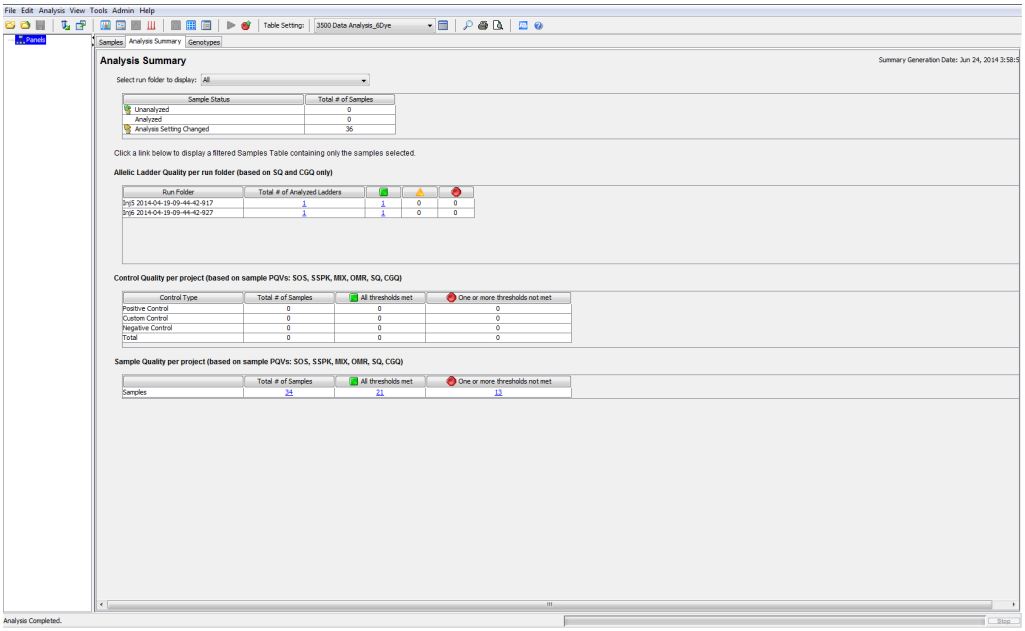
1. In the **Project** window, select **Edit ▸ Add Samples to Project**, then navigate to the disk or directory that contains the sample files.

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

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

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

3. Click **Analyze**, enter a name for the project (in the **Save Project** dialog box), then click **OK** to start analysis.
   - The status bar displays the progress of analysis as a completion bar.
   - The table displays the row of the sample currently being analyzed in green (or red if analysis failed for the sample).
   - The **Analysis Summary** tab is displayed, and the **Genotypes** tab is available when the analysis is complete.



Examine or edit a project

Display electropherogram plots from the Samples and Genotypes tabs of the Project window to examine the data.

For more information on using the GeneMapper™ *ID-X* Software

See “Related documentation” on page 151 for a list of available documents.

5 Experiments and results

Importance of validation

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

Experiment conditions

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

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

Laboratory requirements for internal validation

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

Developmental validation

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

SWGDAM guideline 2.2.1

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

SWGDAM guideline 3.9.2

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

PCR components

We examined the concentration of each component of the kit. We established that the concentration of each component was within the range where data indicated that the amplification met the required performance criteria for specificity, sensitivity, and reproducibility. For example, blood and buccal samples on treated-paper substrates or swab-sample lysates were amplified in the presence of varying concentrations of magnesium chloride, and the results were analyzed on a 3500xL Genetic Analyzer (Figure 6). The performance of the multiplex is most robust within ±20% of the optimal magnesium chloride concentration.

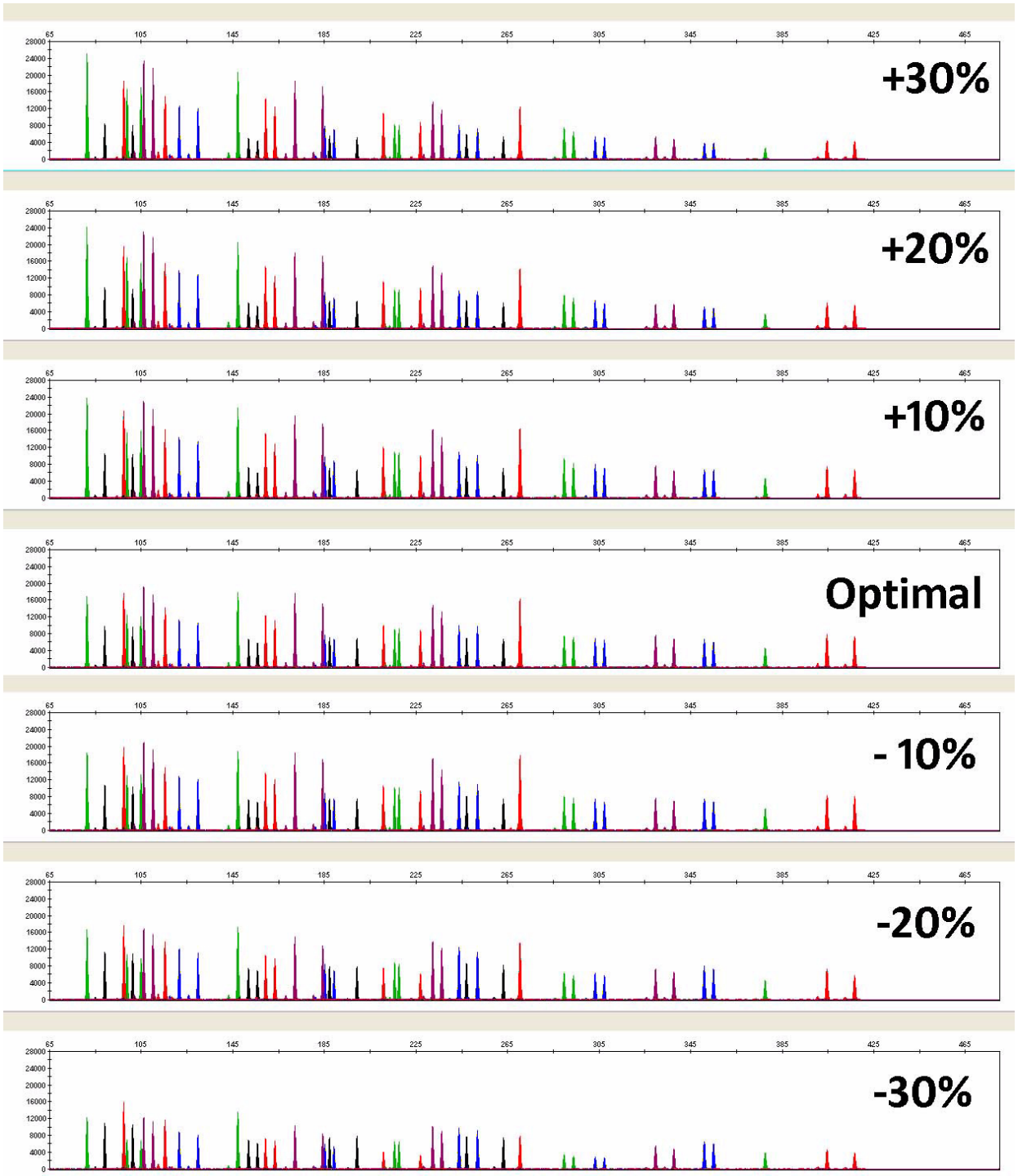


Figure 6 Buccal swab lysate amplified with the GlobalFiler™ Express PCR Amplification Kit in the presence of varying concentrations of magnesium chloride and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 28,000 RFU).

PCR cycle number

Reactions were amplified for 24, 25, 26, and 27 cycles on the Veriti™ Thermal Cycler using a buccal swab lysate. As expected, the amount of PCR product increased with the number of cycles. A full profile was generated for all numbers of thermal cycles (24–27) and off-scale data were collected for several allele peaks at 27 cycle (Figure 7).

None of the cycle numbers tested produced nonspecific peaks.

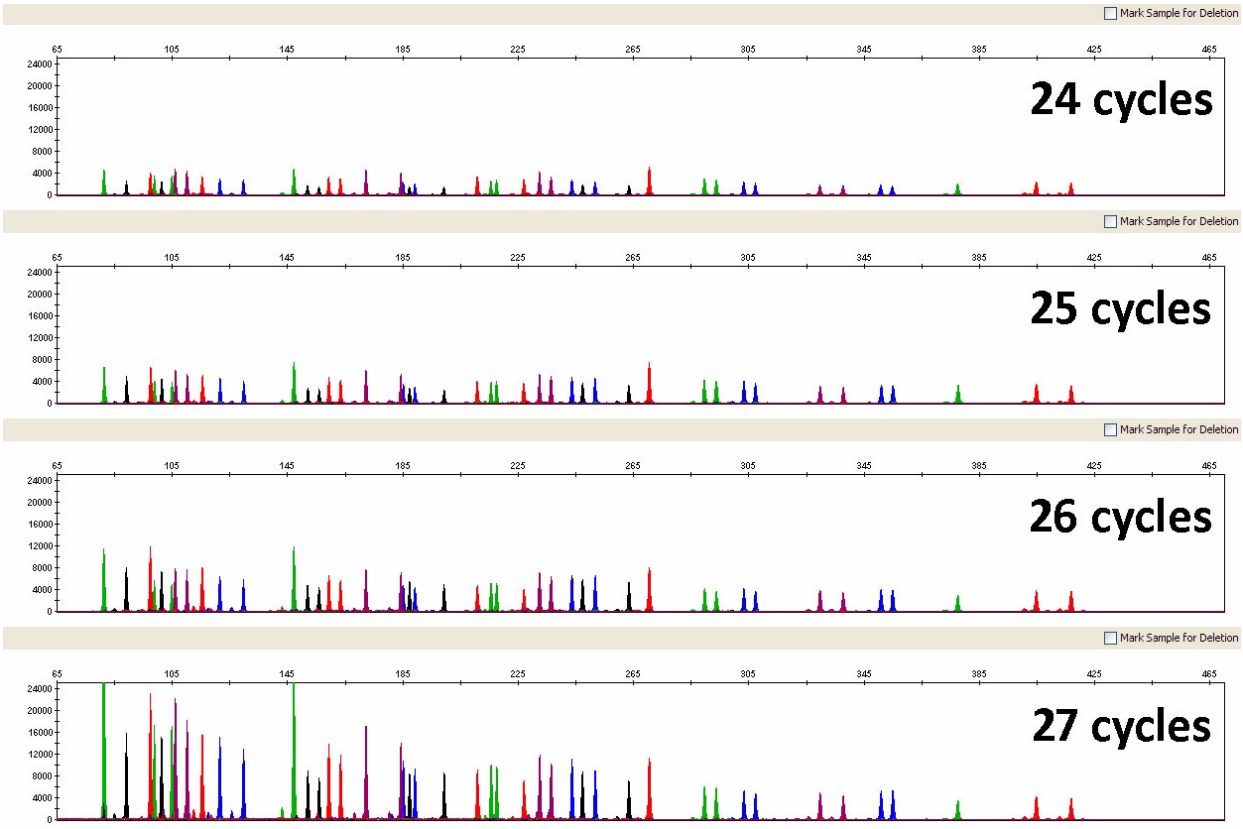


Figure 7 Representative GlobalFiler™ Express PCR Amplification Kit profiles obtained from amplification of buccal swab lysates using 24, 25, 26, and 27 cycles, analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 25,000 RFU).

Thermal cycling temperatures

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

For example, annealing/extension temperatures of 58, 59, 60, 61, and 62°C were tested using a Veriti™ Thermal Cycler (Figure 8). The PCR products were analyzed using a 3500xL Genetic Analyzer.

Of the tested annealing temperatures, 59°C to 61°C produced robust profiles. At 58°C, many smaller amplicons were preferentially amplified relative to the larger amplicons, generating a ski-slope-like STR profile. At 62°C, the yield of most loci was reduced, and the yield of Amelogenin and D7S820 was significantly affected. The optimal combination of specificity, sensitivity, and resistance to PCR

inhibition was observed at 60°C. Thermal cycler temperature is critical to assay performance; therefore, routine, regularly scheduled thermal cycler calibration is recommended.

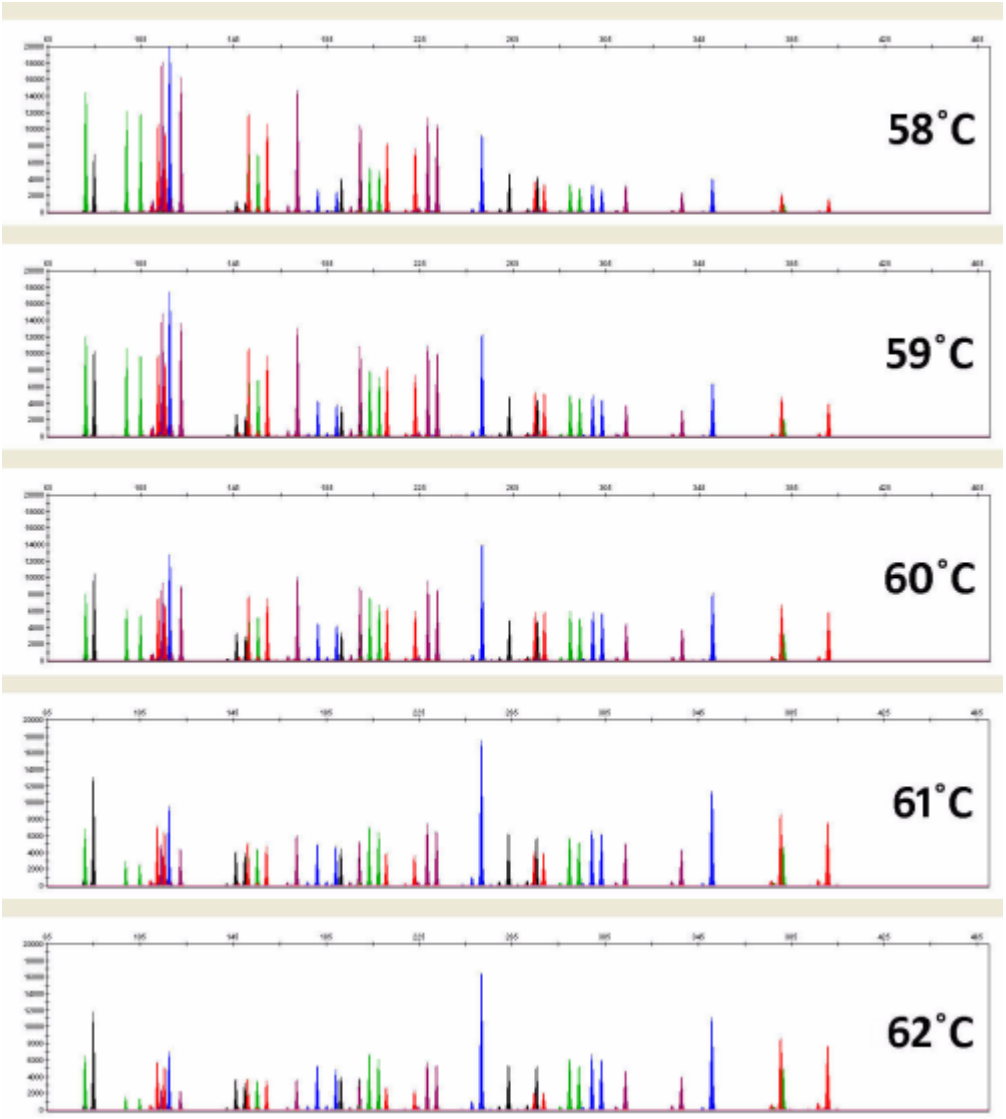


Figure 8 Electropherograms obtained from amplification of blood sample on an FTA™ card at annealing temperatures of 58, 59, 60, 61, and 62°C, analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 20,000 RFU).

Accuracy, precision, and reproducibility

SWGDAM guideline 3.5

"Precision and accuracy of the assay should be demonstrated: Precision characterizes the degree of mutual agreement among a series of individual measurements, values and/or results. Precision depends only on the distribution of random errors and does not relate to the true value or specified value. The measure of precision is usually expressed in terms of imprecision and computed as a

standard deviation of the test results. Accuracy is the degree of conformity of a measured quantity to its actual (true) value. Accuracy of a measuring instrument is the ability of a measuring instrument to give responses close to a true value." (SWGDAM, December 2012)

Accuracy observation

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

The following four figures show the size differences that are typically observed between sample alleles and allelic ladder alleles on the 3130*xl*, 3500, and 3500xL Genetic Analyzers with POP-4™ Polymer and the 3730 Genetic Analyzer with POP-7™ Polymer. The X-axis in the following figures represents the nominal nucleotide sizes for the GlobalFiler™ Express Allelic Ladder. The dashed lines parallel to the X-axis represent the ±0.25-nt windows. The y-axis represents the deviation of each sample allele size from the corresponding Allelic Ladder allele size. All sample alleles are within ±0.5 nt from a corresponding allele in the Allelic Ladder, irrespective of the capillary electrophoresis platforms.

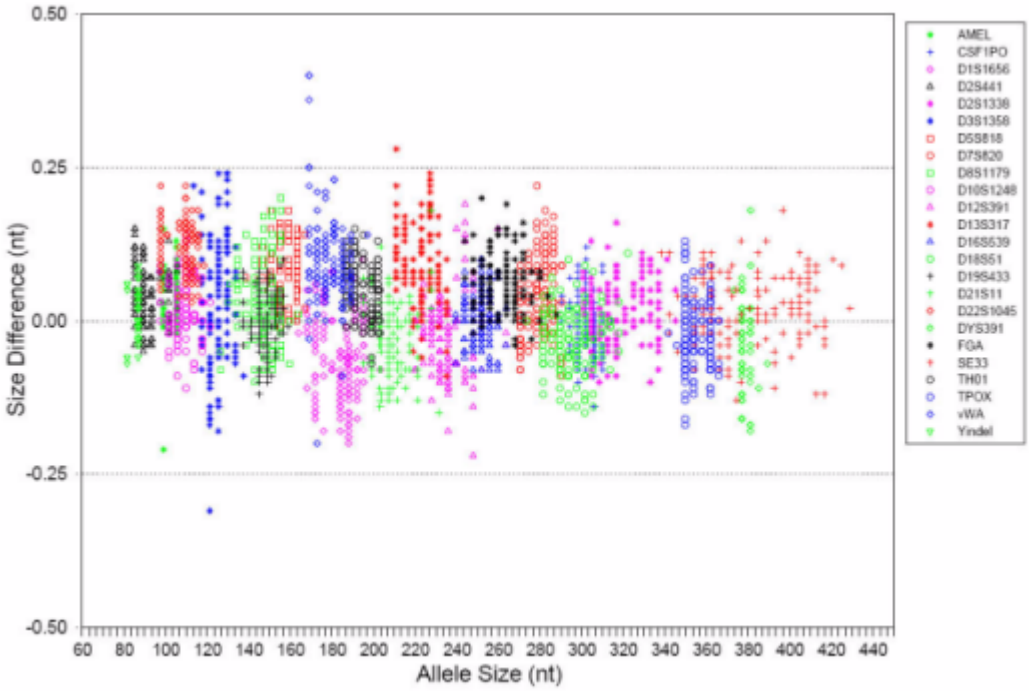


Figure 9 Allele Size vs. Allelic Ladder Sizing for 84 samples analyzed on a 3130*xl* Genetic Analyzer. Size and ladder sizing for the GlobalFiler™ Express PCR Amplification Kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.

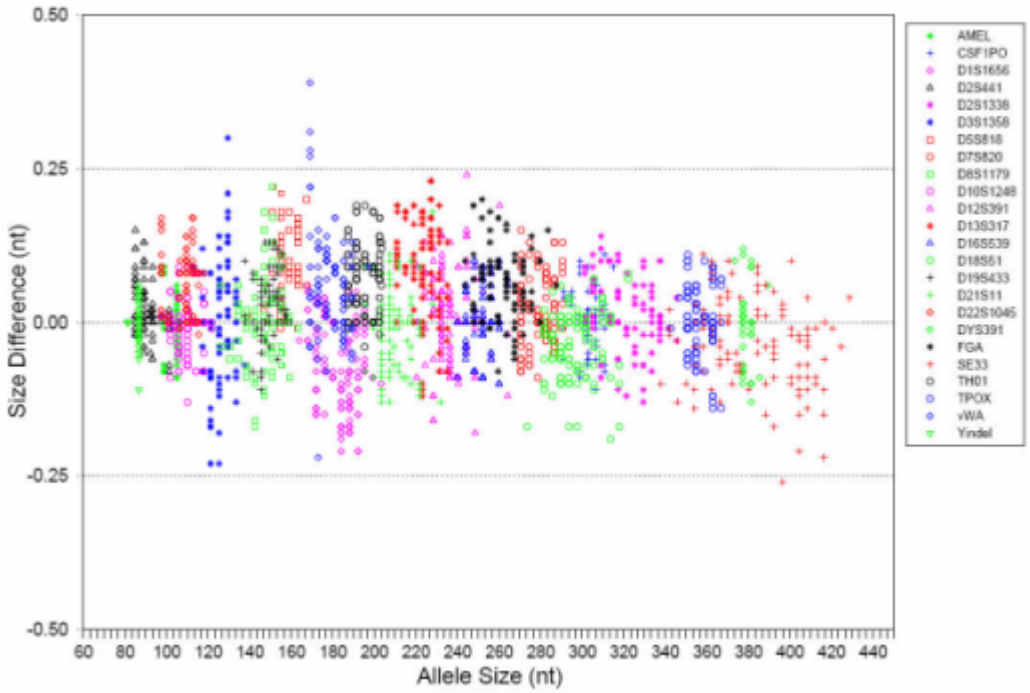


Figure 10 Allele Size vs. Allelic Ladder Sizing for 84 samples analyzed on a 3500 Genetic Analyzer. Size and ladder sizing for the GlobalFiler™ Express kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.

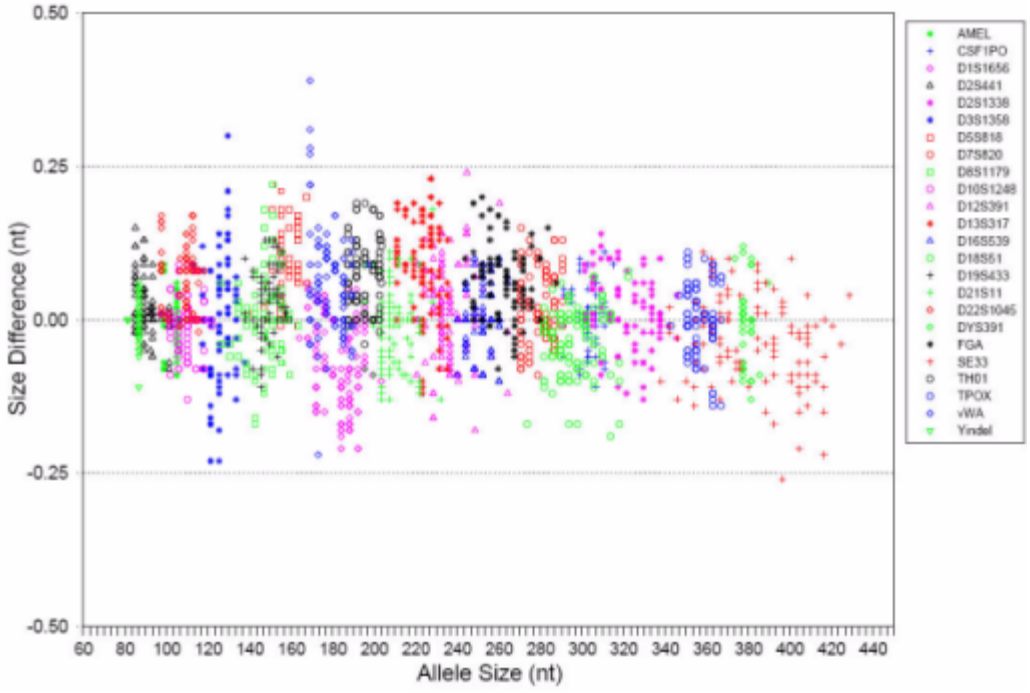


Figure 11 Allele Size vs. Allelic Ladder Sizing for 84 samples analyzed on a 3500xL Genetic Analyzer. Size and ladder sizing for the GlobalFiler™ Express kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.

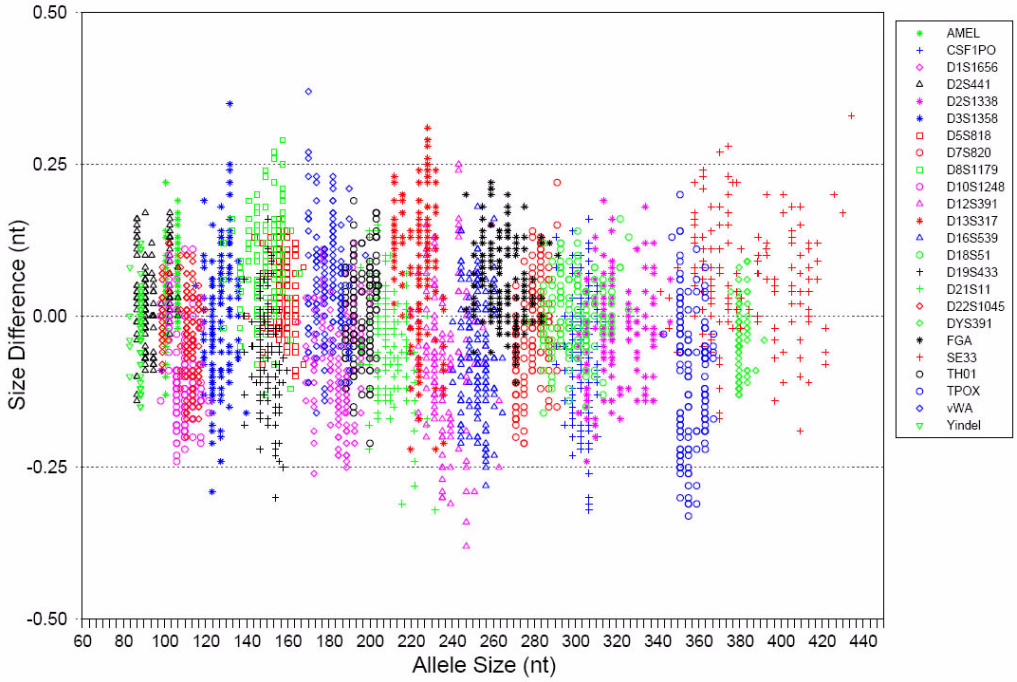


Figure 12 Allele Size vs. Allelic Ladder Sizing for 84 samples analyzed on an 3730 Genetic Analyzer. Size and ladder sizing for the GlobalFiler™ Express kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.

Precision and size window description

Sizing precision enables the determination of accurate and reliable genotypes. The recommended method for genotyping is to use a ±0.5-nt "window" around the size obtained for each allele in the allelic ladder. A ±0.5-nt window allows for the detection and correct assignment of alleles. Any sample allele that sizes outside the specified window could be either:

- An "off-ladder" allele, that is, an allele of a size that is not represented in the allelic ladder.
- An allele that does correspond to an allele in the allelic ladder, but whose size is just outside a window because of measurement error.

The measurement error inherent in any sizing method can be defined by the degree of precision in sizing an allele multiple times. Precision is measured by calculating the standard deviation in the size values obtained for an allele that is run in several injections on a capillary instrument.

Precision observation

Table 2 lists typical precision results obtained from multiple runs of the GlobalFiler™ Express Allelic Ladder using the GeneScan™ 600 LIZ™ Size Standard v2.0. The results were obtained within a set of injections on a single capillary array. The number of repeated injections for each genetic analyzer platform is shown in the following table:

CE platform	Capillaries	Number of injections	Sizing method
3130*xl*	16/injection	5	Local Southern, 60–460 bp
3500	8/injection	12	Local Southern, 60–460 bp
3500xL	24/injection	4	Local Southern, 60–460 bp
3730	48/injection	4	3rd Order Least Square

The mean sizes and the standard deviation for the allele sizing were calculated for all the alleles in each run (Table 2). The mean range and the standard deviation range show the lowest and highest values obtained across multiple runs.

Sample alleles can occasionally size outside of the ±0.5-nt window for a respective Allelic Ladder allele because of measurement error. The frequency of such an occurrence is lowest in detection systems with the smallest standard deviations in sizing. The figures in "Accuracy observation" on page 67 illustrate the tight clustering of allele sizes obtained on the Applied Biosystems™ genetic analyzers, where the standard deviation in sizing is typically less than 0.15 nt. The instance of a sample allele sizing outside the ±0.5-nt window because of measurement error is relatively rare when the standard deviation in sizing is approximately 0.15 nt or less (Smith, 1995).

For sample alleles that do not size within a ±0.5-nt window, the PCR product must be rerun to distinguish between a true off–ladder allele versus measurement error of a sample allele that corresponds to an allele in the Allelic Ladder. Repeat analysis, when necessary, provides an added level of confidence in the final allele assignment.

GeneMapper™ *ID-X* Software automatically flags sample alleles that do not size within the prescribed window around an allelic ladder allele by labeling the allele as OL (off-ladder).

Maximum sizing precision is obtained within the same set of capillary injections. Cross–platform sizing differences occur due to several factors including type and concentration of polymer, run temperature, and electrophoresis conditions. Variations in sizing can also occur between runs on the same instrument and between runs on different instruments of the same platform type because of these factors.

IMPORTANT! To minimize the variation in sizing between runs and to ensure accurate genotyping, follow the guidelines in "Allelic ladder requirements for data analysis" on page 43 and use allelic ladders obtained from the same run as samples to analyze the samples.

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

Table 2 Precision results of multiple runs of the GlobalFiler™ Express Allelic Ladder

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
AMEL								
X	98.86–98.89	0.022–0.027	98.63–98.68	0.031–0.051	98.58–98.62	0.042–0.047	100.47–100.49	0.065–0.069
Y	104.90–104.93	0.025–0.037	104.71–104.77	0.019–0.050	104.67–104.69	0.030–0.043	106.20–106.22	0.050–0.061
CSF1PO								
6	282.58–282.63	0.022–0.042	283.17–283.26	0.017–0.062	283.17–283.26	0.046–0.059	282.49–282.63	0.048–0.079
7	286.54–286.59	0.026–0.046	287.12–287.21	0.026–0.049	287.13–287.25	0.035–0.054	286.48–286.62	0.066–0.079
8	290.49–290.54	0.029–0.045	291.09–291.18	0.031–0.066	291.10–291.22	0.044–0.056	290.47–290.62	0.065–0.070
9	294.44–294.49	0.026–0.039	295.04–295.14	0.030–0.061	295.07–295.18	0.032–0.055	294.47–294.62	0.061–0.081
10	298.38–298.42	0.038–0.052	298.99–299.09	0.006–0.059	299.01–299.12	0.032–0.063	298.49–298.61	0.072–0.076
11	302.28–302.33	0.025–0.043	302.91–302.99	0.016–0.062	302.91–303.03	0.035–0.054	302.46–302.63	0.061–0.082
12	306.19–306.25	0.038–0.044	306.84–306.91	0.025–0.063	306.84–306.97	0.040–0.063	306.46–306.62	0.070–0.080
13	310.15–310.20	0.029–0.043	310.81–310.91	0.042–0.064	310.83–310.96	0.045–0.058	310.46–310.61	0.064–0.091
14	314.18–314.23	0.038–0.042	314.87–314.96	0.005–0.078	314.88–315.01	0.037–0.047	314.46–314.61	0.058–0.074
15	318.40–318.45	0.034–0.039	319.10–319.18	0.004–0.063	319.10–319.25	0.045–0.061	318.45–318.62	0.064–0.081
D10S1248								
8	85.31–85.33	0.025–0.036	85.39–85.46	0.024–0.051	85.37–85.40	0.031–0.046	86.20–86.26	0.060–0.068
9	89.40–89.43	0.025–0.035	89.53–89.58	0.022–0.055	89.49–89.52	0.033–0.045	90.15–90.22	0.062–0.075
10	93.47–93.49	0.030–0.037	93.62–93.68	0.035–0.057	93.58–93.63	0.040–0.053	94.07–94.17	0.066–0.075
11	97.54–97.57	0.026–0.040	97.73–97.79	0.006–0.054	97.70–97.73	0.045–0.050	98.04–98.11	0.057–0.069

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
12	101.62–101.65	0.029–0.036	101.84–101.90	0.017–0.053	101.80–101.86	0.041–0.045	101.96–102.07	0.048–0.074
13	105.70–105.73	0.029–0.037	105.96–106.03	0.034–0.052	105.90–105.96	0.037–0.043	105.90–106.00	0.064–0.068
14	109.75–109.79	0.030–0.038	110.02–110.09	0.014–0.065	109.98–110.05	0.038–0.048	109.85–109.95	0.065–0.074
15	113.74–113.79	0.027–0.039	114.05–114.13	0.001–0.058	114.01–114.08	0.019–0.048	113.77–113.88	0.064–0.068
16	117.65–117.69	0.026–0.035	117.95–118.02	0.001–0.046	117.90–117.97	0.038–0.050	117.70–117.81	0.059–0.070
17	121.52–121.56	0.029–0.043	121.85–121.91	0.004–0.054	121.81–121.86	0.032–0.048	121.64–121.75	0.069–0.083
18	125.40–125.45	0.028–0.041	125.77–125.85	0.022–0.062	125.73–125.79	0.036–0.048	125.56–125.69	0.066–0.082
19	129.32–129.37	0.027–0.045	129.73–129.78	0.028–0.049	129.68–129.75	0.040–0.053	129.47–129.60	0.061–0.071
D12S391								
14	216.01–216.08	0.040–0.040	216.47–216.57	0.019–0.061	216.54–216.61	0.040–0.060	215.51–215.66	0.054–0.071
15	220.06–220.12	0.040–0.045	220.52–220.59	0.039–0.067	220.61–220.67	0.040–0.055	219.47–219.59	0.054–0.074
16	224.08–224.14	0.040–0.047	224.56–224.64	0.018–0.081	224.66–224.72	0.050–0.059	223.48–223.60	0.062–0.078
17	228.02–228.08	0.029–0.047	228.53–228.62	0.030–0.095	228.61–228.69	0.052–0.065	227.36–227.48	0.063–0.072
18	231.99–232.07	0.033–0.043	232.53–232.60	0.017–0.070	232.58–232.68	0.044–0.057	231.30–231.42	0.063–0.076
19	235.95–236.01	0.030–0.044	236.50–236.55	0.026–0.076	236.55–236.64	0.050–0.058	235.20–235.32	0.064–0.083
19.3	239.02–239.08	0.029–0.046	239.52–239.62	0.001–0.071	239.60–239.70	0.048–0.065	238.19–238.32	0.062–0.075
20	239.95–240.03	0.028–0.054	240.51–240.61	0.001–0.068	240.58–240.68	0.036–0.061	239.21–239.33	0.061–0.086
21	244.03–244.09	0.033–0.045	244.55–244.65	0.025–0.061	244.62–244.72	0.047–0.055	243.07–243.20	0.067–0.081
22	247.99–248.04	0.033–0.042	248.51–248.60	0.039–0.063	248.57–248.69	0.046–0.055	246.88–247.01	0.057–0.088
23	251.99–252.05	0.024–0.039	252.50–252.58	0.028–0.056	252.57–252.66	0.051–0.056	250.84–250.97	0.065–0.074

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
24	255.97–256.02	0.031–0.047	256.50–256.58	0.014–0.055	256.54–256.64	0.038–0.053	254.90–255.01	0.064–0.075
25	259.86–259.90	0.033–0.042	260.41–260.52	0.036–0.076	260.46–260.56	0.037–0.059	258.86–258.97	0.067–0.089
26	263.80–263.84	0.032–0.039	264.37–264.46	0.033–0.058	264.41–264.52	0.045–0.061	262.77–262.90	0.064–0.079
27	267.90–267.95	0.033–0.049	268.46–268.55	0.028–0.064	268.54–268.63	0.042–0.052	266.86–266.98	0.069–0.076
D13S317								
5	198.77–198.81	0.023–0.035	198.95–199.01	0.004–0.055	198.96–198.99	0.045–0.055	199.47–199.56	0.046–0.055
6	202.77–202.82	0.024–0.043	202.92–202.97	0.035–0.065	202.94–202.97	0.039–0.048	203.51–203.58	0.047–0.056
7	206.75–206.79	0.022–0.034	206.87–206.93	206.87–206.93	206.89–206.94	0.036–0.046	207.55–207.64	0.044–0.053
8	210.82–210.87	0.027–0.037	210.93–210.99	0.009–0.065	210.96–210.98	0.044–0.050	211.70–211.78	0.046–0.053
9	214.85–214.89	0.028–0.037	214.95–214.99	0.008–0.054	214.97–215.00	0.032–0.049	215.74–215.81	0.045–0.057
10	218.95–218.97	0.022–0.033	219.04–219.09	0.032–0.054	219.08–219.09	0.028–0.053	219.76–219.83	0.042–0.058
11	223.00–223.02	0.022–0.041	223.08–223.15	0.010–0.064	223.13–223.16	0.045–0.054	223.81–223.87	0.045–0.059
12	227.11–227.13	0.024–0.040	227.17–227.25	0.031–0.061	227.24–227.25	0.052–0.067	227.90–227.96	0.047–0.054
13	231.05–231.09	0.029–0.040	231.15–231.20	0.020–0.058	231.19–231.21	0.043–0.050	231.87–231.92	0.040–0.053
14	235.01–235.05	0.029–0.036	235.11–235.17	0.020–0.063	235.15–235.18	0.042–0.057	235.80–235.86	0.043–0.051
15	239.05–239.08	0.031–0.039	239.15–239.21	0.005–0.062	239.19–239.22	0.023–0.055	239.85–239.90	0.041–0.056
16	243.18–243.21	0.025–0.036	243.26–243.31	0.005–0.046	243.29–243.32	0.035–0.049	243.89–243.93	0.048–0.051

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
D16S539								
5	227.17–227.21	0.031–0.038	227.46–227.54	0.029–0.063	227.32–227.40	0.044–0.057	228.05–228.18	0.059–0.073
8	239.33–239.37	0.028–0.044	239.66–239.73	0.001–0.057	239.49–239.58	0.042–0.051	240.14–240.26	0.067–0.077
9	243.46–243.51	0.029–0.038	243.77–243.87	0.005–0.049	243.63–243.72	0.027–0.046	244.18–244.30	0.060–0.073
10	247.59–247.63	0.025–0.045	247.90–247.98	0.001–0.049	247.76–247.84	0.039–0.047	248.20–248.32	0.059–0.070
11	251.65–251.68	0.027–0.039	251.95–252.03	0.008–0.057	251.79–251.89	0.045–0.055	252.23–252.34	0.068–0.073
12	255.61–255.65	0.025–0.037	255.93–256.00	0.006–0.050	255.78–255.86	0.039–0.045	256.26–256.38	0.059–0.074
13	259.55–259.59	0.022–0.036	259.89–259.98	0.001–0.059	259.72–259.84	0.051–0.057	260.28–260.40	0.063–0.069
14	263.55–263.60	0.025–0.038	263.91–264.00	0.015–0.059	263.75–263.86	0.050–0.054	264.30–264.43	0.062–0.076
15	267.59–267.63	0.027–0.041	267.93–268.04	0.025–0.056	267.79–267.89	0.043–0.055	268.32–268.43	0.055–0.077
D18S51								
7	261.06–261.11	0.024–0.038	261.26–261.37	0.005–0.060	261.21–261.29	0.033–0.048	263.60–263.73	0.047–0.064
9	269.20–269.24	0.025–0.041	269.40–269.49	0.028–0.056	269.33–269.39	0.038–0.054	271.85–271.99	0.048–0.057
10	273.27–273.32	0.026–0.043	273.45–273.53	0.024–0.049	273.41–273.45	0.048–0.054	276.01–276.11	0.045–0.064
10.2	275.27–275.32	0.028–0.040	275.43–275.52	0.014–0.050	275.42–275.45	0.034–0.045	277.97–278.10	0.053–0.060
11	277.35–277.37	0.022–0.035	277.50–277.59	0.009–0.058	277.48–277.52	0.043–0.059	280.14–280.26	0.050–0.058
12	281.39–281.44	0.026–0.037	281.55–281.64	0.008–0.054	281.51–281.54	0.050–0.053	284.28–284.40	0.054–0.058
13	285.42–285.46	0.027–0.038	285.58–285.66	0.018–0.056	285.52–285.55	0.042–0.047	288.40–288.53	0.044–0.064
13.2	287.38–287.42	0.028–0.037	287.54–287.62	0.025–0.052	287.49–287.53	0.049–0.053	290.40–290.51	0.051–0.059
14	289.43–289.46	0.033–0.037	289.60–289.67	0.025–0.058	289.52–289.57	0.042–0.051	292.56–292.68	0.048–0.062

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
14.2	291.41–291.43	0.027–0.035	291.56–291.64	0.023–0.060	291.50–291.54	0.041–0.052	294.55–294.65	0.054–0.059
15	293.43–293.47	0.022–0.032	293.57–293.66	0.035–0.063	293.53–293.57	0.041–0.045	296.70–296.82	0.051–0.060
16	297.44–297.47	0.024–0.039	297.58–297.69	0.008–0.055	297.54–297.57	0.034–0.048	300.86–300.95	0.049–0.056
17	301.40–301.43	0.025–0.035	301.55–301.64	0.009–0.058	301.49–301.55	0.052–0.056	304.98–305.09	0.051–0.060
18	305.36–305.39	0.028–0.046	305.51–305.60	0.028–0.065	305.47–305.52	0.043–0.045	309.12–309.23	0.048–0.054
19	309.38–309.41	0.021–0.039	309.50–309.60	0.019–0.065	309.47–309.54	0.037–0.063	313.27–313.38	0.052–0.062
20	313.44–313.48	0.030–0.040	313.55–313.66	0.000–0.065	313.53–313.59	0.033–0.063	317.41–317.52	0.050–0.058
21	317.69–317.71	0.027–0.040	317.78–317.91	0.021–0.065	317.78–317.83	0.038–0.057	321.56–321.65	0.057–0.062
22	322.00–322.01	0.028–0.044	322.07–322.20	0.012–0.067	322.06–322.10	0.026–0.056	325.74–325.85	0.050–0.061
23	326.04–326.05	0.020–0.036	326.12–326.23	0.030–0.060	326.11–326.15	0.052–0.063	329.85–329.97	0.044–0.055
24	330.13–330.15	0.024–0.035	330.21–330.30	0.030–0.055	330.19–330.25	0.036–0.058	333.99–334.10	0.051–0.061
25	334.18–334.22	0.030–0.037	334.27–334.36	0.027–0.072	334.28–334.30	0.046–0.057	338.14–338.26	0.050–0.056
26	338.22–338.26	0.025–0.049	338.29–338.41	0.008–0.052	338.30–338.35	0.036–0.053	342.29–342.41	0.052–0.063
27	342.29–342.34	0.026–0.038	342.37–342.47	0.011–0.058	342.38–342.42	0.045–0.063	346.45–346.57	0.047–0.057
D19S433								
6	118.28–118.35	0.024–0.047	118.50–118.61	0.026–0.068	118.52–118.55	0.042–0.048	120.32–120.46	0.056–0.091
7	122.03–122.10	0.024–0.045	122.28–122.39	0.042–0.085	122.33–122.34	0.040–0.052	124.04–124.19	0.056–0.089
8	125.82–125.89	0.022–0.050	126.11–126.21	0.025–0.063	126.15–126.16	0.046–0.061	127.78–127.91	0.064–0.092
9	129.64–129.68	0.031–0.049	129.94–130.05	0.040–0.067	129.99–130.01	0.051–0.054	131.52–131.67	0.063–0.107
10	133.46–133.52	0.033–0.047	133.81–133.93	0.034–0.073	133.86–133.89	0.039–0.058	135.27–135.42	0.068–0.089

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
11	137.33–137.38	0.036–0.048	137.69–137.82	0.044–0.072	137.74–137.78	0.047–0.058	139.00–139.17	0.062–0.085
12	141.22–141.27	0.021–0.044	141.63–141.76	0.044–0.068	141.67–141.73	0.042–0.058	142.78–142.93	0.072–0.091
12.2	143.21–143.27	0.030–0.051	143.63–143.75	0.042–0.084	143.69–143.74	0.038–0.055	144.68–144.85	0.064–0.085
13	145.17–145.22	0.028–0.047	145.60–145.73	0.032–0.068	145.65–145.71	0.044–0.058	146.52–146.71	0.065–0.084
13.2	147.16–147.23	0.031–0.04	147.61–147.73	0.037–0.065	147.67–147.71	0.034–0.060	148.45–148.61	0.072–0.077
14	149.11–149.17	0.036–0.045	149.56–149.69	0.041–0.076	149.64–149.69	0.047–0.062	150.30–150.48	0.059–0.082
14.2	151.11–151.18	0.029–0.051	151.58–151.71	0.035–0.074	151.63–151.71	0.046–0.068	152.22–152.40	0.066–0.093
15	153.07–153.13	0.029–0.053	153.56–153.67	0.032–0.072	153.61–153.67	0.047–0.056	154.08–154.25	0.062–0.092
15.2	155.06–155.15	0.030–0.040	155.57–155.68	0.036–0.075	155.63–155.69	0.050–0.057	156.00–156.17	0.057–0.089
16	157.02–157.11	0.032–0.052	157.53–157.66	0.031–0.077	157.60–157.66	0.046–0.063	157.86–158.04	0.070–0.086
16.2	159.03–159.12	0.038–0.050	159.57–159.67	0.036–0.071	159.61–159.68	0.030–0.060	159.79–159.96	0.069–0.087
17	160.98–161.07	0.038–0.048	161.49–161.61	0.007–0.072	161.56–161.62	0.040–0.070	161.63–161.82	0.070–0.094
17.2	162.97–163.04	0.036–0.047	163.50–163.62	0.015–0.073	163.57–163.63	0.051–0.061	163.58–163.75	0.064–0.100
18.2	166.99–167.07	0.030–0.045	167.55–167.66	0.024–0.067	167.61–167.68	0.052–0.060	167.48–167.67	0.065–0.094
19.2	170.89–170.97	0.028–0.044	171.45–171.56	0.034–0.074	171.52–171.61	0.038–0.059	171.28–171.46	0.068–0.095
D1S1656								
9	159.91–159.94	0.018–0.037	159.94–160.03	0.000–0.054	159.98–160.00	0.030–0.052	160.85–160.91	0.046–0.058
10	163.93–163.97	0.023–0.036	163.97–164.04	0.016–0.054	163.99–164.02	0.036–0.043	164.81–164.88	0.047–0.058
11	167.94–167.97	0.026–0.036	167.97–168.05	0.028–0.059	168.03–168.03	0.031–0.046	168.78–168.84	0.051–0.061
12	171.94–171.98	0.025–0.033	172.02–172.07	0.018–0.057	172.03–172.05	0.034–0.048	172.72–172.80	0.050–0.059

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
13	175.95–175.99	0.023–0.033	176.01–176.08	0.012–0.056	176.05–176.07	0.040–0.044	176.69–176.76	0.046–0.066
14	180.02–180.06	0.031–0.038	180.11–180.16	0.004–0.059	180.14–180.16	0.046–0.055	180.71–180.80	0.049–0.060
14.3	183.09–183.12	0.022–0.041	183.17–183.21	0.032–0.056	183.19–183.22	0.027–0.051	183.70–183.78	0.049–0.062
15	184.00–184.04	0.029–0.035	184.10–184.16	0.032–0.052	184.12–184.15	0.035–0.054	184.61–184.67	0.050–0.055
15.3	187.15–187.18	0.020–0.034	187.23–187.30	0.024–0.059	187.28–187.30	0.042–0.049	187.65–187.72	0.049–0.053
16	188.05–188.08	0.021–0.030	188.16–188.22	0.031–0.068	188.19–188.22	0.027–0.045	188.56–188.63	0.056–0.060
16.3	191.19–191.24	0.017–0.035	191.30–191.36	0.023–0.063	191.35–191.37	0.032–0.054	191.62–191.69	0.047–0.055
17	192.10–192.14	0.030–0.039	192.22–192.30	0.021–0.063	192.26–192.28	0.038–0.050	192.52–192.58	0.052–0.063
17.3	195.23–195.26	0.021–0.038	195.37–195.42	0.011–0.060	195.40–195.42	0.035–0.055	195.56–195.64	0.053–0.060
18.3	199.28–199.31	0.020–0.035	199.40–199.47	0.036–0.056	199.44–199.47	0.023–0.049	199.54–199.59	0.050–0.067
19.3	203.24–203.27	0.025–0.038	203.37–203.46	0.013–0.057	203.40–203.41	0.035–0.047	203.50–203.56	0.044–0.060
20.3	207.19–207.22	0.029–0.034	207.32–207.39	0.022–0.057	207.34–207.38	0.041–0.049	207.46–207.52	0.051–0.064
D21S11								
24	182.84–182.89	0.020–0.038	183.09–183.14	0.018–0.056	182.98–183.05	0.024–0.049	183.41–183.58	0.060–0.075
24.2	184.92–184.98	0.029–0.033	185.17–185.23	0.026–0.055	185.06–185.14	0.039–0.055	185.42–185.58	0.051–0.076
25	186.94–187.00	0.027–0.038	187.21–187.26	0.021–0.057	187.11–187.17	0.041–0.054	187.45–187.60	0.053–0.074
26	191.04–191.10	0.019–0.033	191.33–191.37	0.024–0.055	191.21–191.29	0.037–0.059	191.47–191.63	0.060–0.072
27	195.16–195.20	0.025–0.036	195.45–195.52	0.014–0.058	195.35–195.41	0.033–0.051	195.55–195.69	0.059–0.067
28	199.20–199.24	0.029–0.039	199.49–199.57	0.007–0.059	199.39–199.46	0.021–0.056	199.51–199.66	0.063–0.070
28.2	201.21–201.26	0.027–0.039	201.48–201.53	0.012–0.058	201.38–201.45	0.042–0.054	201.52–201.66	0.053–0.072

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
29	203.16–203.22	0.022–0.037	203.46–203.55	0.004–0.059	203.34–203.41	0.029–0.044	203.48–203.64	0.055–0.070
29.2	205.22–205.27	0.022–0.034	205.49–205.56	0.008–0.067	205.38–205.45	0.039–0.054	205.58–205.74	0.065–0.073
30	207.19–207.25	0.022–0.038	207.48–207.55	0.008–0.060	207.36–207.44	0.039–0.047	207.54–207.68	0.065–0.067
30.2	209.17–209.23	0.025–0.041	209.45–209.51	0.020–0.060	209.34–209.41	0.038–0.051	209.55–209.70	0.062–0.086
31	211.20–211.24	0.021–0.039	211.48–211.54	0.012–0.061	211.37–211.45	0.048–0.051	211.54–211.71	0.066–0.083
31.2	213.17–213.23	0.021–0.041	213.45–213.53	0.004–0.054	213.33–213.41	0.051–0.057	213.56–213.71	0.059–0.063
32	215.22–215.27	0.020–0.036	215.52–215.57	0.035–0.055	215.40–215.48	0.031–0.044	215.56–215.70	0.057–0.074
32.2	217.26–217.31	0.020–0.034	217.54–217.59	0.031–0.052	217.44–217.51	0.026–0.053	217.57–217.72	0.061–0.075
33	219.33–219.38	0.025–0.041	219.61–219.71	0.006–0.056	219.54–219.60	0.044–0.060	219.58–219.75	0.063–0.074
33.2	221.30–221.34	0.024–0.037	221.60–221.68	0.039–0.061	221.52–221.58	0.028–0.046	221.53–221.70	0.053–0.074
34	223.44–223.48	0.027–0.038	223.75–223.81	0.038–0.058	223.65–223.72	0.050–0.059	223.71–223.86	0.067–0.075
34.2	225.37–225.41	0.033–0.040	225.68–225.76	0.038–0.072	225.59–225.66	0.048–0.063	225.62–225.75	0.062–0.074
35	227.46–227.50	0.032–0.043	227.79–227.86	0.028–0.067	227.68–227.75	0.045–0.058	227.70–227.84	0.069–0.076
35.2	229.41–229.44	0.025–0.042	229.74–229.80	0.024–0.058	229.64–229.72	0.043–0.068	229.63–229.76	0.065–0.077
36	231.40–231.45	0.037–0.045	231.76–231.83	0.026–0.065	231.66–231.75	0.041–0.054	231.53–231.66	0.056–0.075
37	235.50–235.54	0.025–0.044	235.84–235.93	0.019–0.065	235.75–235.84	0.042–0.054	235.63–235.77	0.061–0.076
38	239.48–239.53	0.026–0.042	239.83–239.89	0.001–0.059	239.71–239.82	0.047–0.059	239.53–239.69	0.057–0.078

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
D22S1045								
8	88.49–88.51	0.024–0.027	88.33–88.40	0.006–0.045	88.29–88.33	0.029–0.038	90.00–90.01	0.057–0.063
9	91.47–91.48	0.023–0.032	91.33–91.39	0.006–0.047	91.29–91.32	0.026–0.043	92.94–92.96	0.056–0.069
10	94.46–94.47	0.027–0.037	94.31–94.38	0.004–0.051	94.28–94.31	0.034–0.042	95.88–95.91	0.059–0.065
11	97.44–97.46	0.027–0.033	97.30–97.37	0.006–0.050	97.28–97.29	0.030–0.040	98.83–98.87	0.051–0.068
12	100.44–100.46	0.018–0.031	100.31–100.36	0.032–0.053	100.28–100.29	0.027–0.043	101.79–101.83	0.051–0.055
13	103.47–103.49	0.018–0.035	103.36–103.39	0.011–0.070	103.31–103.33	0.028–0.050	104.76–104.78	0.058–0.065
14	106.49–106.50	0.022–0.034	106.36–106.43	0.001–0.054	106.32–106.34	0.034–0.041	107.71–107.77	0.050–0.059
15	109.48–109.49	0.028–0.039	109.36–109.41	0.014–0.053	109.33–109.34	0.040–0.049	110.67–110.72	0.052–0.059
16	112.46–112.48	0.032–0.041	112.34–112.39	0.006–0.065	112.30–112.33	0.044–0.051	113.63–113.68	0.054–0.062
17	115.39–115.41	0.023–0.034	115.27–115.34	0.001–0.051	115.24–115.26	0.024–0.037	116.60–116.64	0.048–0.059
18	118.28–118.30	0.025–0.028	118.15–118.23	0.001–0.052	118.10–118.13	0.014–0.033	119.56–119.62	0.046–0.064
19	121.15–121.17	0.029–0.037	121.06–121.10	0.014–0.051	120.98–121.00	0.029–0.048	122.53–122.60	0.044–0.059
D2S1338								
11	280.99–281.04	0.027–0.035	281.61–281.74	0.009–0.060	281.68–281.78	0.049–0.055	281.21–281.39	0.062–0.066
12	284.94–284.99	0.031–0.042	285.60–285.67	0.031–0.059	285.60–285.71	0.048–0.052	285.24–285.42	0.058–0.069
13	288.88–288.95	0.028–0.036	289.53–289.60	0.026–0.062	289.53–289.66	0.037–0.050	289.29–289.45	0.060–0.075
14	292.82–292.88	0.027–0.045	293.46–293.53	0.019–0.060	293.46–293.58	0.046–0.051	293.33–293.50	0.060–0.075
15	296.70–296.76	0.033–0.041	297.27–297.34	0.037–0.057	297.29–297.41	0.034–0.060	297.42–297.56	0.059–0.064
16	300.63–300.69	0.026–0.042	301.22–301.30	0.009–0.061	301.26–301.37	0.045–0.052	301.44–301.59	0.060–0.072

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
17	304.53–304.58	0.029–0.041	305.12–305.20	0.027–0.064	305.14–305.27	0.038–0.054	305.48–305.65	0.062–0.074
18	308.46–308.52	0.024–0.036	309.05–309.14	0.015–0.064	309.11–309.22	0.040–0.061	309.50- 309.67	0.057–0.066
19	312.46–312.51	0.029–0.042	313.06–313.16	0.008–0.061	313.12–313.25	0.043–0.059	313.54–313.72	0.062–0.066
20	316.62–316.67	0.020–0.037	317.21–317.30	0.026–0.065	317.27–317.39	0.047–0.057	317.60–317.76	0.057–0.064
21	320.81–320.86	0.025–0.043	321.40–321.49	0.042–0.064	321.45–321.58	0.038–0.063	321.65–321.82	0.061–0.074
22	324.89–324.95	0.029–0.045	325.44–325.53	0.016–0.058	325.50–325.63	0.049–0.075	325.72–325.88	0.053–0.068
23	328.91–328.99	0.029–0.038	329.48–329.58	0.031–0.066	329.57–329.67	0.050–0.062	329.76–329.93	0.057–0.077
24	332.95–333.00	0.025–0.034	333.53–333.61	0.018–0.056	333.58–333.69	0.049–0.060	333.80–333.99	0.060–0.073
25	336.94–337.00	0.024–0.035	337.51–337.59	0.016–0.065	337.57–337.67	0.050–0.058	337.85–338.05	0.057–0.065
26	340.96–341.00	0.026–0.037	341.50–341.57	0.009–0.060	341.57–341.68	0.033–0.061	341.91–342.10	0.060–0.079
27	345.05–345.09	0.023–0.036	345.58–345.67	0.023–0.057	345.64–345.74	0.047–0.054	345.98–346.18	0.060–0.067
28	349.37–349.42	0.026–0.038	349.79–349.89	0.032–0.062	349.90–349.95	0.043–0.050	350.21–350.38	0.050–0.063
D2S441								
8	76.79–76.81	0.019–0.034	76.57–76.62	0.001–0.046	76.55–76.60	0.026–0.047	78.52–78.54	0.070–0.078
9	80.93–80.95	0.026–0.034	80.74–80.77	0.000–0.047	80.70–80.75	0.035–0.048	82.51–82.55	0.067–0.072
10	85.04–85.08	0.018–0.033	84.87–84.91	0.006–0.052	84.84–84.88	0.029–0.036	86.55–86.57	0.061–0.066
11	89.16–89.18	0.023–0.031	89.01–89.05	0.015–0.048	88.97–89.02	0.036–0.043	90.56–90.59	0.050–0.068
11.3	92.32–92.35	0.025–0.033	92.19–92.24	0.018–0.049	92.16–92.18	0.032–0.040	93.68–93.70	0.058–0.064
12	93.25–93.27	0.020–0.029	93.12–93.17	0.015–0.050	93.09–93.12	0.033–0.041	94.57–94.59	0.052–0.068
13	97.18–97.20	0.022–0.031	97.07–97.11	0.005–0.048	97.03–97.06	0.032–0.046	98.44–98.47	0.047–0.061

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
14	101.30–101.32	0.025–0.033	101.18–101.23	0.011–0.053	101.17–101.18	0.011–0.045	102.46–102.49	0.053–0.067
15	105.43–105.45	0.028–0.033	105.33–105.38	0.001–0.055	105.32–105.34	0.038–0.047	106.50–106.53	0.047–0.061
16	109.55–109.56	0.027–0.037	109.46–109.51	0.030–0.055	109.45–109.46	0.031–0.046	110.51–110.56	0.053–0.061
17	113.74–113.76	0.031–0.038	113.67–113.72	0.005–0.052	113.65–113.68	0.048–0.051	114.64–114.70	0.053–0.056
D3S1358								
9	96.60–96.64	0.022–0.033	96.49–96.55	0.008–0.047	96.47–96.49	0.039–0.049	98.32–98.38	0.061–0.073
10	100.76–100.79	0.026–0.037	100.66–100.71	0.004–0.052	100.63–100.65	0.044–0.051	102.48–102.54	0.060–0.072
11	104.95–104.97	0.026–0.037	104.81–104.90	0.016–0.051	104.80–104.83	0.040–0.048	106.63–106.71	0.060–0.064
12	108.98–109.00	0.024–0.039	108.83–108.92	0.023–0.046	108.83–108.85	0.040–0.044	110.66–110.74	0.054–0.067
13	113.23–113.25	0.032–0.037	113.08–113.17	0.000–0.061	113.07–113.09	0.047–0.052	114.94–115.00	0.055–0.063
14	117.21–117.24	0.024–0.041	117.09–117.14	0.015–0.047	117.06–117.07	0.015–0.035	119.07–119.13	0.051–0.066
15	121.09–121.12	0.023–0.034	120.96–121.04	0.004–0.053	120.92–120.94	0.031–0.050	123.11–123.19	0.049–0.069
16	125.20–125.24	0.030–0.036	125.09–125.15	0.024–0.061	125.05–125.06	0.033–0.047	127.36–127.41	0.053–0.065
17	129.33–129.37	0.023–0.038	129.22–129.29	0.013–0.063	129.18–129.20	0.037–0.047	131.58–131.63	0.051–0.061
18	133.36–133.40	0.020–0.035	133.26–133.32	0.018–0.055	133.22–133.23	0.044–0.052	135.68–135.75	0.051–0.065
19	137.35–137.39	0.026–0.036	137.24–137.31	0.014–0.058	137.21–137.21	0.041–0.048	139.73–139.80	0.049–0.055
20	141.66–141.71	0.027–0.036	141.56–141.62	0.005–0.054	141.52–141.54	0.038–0.046	144.02–144.09	0.058–0.061

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
D5S818								
7	138.53–138.55	0.025–0.039	138.60–138.68	0.004–0.050	138.58–138.60	0.038–0.056	139.69–139.76	0.051–0.061
8	142.59–142.60	0.027–0.034	142.66–142.71	0.034–0.053	142.63–142.66	0.040–0.051	143.72–143.77	0.052–0.060
9	146.68–146.71	0.013–0.034	146.75–146.82	0.017–0.048	146.74–146.75	0.036–0.048	147.69–147.77	0.045–0.063
10	150.82–150.83	0.022–0.032	150.90–150.97	0.020–0.054	150.89–150.90	0.030–0.042	151.67–151.76	0.047–0.052
11	154.91–154.93	0.029–0.039	155.00–155.06	0.015–0.057	154.98–155.02	0.041–0.052	155.65–155.74	0.045–0.064
12	159.00–159.03	0.025–0.038	159.10–159.17	0.038–0.056	159.09–159.12	0.037–0.053	159.64–159.70	0.043–0.055
13	163.04–163.09	0.019–0.032	163.16–163.21	0.015–0.051	163.13–163.17	0.037–0.053	163.61–163.69	0.050–0.061
14	167.07–167.10	0.026–0.034	167.16–167.24	0.030–0.055	167.17–167.20	0.040–0.044	167.57–167.66	0.056–0.064
15	171.08–171.12	0.021–0.032	171.20–171.26	0.025–0.051	171.20–171.22	0.034–0.056	171.56–171.64	0.055–0.064
16	175.09–175.13	0.029–0.032	175.22–175.27	0.038–0.062	175.22–175.25	0.038–0.055	175.54–175.61	0.052–0.061
17	179.10–179.14	0.021–0.039	179.25–179.31	0.005–0.052	179.25–179.27	0.005–0.050	179.50–179.59	0.054–0.066
18	183.16–183.20	0.021–0.035	183.32–183.39	0.026–0.062	183.30–183.37	0.027–0.049	183.49–183.58	0.053–0.070
D7S820								
6	262.40–262.42	0.025–0.040	262.54–262.60	0.034–0.057	262.55–262.60	0.035–0.051	262.97–263.01	0.049–0.058
7	266.41–266.43	0.027–0.044	266.56–266.62	0.020–0.058	266.55–266.61	0.043–0.052	266.96–266.99	0.050–0.057
8	270.41–270.43	0.025–0.033	270.57–270.63	0.027–0.068	270.58–270.62	0.037–0.053	270.92–270.98	0.050–0.057
9	274.43–274.45	0.029–0.034	274.59–274.64	0.027–0.051	274.62–274.64	0.035–0.050	274.92–274.97	0.052–0.060
10	278.44–278.46	0.026–0.032	278.61–278.69	0.007–0.060	278.63–278.66	0.038–0.059	278.92–278.97	0.051–0.058
11	282.43–282.45	0.023–0.032	282.62–282.67	0.011–0.066	282.60–282.64	0.035–0.048	282.91–282.96	0.043–0.068

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
12	286.40–286.41	0.028–0.036	286.58–286.64	0.033–0.057	286.57–286.60	0.042–0.054	286.91–286.96	0.051–0.068
13	290.35–290.38	0.026–0.032	290.54–290.61	0.025–0.057	290.54–290.58	0.036–0.045	290.91–290.95	0.052–0.059
14	294.30–294.33	0.027–0.037	294.49–294.57	0.015–0.062	294.51–294.54	0.033–0.054	294.89–294.94	0.050–0.053
15	298.25–298.26	0.026–0.051	298.46–298.54	0.006–0.060	298.46–298.49	0.042–0.052	298.89–298.94	0.058–0.060
D8S1179								
5	114.45–114.46	0.029–0.036	114.21–114.27	0.032–0.052	114.15–114.18	0.031–0.046	116.07–116.10	0.052–0.064
6	118.42–118.43	0.024–0.035	118.20–118.25	0.000–0.054	118.12–118.16	0.029–0.044	120.19–120.24	0.056–0.062
7	122.38–122.40	0.022–0.036	122.17–122.21	0.034–0.057	122.09–122.12	0.035–0.050	124.33–124.37	0.057–0.065
8	126.39–126.41	0.024–0.031	126.18–126.23	0.008–0.052	126.08–126.12	0.035–0.049	128.45–128.50	0.049–0.070
9	130.41–130.43	0.030–0.038	130.21–130.25	0.018–0.062	130.11–130.13	0.039–0.047	132.58–132.61	0.051–0.059
10	134.45–134.49	0.024–0.035	134.25–134.30	0.015–0.049	134.17–134.19	0.037–0.054	136.68–136.75	0.048–0.062
11	138.52–138.57	0.028–0.032	138.32–138.36	0.004–0.054	138.24–138.27	0.037–0.050	140.81–140.86	0.053–0.068
12	142.66–142.70	0.027–0.040	142.45–142.50	0.012–0.053	142.37–142.39	0.032–0.040	144.93–144.98	0.051–0.060
13	146.89–146.93	0.017–0.039	146.71–146.74	0.023–0.054	146.61–146.64	0.034–0.042	149.11–149.16	0.053–0.060
14	151.05–151.08	0.025–0.035	150.85–150.89	0.025–0.053	150.76–150.78	0.034–0.040	153.21–153.26	0.053–0.067
15	155.21–155.24	0.025–0.038	155.00–155.04	0.015–0.057	154.90–154.93	0.036–0.046	157.31–157.36	0.046–0.059
16	159.37–159.40	0.028–0.035	159.16–159.19	0.004–0.057	159.07–159.08	0.032–0.046	161.39–161.45	0.052–0.066
17	163.46–163.49	0.020–0.035	163.25–163.28	0.031–0.051	163.16–163.19	0.030–0.048	165.50–165.54	0.054–0.062
18	167.54–167.57	0.022–0.037	167.32–167.36	0.031–0.048	167.23–167.26	0.037–0.050	169.59–169.62	0.050–0.062
19	171.62–171.64	0.025–0.034	171.39–171.42	0.024–0.063	171.30–171.33	0.036–0.051	173.66–173.72	0.057–0.062

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
DYS391								
7	364.73–364.78	0.028–0.044	365.17–365.27	0.028–0.062	365.11–365.19	0.045–0.066	367.09–367.32	0.053–0.065
8	368.80–368.85	0.027–0.045	369.25–369.35	0.025–0.060	369.18–369.28	0.043–0.061	371.22–371.44	0.054–0.068
9	372.81–372.85	0.027–0.036	373.25–373.34	0.022–0.064	373.18–373.28	0.046–0.057	375.29–375.52	0.043–0.063
10	376.80–376.86	0.029–0.039	377.24–377.34	0.011–0.067	377.17–377.27	0.044–0.063	379.37–379.59	0.050–0.066
11	380.85–380.89	0.030–0.042	381.26–381.34	0.005–0.057	381.21–381.30	0.044–0.054	383.46–383.67	0.056–0.067
12	384.90–384.95	0.030–0.038	385.24–385.35	0.024–0.064	385.21–385.29	0.049–0.059	387.45–387.65	0.055–0.074
13	389.00–389.04	0.023–0.046	389.33–389.43	0.027–0.065	389.30–389.37	0.046–0.056	391.61–391.80	0.055–0.070
FGA								
13	223.48–223.53	0.028–0.038	223.35–223.42	0.038–0.080	223.43–223.50	0.036–0.060	226.22–226.30	0.050–0.069
14	227.48–227.55	0.037–0.043	227.38–227.46	0.029–0.079	227.47–227.53	0.045–0.061	230.28–230.34	0.051–0.073
15	231.52–231.59	0.024–0.041	231.40–231.48	0.034–0.073	231.50–231.56	0.050–0.065	234.36–234.41	0.059–0.078
16	235.55–235.62	0.031–0.039	235.43–235.49	0.024–0.077	235.53–235.59	0.047–0.062	238.42–238.47	0.057–0.073
17	239.58–239.64	0.017–0.036	239.45–239.52	0.004–0.071	239.55–239.60	0.025–0.052	242.50–242.54	0.053–0.080
18	243.70–243.75	0.027–0.035	243.55–243.64	0.039–0.087	243.65–243.72	0.042–0.051	246.55–246.61	0.051–0.073
19	247.82–247.87	0.022–0.036	247.63–247.71	0.011–0.065	247.76–247.81	0.053–0.056	250.63–250.67	0.053–0.074
20	251.86–251.89	0.023–0.034	251.68–251.75	0.004–0.070	251.78–251.83	0.024–0.046	254.71–254.76	0.057–0.074
21	255.81–255.84	0.027–0.039	255.61–255.70	0.006–0.070	255.74–255.79	0.032–0.047	258.77–258.84	0.060–0.074
22	259.73–259.77	0.031–0.039	259.55–259.61	0.000–0.072	259.66–259.71	0.024–0.056	262.85–262.91	0.061–0.073
23	263.74–263.78	0.028–0.041	263.55–263.62	0.031–0.078	263.68–263.72	0.050–0.056	266.92–266.98	0.060–0.079

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
24	267.74–267.79	0.022–0.029	267.55–267.62	0.025–0.067	267.65–267.73	0.038–0.055	270.94–271.01	0.058–0.081
25	271.79–271.84	0.031–0.038	271.58–271.66	0.024–0.067	271.72–271.77	0.041–0.053	275.08–275.15	0.060–0.081
26	275.80–275.87	0.024–0.032	275.61–275.67	0.027–0.063	275.73–275.80	0.044–0.054	279.16–279.20	0.055–0.074
26.2	277.84–277.88	0.026–0.035	277.62–277.70	0.009–0.064	277.76–277.84	0.044–0.059	281.25–281.29	0.059–0.081
27	279.82–279.85	0.028–0.038	279.59–279.66	0.040–0.059	279.70–279.78	0.025–0.060	283.20–283.26	0.059–0.075
28	283.81–283.84	0.029–0.038	283.59–283.65	0.008–0.064	283.68–283.74	0.042–0.053	287.29–287.33	0.051–0.082
29	287.79–287.84	0.030–0.048	287.57–287.63	0.027–0.048	287.67–287.75	0.040–0.055	291.39–291.45	0.062–0.082
30	291.80–291.85	0.034–0.046	291.57–291.63	0.029–0.065	291.69–291.75	0.044–0.051	295.53–295.60	0.063–0.083
30.2	293.58–293.63	0.027–0.038	293.35–293.42	0.019–0.057	293.46–293.53	0.043–0.050	297.22–297.28	0.063–0.072
31.2	297.55–297.61	0.034–0.037	297.31–297.37	0.008–0.055	297.42–297.50	0.044–0.053	301.32–301.38	0.056–0.085
32.2	301.49–301.54	0.032–0.044	301.24–301.33	0.006–0.066	301.38–301.44	0.031–0.057	305.43–305.49	0.062–0.078
33.2	305.42–305.46	0.028–0.040	305.17–305.25	0.028–0.064	305.31–305.37	0.036–0.056	309.52–309.57	0.059–0.085
42.2	342.26–342.29	0.028–0.049	341.96–342.00	0.008–0.060	342.11–342.18	0.036–0.052	346.61–346.66	0.060–0.086
43.2	346.34–346.37	0.033–0.042	346.01–346.09	0.022–0.071	346.17–346.27	0.036–0.060	350.71–350.78	0.063–0.085
44.2	350.42–350.45	0.025–0.047	350.10–350.16	0.027–0.077	350.24–350.34	0.042–0.047	354.79–354.84	0.061–0.078
45.2	354.49–354.55	0.022–0.041	354.18–354.24	0.028–0.077	354.34–354.42	0.050–0.062	358.91–358.96	0.065–0.089
46.2	358.43–358.46	0.027–0.053	358.12–358.18	0.008–0.064	358.27–358.37	0.028–0.060	362.98–363.02	0.060–0.097
47.2	362.45–362.49	0.030–0.043	362.13–362.19	0.046–0.066	362.29–362.37	0.051–0.058	367.09–367.14	0.072–0.094
48.2	366.52–366.54	0.027–0.041	366.21–366.24	0.017–0.065	366.37–366.45	0.044–0.060	371.22–371.30	0.062–0.092

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
50.2	374.50–374.53	0.026–0.038	374.16–374.21	0.029–0.074	374.32–374.41	0.037–0.062	379.46–379.51	0.068–0.098
51.2	378.51–378.57	0.027–0.040	378.13–378.21	0.042–0.065	378.32–378.41	0.047–0.066	383.56–383.62	0.063–0.095
SE33								
4.2	307.08–307.10	0.024–0.037	307.12–307.19	0.031–0.080	307.19–307.22	0.038–0.053	310.30–310.35	0.047–0.057
6.3	316.31–316.34	0.028–0.036	316.31–316.39	0.018–0.057	316.40–316.43	0.036–0.051	319.58–319.65	0.045–0.063
8	321.56–321.58	0.024–0.037	321.54–321.62	0.006–0.065	321.65–321.67	0.041–0.067	324.71–324.77	0.047–0.057
9	325.67–325.70	0.024–0.038	325.63–325.72	0.017–0.063	325.76–325.77	0.047–0.062	328.83–328.88	0.056–0.062
11	333.77–333.80	0.029–0.039	333.74–333.83	0.032–0.053	333.86–333.88	0.041–0.049	337.06–337.12	0.051–0.063
12	337.85–337.87	0.030–0.040	337.83–337.88	0.016–0.060	337.93–337.94	0.048–0.054	341.22–341.28	0.052–0.061
13	341.90–341.93	0.029–0.038	341.85–341.93	0.011–0.062	341.98–342.00	0.025–0.052	345.34–345.43	0.053–0.061
14	346.01–346.03	0.032–0.048	345.93–346.02	0.026–0.055	346.07–346.09	0.044–0.053	349.47–349.55	0.051–0.063
15	350.07–350.09	0.031–0.041	349.99–350.10	0.022–0.055	350.11–350.14	0.037–0.051	353.55–353.63	0.050–0.058
16	354.17–354.20	0.030–0.043	354.11–354.20	0.026–0.060	354.22–354.25	0.043–0.060	357.75–357.82	0.054–0.067
17	358.26–358.29	0.031–0.038	358.20–358.29	0.008–0.060	358.34–358.36	0.055–0.065	361.90–361.97	0.057–0.062
18	362.32–362.33	0.026–0.042	362.26–362.35	0.010–0.065	362.41–362.43	0.024–0.059	366.01–366.09	0.059–0.065
19	366.28–366.29	0.026–0.041	366.20–366.31	0.030–0.064	366.34–366.36	0.042–0.052	370.09–370.16	0.059–0.068
20	370.32–370.34	0.026–0.036	370.24–370.33	0.031–0.065	370.38–370.40	0.036–0.050	374.26–374.33	0.059–0.069
20.2	372.32–372.33	0.028–0.041	372.23–372.31	0.026–0.054	372.37–372.39	0.048–0.059	376.32–376.39	0.051–0.067
21	374.33–374.34	0.027–0.036	374.25–374.33	0.026–0.065	374.39–374.40	0.041–0.049	378.37–378.44	0.059–0.072
21.2	376.34–376.35	0.025–0.035	376.23–376.32	0.033–0.064	376.39–376.41	0.047–0.051	380.47–380.51	0.059–0.070

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
22.2	370.32–370.34	0.026–0.036	380.22–380.30	0.040–0.065	370.38–370.40	0.036–0.050	384.54–384.60	0.042–0.072
23.2	372.32–372.33	0.028–0.041	384.30–384.41	0.034–0.074	372.37–372.39	0.048–0.059	388.72–388.78	0.061–0.069
24.2	374.33–374.34	0.027–0.036	388.36–388.45	0.023–0.059	374.39–374.40	0.041–0.049	392.84–392.88	0.061–0.072
25.2	376.34–376.35	0.025–0.035	392.34–392.42	0.033–0.062	376.39–376.41	0.047–0.051	396.89–396.93	0.061–0.071
26.2	380.33–380.36	0.028–0.044	396.47–396.54	0.010–0.072	380.38–380.40	0.003–0.055	401.09–401.14	0.062–0.075
27.2	384.45–384.47	0.024–0.039	400.48–400.57	0.041–0.065	384.48–384.52	0.050–0.056	405.22–405.25	0.062–0.081
28.2	388.50–388.51	0.032–0.043	404.41–404.50	0.021–0.067	388.52–388.57	0.042–0.059	409.28–409.32	0.061–0.070
29.2	392.49–392.52	0.030–0.046	408.37–408.49	0.037–0.076	392.51–392.54	0.046–0.056	413.39–413.45	0.062–0.075
30.2	396.61–396.65	0.035–0.043	412.39–412.49	0.010–0.071	396.62–396.66	0.030–0.055	417.54–417.60	0.064–0.077
31.2	400.64–400.67	0.036–0.050	416.40–416.49	0.026–0.058	400.65–400.70	0.047–0.054	421.59–421.65	0.060–0.074
32.2	404.58–404.60	0.037–0.046	420.56–420.65	0.006–0.063	404.58–404.61	0.050–0.061	425.81–425.85	0.060–0.077
33.2	408.56–408.60	0.033–0.050	424.57–424.68	0.027–0.077	408.55–408.60	0.049–0.056	429.91–429.96	0.063–0.075
34.2	412.58–412.60	0.024–0.049	428.62–428.72	0.033–0.063	412.57–412.58	0.043–0.057	434.03–434.07	0.064–0.085
35	416.59–416.60	0.019–0.042	430.63–430.72	0.033–0.074	416.57–416.60	0.048–0.057	436.09–436.13	0.069–0.094
35.2	420.74–420.77	0.037–0.051	432.62–432.72	0.038–0.068	420.71–420.74	0.042–0.066	438.11–438.15	0.069–0.082
36	424.76–424.81	0.030–0.055	434.62–434.74	0.031–0.072	424.73–424.78	0.048–0.066	440.17–440.20	0.068–0.084
37	428.79–428.82	0.030–0.050	438.57–438.71	0.039–0.070	428.75–428.79	0.054–0.059	444.19–444.23	0.068–0.084

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
TH01								
4	178.72–178.81	0.022–0.042	179.17–179.24	0.001–0.080	179.17–179.24	0.046–0.059	180.08–180.29	0.056–0.075
5	182.76–182.83	0.030–0.047	183.21–183.29	0.034–0.070	183.21–183.28	0.045–0.054	184.03–184.25	0.053–0.081
6	186.80–186.87	0.031–0.042	187.28–187.36	0.034–0.068	187.29–187.36	0.043–0.054	187.99–188.19	0.056–0.078
7	190.85–190.92	0.023–0.042	191.34–191.43	0.025–0.064	191.35–191.42	0.045–0.052	191.95–192.15	0.049–0.071
8	194.88–194.96	0.026–0.040	195.39–195.49	0.031–0.070	195.39–195.46	0.042–0.047	195.91–196.13	0.065–0.069
9	198.91–198.99	0.029–0.043	199.44–199.53	0.000–0.080	199.43–199.50	0.050–0.057	199.90–200.08	0.054–0.074
9.3	201.95–202.04	0.025–0.041	202.46–202.55	0.010–0.067	202.47–202.53	0.047–0.053	202.92–203.11	0.060–0.078
10	202.86–202.94	0.027–0.044	203.38–203.48	0.013–0.065	203.36–203.44	0.039–0.053	203.84–204.04	0.052–0.078
11	206.83–206.90	0.031–0.041	207.31–207.45	0.026–0.071	207.32–207.40	0.047–0.057	207.81–208.00	0.058–0.075
13.3	217.84–217.91	0.029–0.042	218.35–218.43	0.017–0.088	218.37–218.44	0.041–0.052	218.72–218.92	0.057–0.068
TPOX								
5	337.43–337.50	0.024–0.046	338.35–338.51	0.008–0.061	338.30–338.51	0.049–0.060	338.62–338.92	0.078–0.089
6	341.43–341.54	0.030–0.052	342.41–342.59	0.012–0.065	342.35–342.56	0.055–0.065	342.67–342.96	0.071–0.095
7	345.60–345.67	0.030–0.050	346.57–346.71	0.021–0.053	346.49–346.69	0.050–0.059	346.76–347.04	0.081–0.092
8	349.60–349.66	0.035–0.042	350.55–350.76	0.021–0.061	350.51–350.72	0.040–0.055	350.74–351.05	0.075–0.102
9	353.66–353.71	0.026–0.048	354.66–354.79	0.028–0.078	354.58–354.79	0.046–0.064	354.78–355.08	0.084–0.110
10	357.69–357.76	0.035–0.051	358.71–358.85	0.006–0.061	358.64–358.84	0.051–0.068	358.81–359.12	0.082–0.101
11	361.72–361.77	0.031–0.045	362.73–362.88	0.011–0.066	362.66–362.89	0.047–0.075	362.85–363.16	0.082–0.098
12	365.71–365.78	0.035–0.056	366.73–366.89	0.028–0.075	366.68–366.89	0.055–0.075	366.88–367.18	0.085–0.094

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
13	369.72–369.79	0.035–0.046	370.76–370.92	0.030–0.062	370.70–370.91	0.054–0.066	370.94–371.22	0.089–0.101
14	373.71–373.79	0.035–0.052	374.77–374.93	0.022–0.071	374.70–374.91	0.055–0.077	374.96–375.25	0.087–0.103
15	377.72–377.79	0.037–0.050	378.78–378.94	0.005–0.065	378.70–378.92	0.059–0.067	379.02–379.30	0.076–0.097
Y indel								
1	81.26–81.31	0.022–0.035	81.08–81.17	0.015–0.049	81.05–81.10	0.033–0.045	82.91–82.99	0.067–0.077
2	86.53–86.58	0.028–0.034	86.38–86.43	0.011–0.060	86.34–86.35	0.028–0.046	88.23–88.31	0.064–0.072
vWA								
11	156.50–156.52	0.023–0.033	156.60–156.65	0.015–0.048	156.55–156.59	0.040–0.048	157.91–158.01	0.042–0.064
12	160.56–160.59	0.019–0.036	160.67–160.71	0.005–0.053	160.64–160.66	0.038–0.055	161.82–161.93	0.054–0.059
13	164.61–164.65	0.021–0.035	164.73–164.77	0.011–0.047	164.68–164.72	0.037–0.052	165.83–165.93	0.053–0.059
14	168.81–168.86	0.023–0.032	168.95–169.00	0.029–0.052	168.93–168.97	0.040–0.047	169.96–170.07	0.049–0.057
15	172.75–172.77	0.026–0.033	172.86–172.91	0.017–0.055	172.84–172.87	0.037–0.040	173.90–174.01	0.054–0.064
16	176.76–176.80	0.021–0.034	176.91–176.95	0.008–0.049	176.87–176.90	0.037–0.049	177.90–177.99	0.051–0.060
17	180.81–180.83	0.019–0.033	180.95–180.99	0.004–0.056	180.90–180.96	0.045–0.053	181.87–181.98	0.054–0.063
18	184.84–184.89	0.025–0.037	185.02–185.06	0.010–0.050	184.97–185.01	0.038–0.053	185.78–185.91	0.056–0.062
19	188.93–188.95	0.024–0.034	189.10–189.16	0.027–0.054	189.08–189.12	0.043–0.052	189.77–189.88	0.055–0.056
20	193.00–193.02	0.028–0.039	193.19–193.24	0.023–0.059	193.16–193.20	0.036–0.057	193.76–193.86	0.049–0.055
21	197.02–197.05	0.029–0.037	197.23–197.28	0.008–0.060	197.19–197.24	0.046–0.052	197.67–197.79	0.054–0.066
22	201.03–201.06	0.026–0.035	201.25–201.31	0.035–0.056	201.21–201.26	0.044–0.054	201.66–201.76	0.058–0.076

Allele	3130*xl*		3500		3500xL		3730	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
23	204.91–204.95	0.024–0.036	205.12–205.19	0.015–0.047	205.08–205.13	0.041–0.048	205.41–205.53	0.048–0.065
24	209.23–209.26	0.024–0.040	209.43–209.50	0.028–0.063	209.40–209.44	0.041–0.053	209.89–209.99	0.051–0.061

Extra peaks in the electropherogram

Causes of extra peaks

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

Extra peaks: Stutter

Stutter definition

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

Contact HID Support for more information on plus stutter.

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

Stutter observations

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

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

Marker-specific plus stutter observed in the population study with the GlobalFiler™ Express PCR Amplification Kit is shown in Figure 13 through Figure 19 and listed in Table 3.

Additional marker-specific plus stutter observed in the population study with the GlobalFiler™ Express PCR Amplification Kit is listed in “Stutter observations” on page 91. Examples of non-standard stutter peaks at two loci are shown in “Example of non-standard stutter peaks observed at the D22S1045 and SE33 loci” on page 96.

The stutter filter settings that are derived from this data are listed in “Stutter percentage filter settings that are provided with the GeneMapper™ ID-X Software” on page 98.

Workflow	Number of samples
Treated paper	284 blood samples on FTA™ Classic Cards 272 buccal samples on Indicating FTA™ Cards
Untreated paper	45 blood samples on FTA™ Bloodstain Cards 90 buccal samples on Bode Buccal DNA Collector™ Device
Buccal swab	45 buccal swab samples lysed in Prep-n-Go™ Buffer

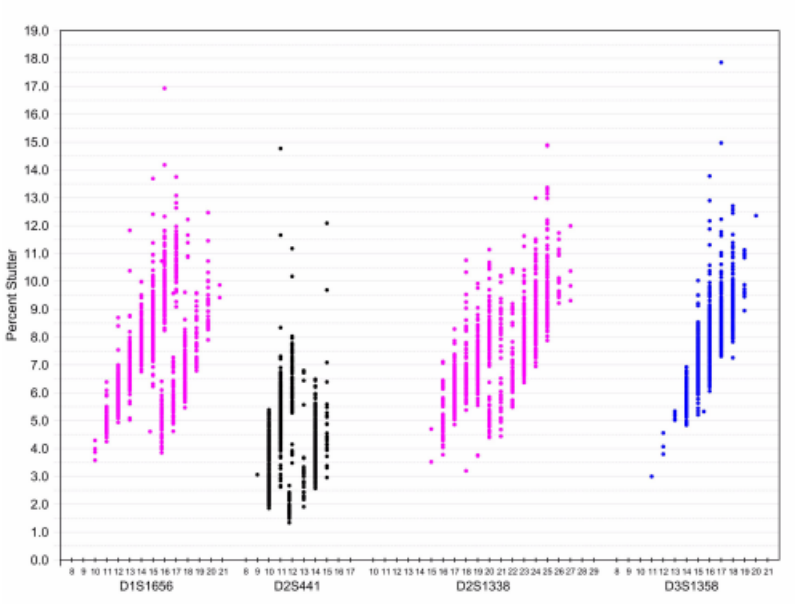


Figure 13 Stutter percentages for D1S1656, D2S441, D2S1338, and D3S1358 loci (Blue=FAM™ dye, black=NED™ dye, purple=SID™ dye)

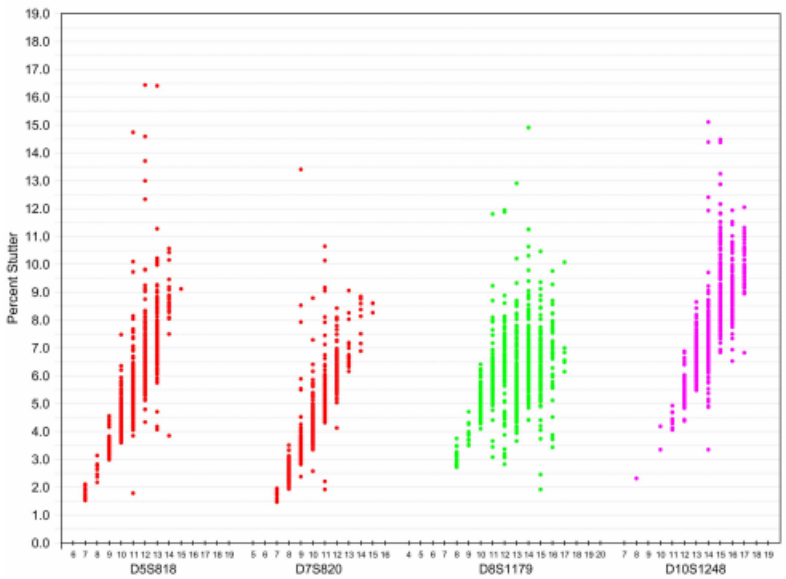


Figure 14 Stutter percentages for D5S818, D7S820, D8S1179, and D10S1248 (Green=VIC™ dye, red=TAZ™ dye, purple=SID™ dye)

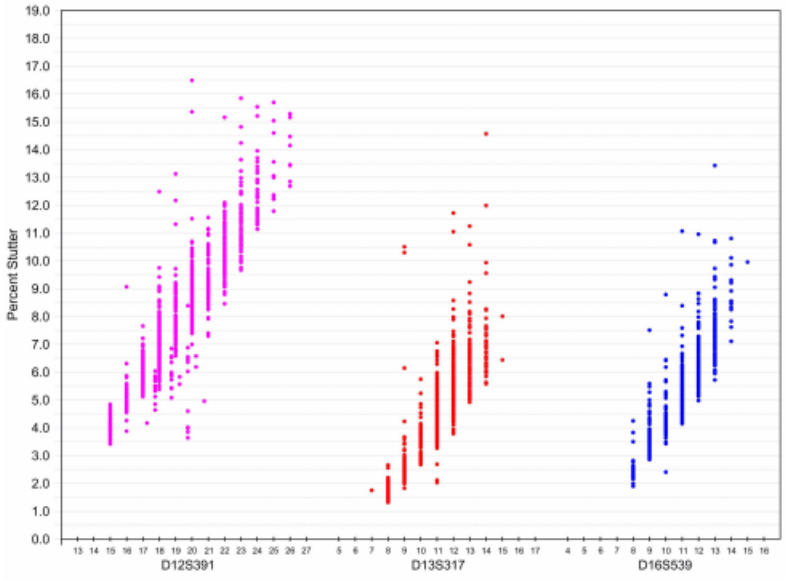


Figure 15 Stutter percentages for D12S391, D13S317, and D16S539 loci (Blue=FAM™ dye, red=TAZ™ dye, purple=SID™ dye)

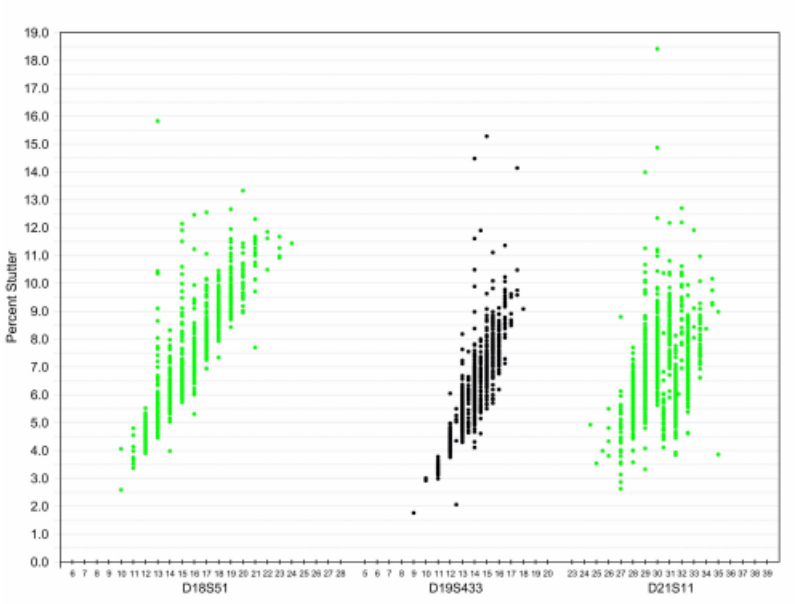


Figure 16 Stutter percentages for D18S51, D19S433, and D21S11 loci (Green=VIC™ dye, black=NED™ dye)

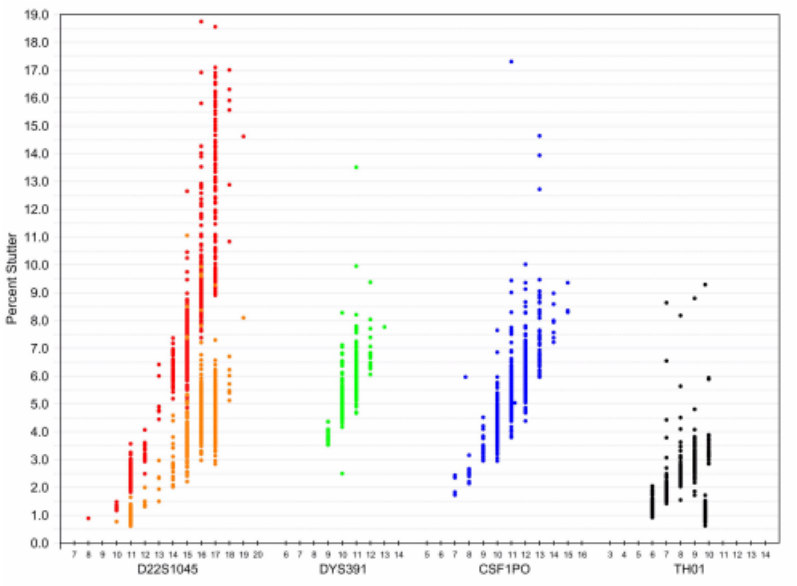


Figure 17 Stutter percentages for D22S1045, DYS391, CSF1PO, and TH01 loci (Blue=FAM™ dye, green=VIC™ dye, black=NED™ dye, red=TAZ™ dye). Red and orange data points associated with D22S1045 locus indicate minus and plus stutter, respectively.

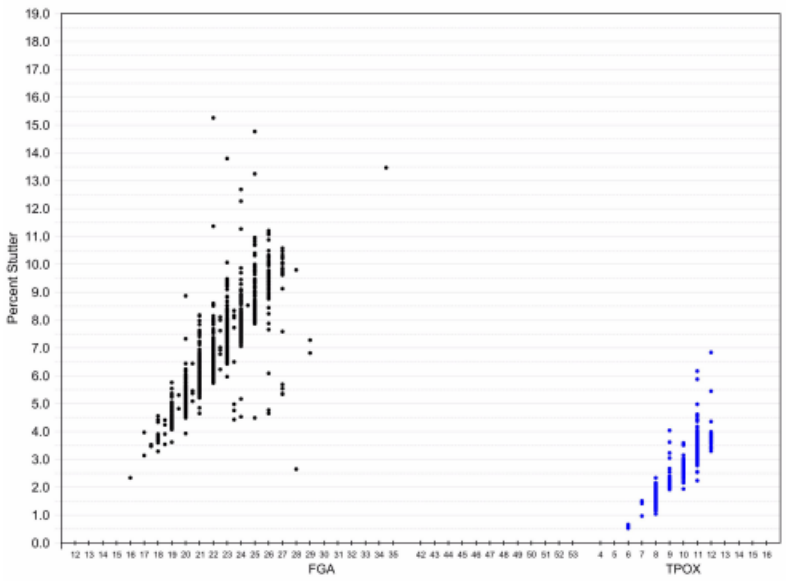


Figure 18 Stutter percentages for FGA and TPOX loci (Blue=FAM™ dye, black=NED™ dye)

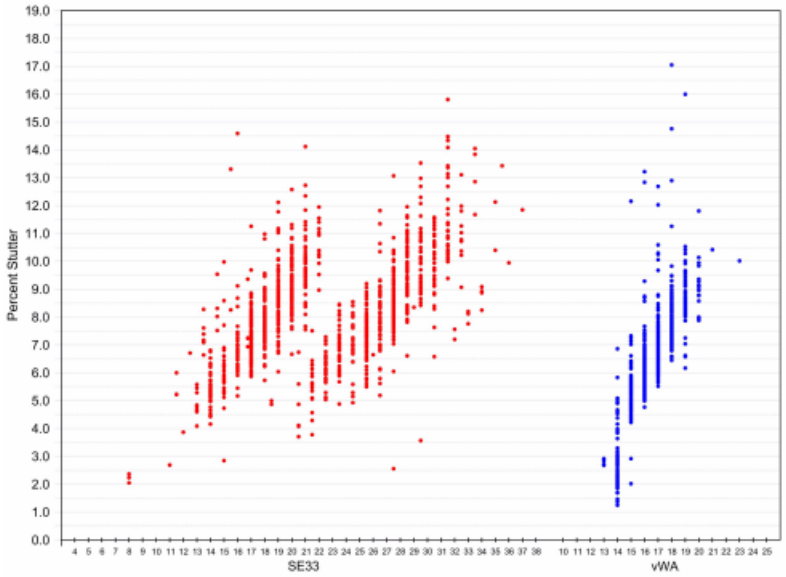


Figure 19 Stutter percentages for SE33 and vWA loci (Blue=FAM™ dye, red=TAZ™ dye)

Table 3 Marker-specific plus stutter observed in the population study with the GlobalFiler™ Express PCR Amplification Kit. Data produced on a 3500xL Genetic Analyzer using a 50-RFU threshold cutoff. Markers that showed negligible plus stutter are omitted.

Loci	Observations	Mean	Standard deviation	Minimum	Maximum
D10S1248	156	1.2437	1.0038	0.17	4.58
D12S391	107	0.8623	0.9123	0.22	5.91
D16S539	185	0.859	0.7155	0.19	6.4
D18S51	137	1.0282	0.7157	0.25	5.9
D19S433	31	1.944	1.322	0.27	5.2
D1S1656	448	0.9966	0.6624	0.3	7.26
D21S11	264	1.0044	0.5295	0.42	4.66
D22S1045	787	3.68075	1.53	0.61	11.06
D2S1338	28	1.593	1.75	0.19	7.13
D2S441	259	0.9106	0.6228	0.31	4.65
D3S1358	214	1.282	1.707	0.28	10.3
D8S1179	156	1.2453	1.1121	0.36	7.15
FGA	109	1	1.157	0.32	8.82
TH01	16	1.42	1.5	0.24	6.09
vWA	80	1.069	1.027	0.2	5.01

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

STR loci such as D1S1656 (Figure 20) and SE33 (Figure 21) include more complex nucleotide sequences including regions of dinucleotide repeats which can yield additional stutter peaks. If these stutter peaks exceed the peak amplitude threshold (typically 175 RFU), they may be detected as additional alleles in the profile. The stutter file that is provided with the GeneMapper™ *ID-X* Software for analysis of GlobalFiler™ kit data contain a minus 2–nt stutter filter for SE33 and D1S1656 to prevent these peaks from being called in normal profiles (see “Stutter percentage filter settings that are provided with the GeneMapper™ ID-X Software” on page 98).

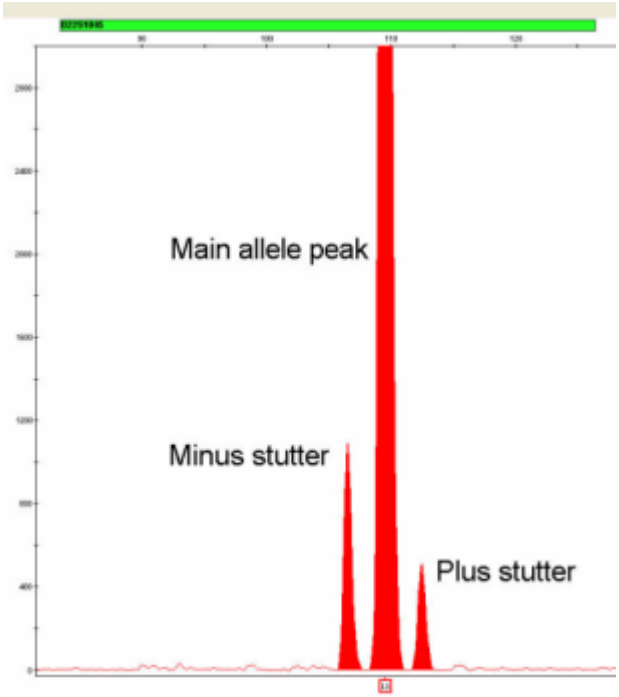


Figure 20 GlobalFiler™ Express PCR Amplification Kit electropherogram showing plus stutter associated with the D22S1045 STR locus. Data produced on a 3500xL Genetic Analyzer.

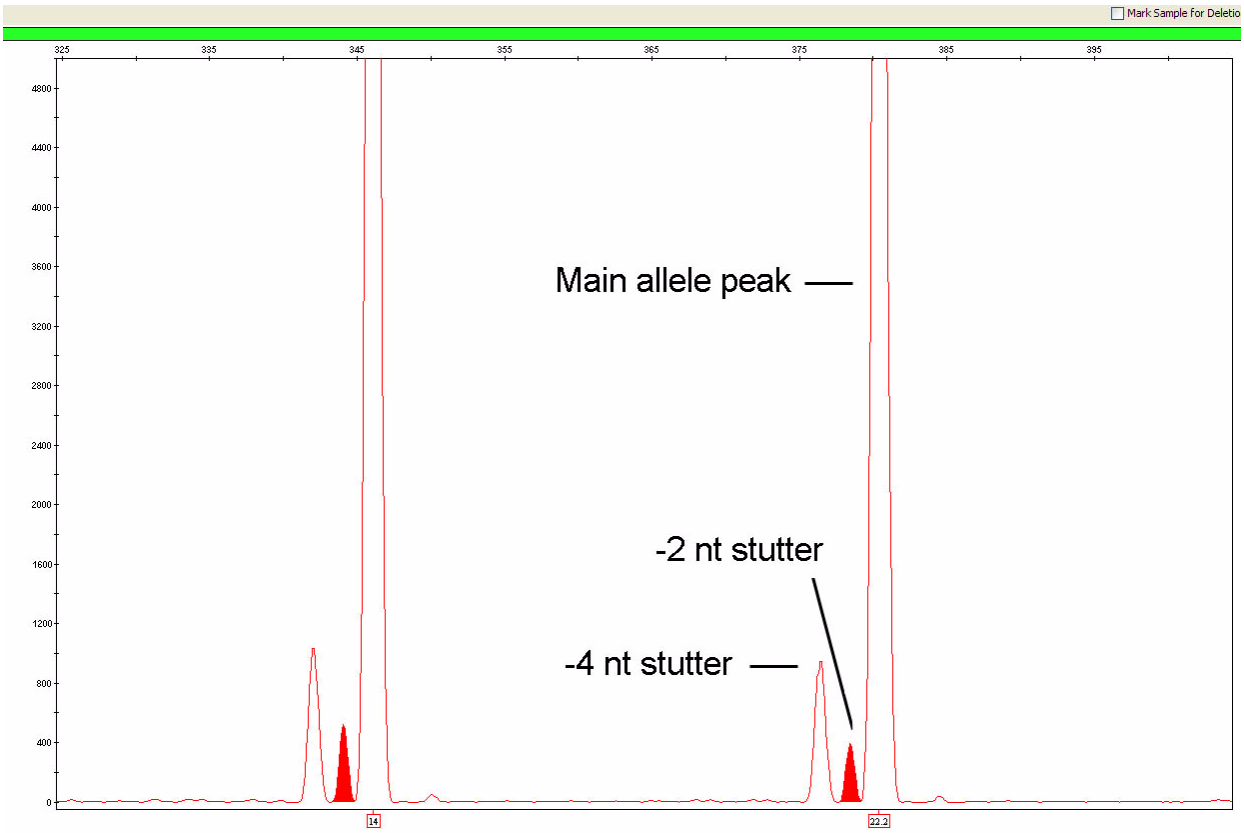


Figure 21 Example of a –2 nt reproducible artifact at the SE33 locus. Data produced on a 3500xL Genetic Analyzer.

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

The settings in Table 4 were derived using the data shown earlier in this section. The proportion of the stutter product relative to the main allele (stutter percent) is measured by dividing the height of the stutter peak by the height of the main allele peak.

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

Table 4 Marker-specific stutter filter percentages for GlobalFiler™ Express kit loci

Locus [1]	% Stutter
CSF1PO	11.40
D10S1248	12.50
D12S391	15.08
D13S317	9.98
D16S539	10.17
D18S51	13.47
D19S433	10.58
D1S1656	13.08
D1S1656 (–2 nt)	1.79
D21S11	11.42
D22S1045	17.30
D22S1045 (+3 nt)	8.27
D2S1338	13.14
D2S441	8.75
D3S1358	12.45
D5S818	10.84
D7S820	10.21
D8S1179	10.20
DYS391	8.54
FGA	11.96
SE33	14.42

Table 4 Marker-specific stutter filter percentages for GlobalFiler Express kit loci *(continued)*

Locus [1]	% Stutter
SE33 (–2 nt)	4.97
TH01	5.24
TPOX	5.43
vWA	12.33

[1] These percentages are used as stutter filters in AmpFLSTR_Stutter.txt

Extra peaks: Addition of 3' A nucleotide

3′ A nucleotide addition definition

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

3′ A observations

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

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

- The primer sequences have been optimized to encourage +A addition.
- The PCR chemistry allows complete +A addition with a short final incubation at The new, highly robust PCR chemistry allows complete +A addition with a short final incubation at 60°C for 8 minutes. .

This final extension step gives the DNA polymerase additional time to complete +A addition to all double-stranded PCR products. Figure 22 shows examples of incomplete and normal +A addition. Final extension incubation for longer than the recommended time can result in double +A addition, in which two nontemplate adenosine residues are added to the PCR product. Double +A addition can cause "shoulders" on the right side of main allele peaks, and is therefore to be avoided.

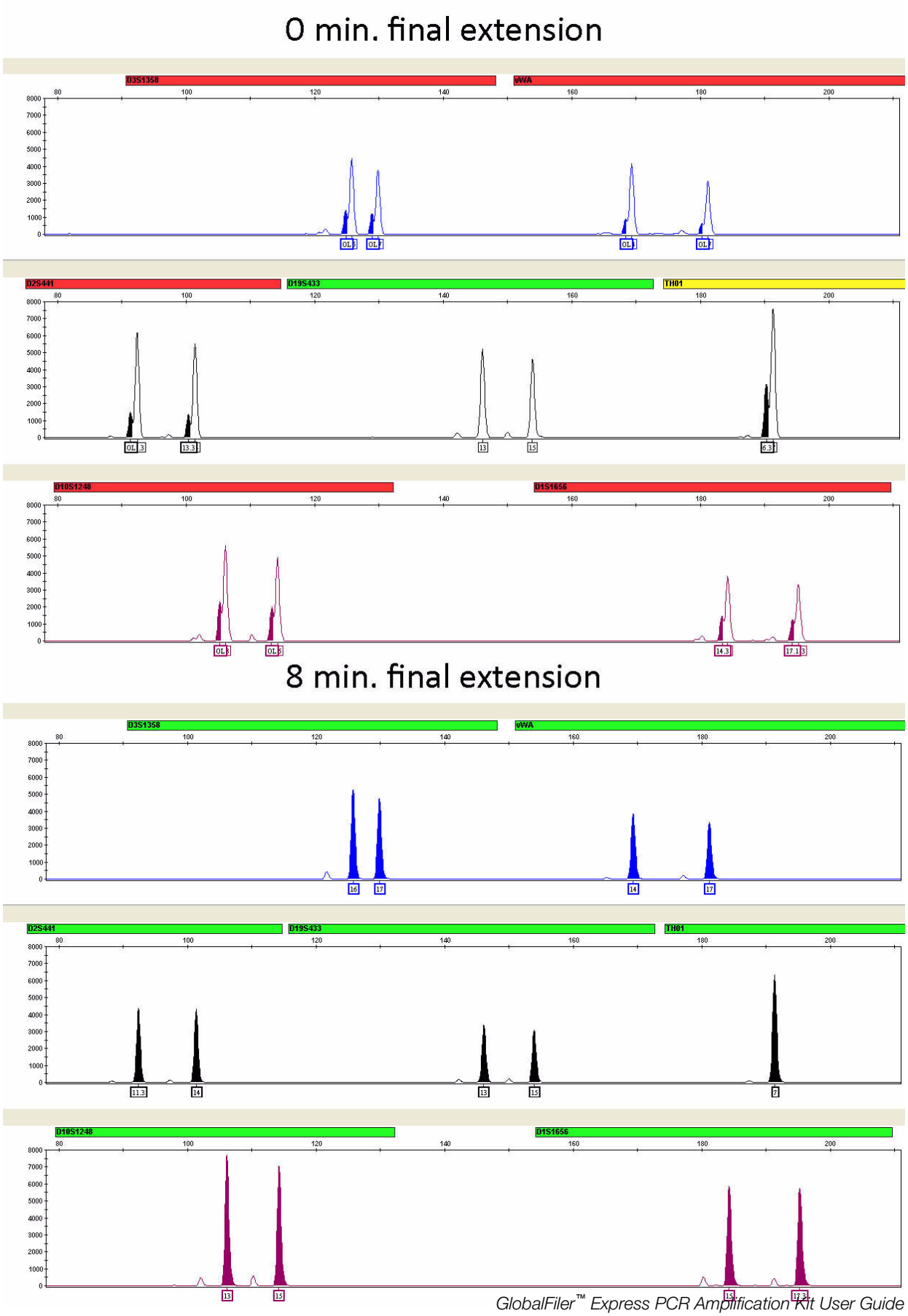


Figure 22 Omitting the final extension step results in shoulders on main allele peaks due to incomplete A nucleotide addition. Examples shown are the smaller amplicons of FAM™, NED™, and SID™ dye channel data from a 3500xL Genetic Analyzer using the GlobalFiler™ Express PCR Amplification Kit.

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

Extra peaks: Artifacts

Artifact definition

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

Artifact observation

Due to improvements in PCR primer manufacturing processes, the incidence of artifacts has been greatly reduced in the GlobalFiler™ Express kit. Kit electropherograms are free of reproducible dye artifacts in the kit read region of 74–444 nt for commonly used analytical thresholds. Figure 23 shows the low baseline-level fluorescence that is observed in a typical negative control PCR.

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

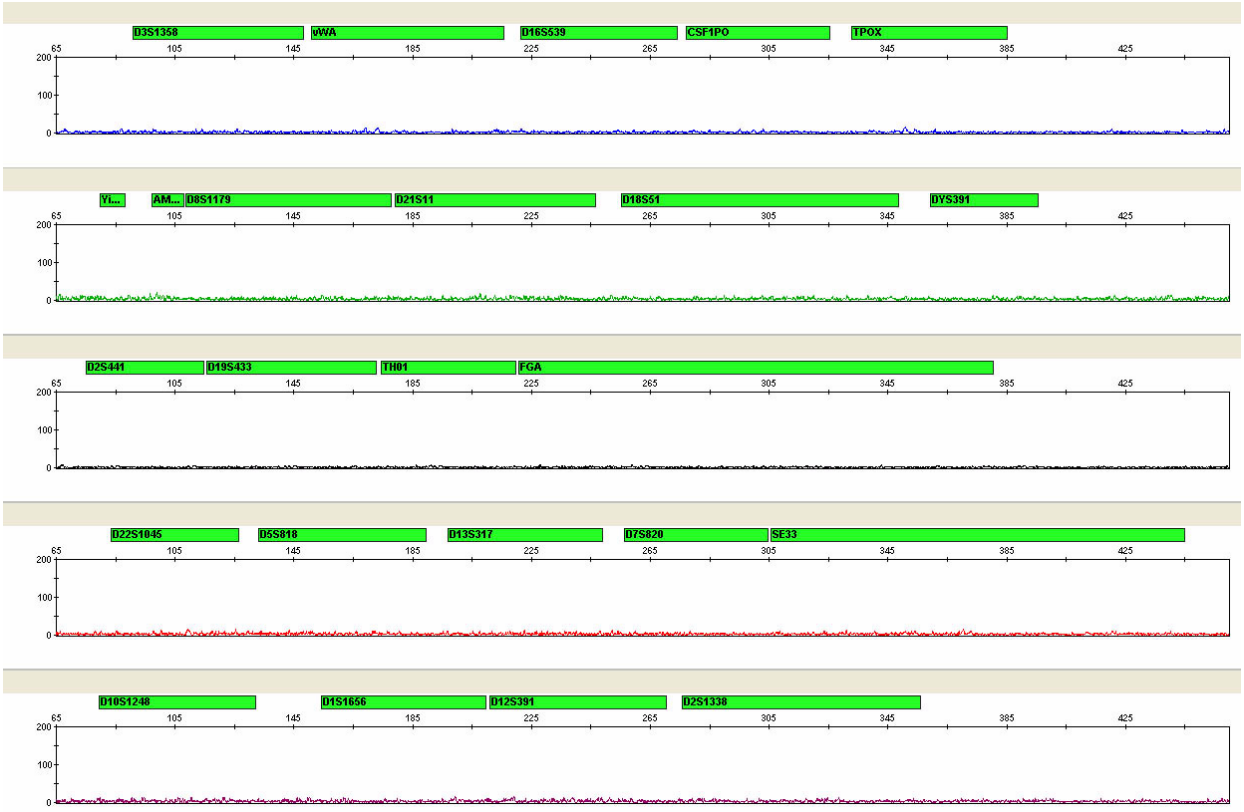


Figure 23 Examples of fluorescence background in data produced on a 3500xL Genetic Analyzer (Y-axis scale 0 to 200 RFU).

Characterization of loci

SWGDAM guideline 3.1

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

Loci in this kit

This section describes basic characteristics of the 21 autosomal STR loci, Y STR locus, Y indel locus, and sex-determining marker (Amelogenin), that are amplified with the GlobalFiler™ Express PCR Amplification Kit. Most of these loci have been extensively characterized by other laboratories.

Nature of polymorphisms

The primers for the Amelogenin locus flank a 6-nucleotide deletion in intron 1 of the X homolog. Amplification generates 99-nt and 105-nt products from the X and Y chromosomes, respectively. (Sizes are the actual nucleotide size according to sequencing results, including 3´ A nucleotide addition, and size may not correspond exactly to allele mobility observed on capillary electrophoresis platforms.) Except for D22S1045, a trinucleotide STR, the remaining loci are tetranucleotide short tandem repeat (STR) loci. The length differences among alleles of a particular locus are caused by differences in the number of repeat units.

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

Inheritance

The Centre d'Etude du Polymorphisme Humain (CEPH) has collected DNA from families of Utah Mormon, French Venezuelan, and Amish descent. These DNA sets have been extensively studied all over the world and are routinely used to characterize the mode of inheritance of various DNA loci. Each family set contains three generations, generally including four grandparents, two parents, and several offspring. Consequently, the CEPH family DNA sets are ideal for studying inheritance patterns (Begovich *et al*., 1992).

Mapping

The GlobalFiler™ Express PCR Amplification Kit loci have been mapped, and the chromosomal locations have been published (Nakahori *et al.*, 1991; Edwards *et al.*, 1992; Kimpton *et al.*, 1992; Mills *et al.*, 1992; Sharma and Litt, 1992; Li *et al.*, 1993; Straub *et al.*, 1993; Barber and Parkin, 1996; and Lareu, *et al.*, 1996).

Genetic linkage

Two sets of STR loci in the GlobalFiler™ Express PCR Amplification Kit are located on the same chromosomes. vWA and D12S391 are located approximately 6.3 million bp apart on the p arm of chromosome 12; D2S1338 and D2S441 are located approximately 150 million bp apart on opposite arms of chromosome 2. Linkage disequilibrium analysis was conducted on the genotype results from 1,034 individuals of three ethnic groups (350 African American, 349 Caucasian, and 335 Hispanic). STR locus genotype results from the population study were analyzed using the Linkage Disequilibrium module of GenePop software version 4.0.10 (Raymond and Rousset, 1995; Rousset, 2008). See Table 5 for results.

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

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

Table 5 GenePop software LD Result (p-value for pairwise analysis of loci)

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

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

Species specificity

SWGDAM Guideline 3.2

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

Nonhuman study observation

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

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

- Primates: gorilla, chimpanzee, and macaque (1.0 ng each)
- Non-primates: mouse, dog, sheep, pig, rabbit, cat, horse, hamster, rat, chicken, and cow (10.0 ng each)
- Microorganisms: *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Fusobacterium nucleatum*, *Lactobacillus casei*, *Staphylococcus aureus*, *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus salivarius*, and *Streptococcus viridans* (equivalent to 105 copies) (These microorganisms are commonly found in the oral cavity (Suido *et al.*, 1986; Guthmiller *et al.*, 2001).)

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

Figure 24 shows example electropherogram results from the species specificity tests. The chimpanzee and gorilla DNA samples produced partial profiles in the 70–400 nucleotide region (gorilla data not shown). Macaque DNA produced an Amelogenin X peak, a 6-FAM™ dye peak at 359 bp, a NED™ dye peak at 278 bp, and two small SID™ dye peaks at 304 bp and 328 bp.

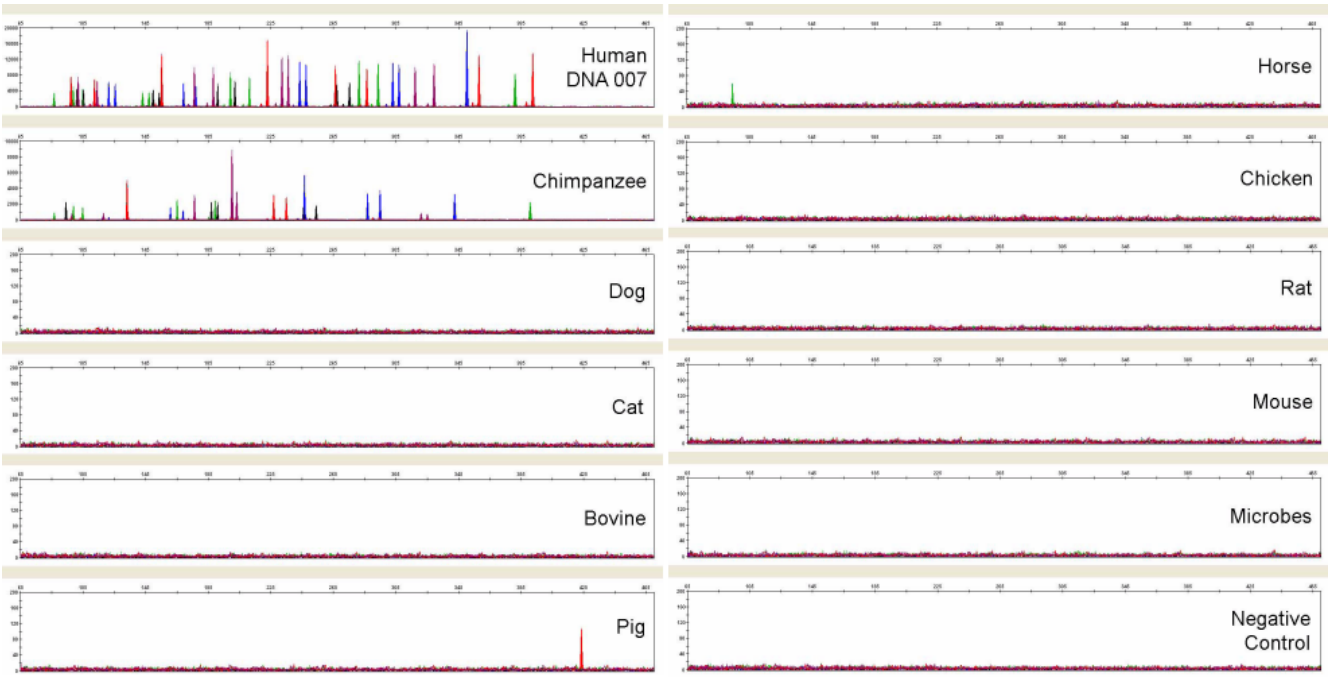


Figure 24 Representative electropherograms for some species tested in a species specificity study. Data produced on a 3500xL Genetic Analyzer.

Among the non-primate species, most produced no peaks over a threshold of 175 RFU. Horse yielded reproducible VIC™ dye peaks at 94 bp (<100 RFU) due to Amelogenin cross-reactivity. Pig yielded reproducible TAZ™ dye peaks at 424 bp (<200 RFU). Individual replicate PCRs of dog, mouse, and chicken yielded single, small (<50 RFU), non-reproducible peaks. These non-reproducible

cross-reactivities were not detectable when the dog, mouse, or chicken DNA were amplified in the presence of human blood or buccal samples on an FTA™ card (data not shown).

Sensitivity

SWGDAM guideline 3.3

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

Sample collection factors that can affect DNA quantity

The GlobalFiler™ Express PCR Amplification Kit has been optimized at 15 µL PCR reaction volume to overcome the PCR inhibition expected when amplifying:

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

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

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

It is essential to optimize the PCR conditions for types of blood samples and spotting protocol. See "Optimize PCR cycle number (before first use of the kit)" on page 18.

Effect of DNA quantity on results

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

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

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

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

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

Sensitivity observation

Figure 25 shows the results of amplification of different input DNA amounts. The y-axis is magnified for the lower amounts of DNA. All data was collected using the 3500xL Genetic Analyzer. The amount of DNA was calculated based on the assumptions of 100% cell lysis efficiency and that each cell contains 6 pg of DNA.

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

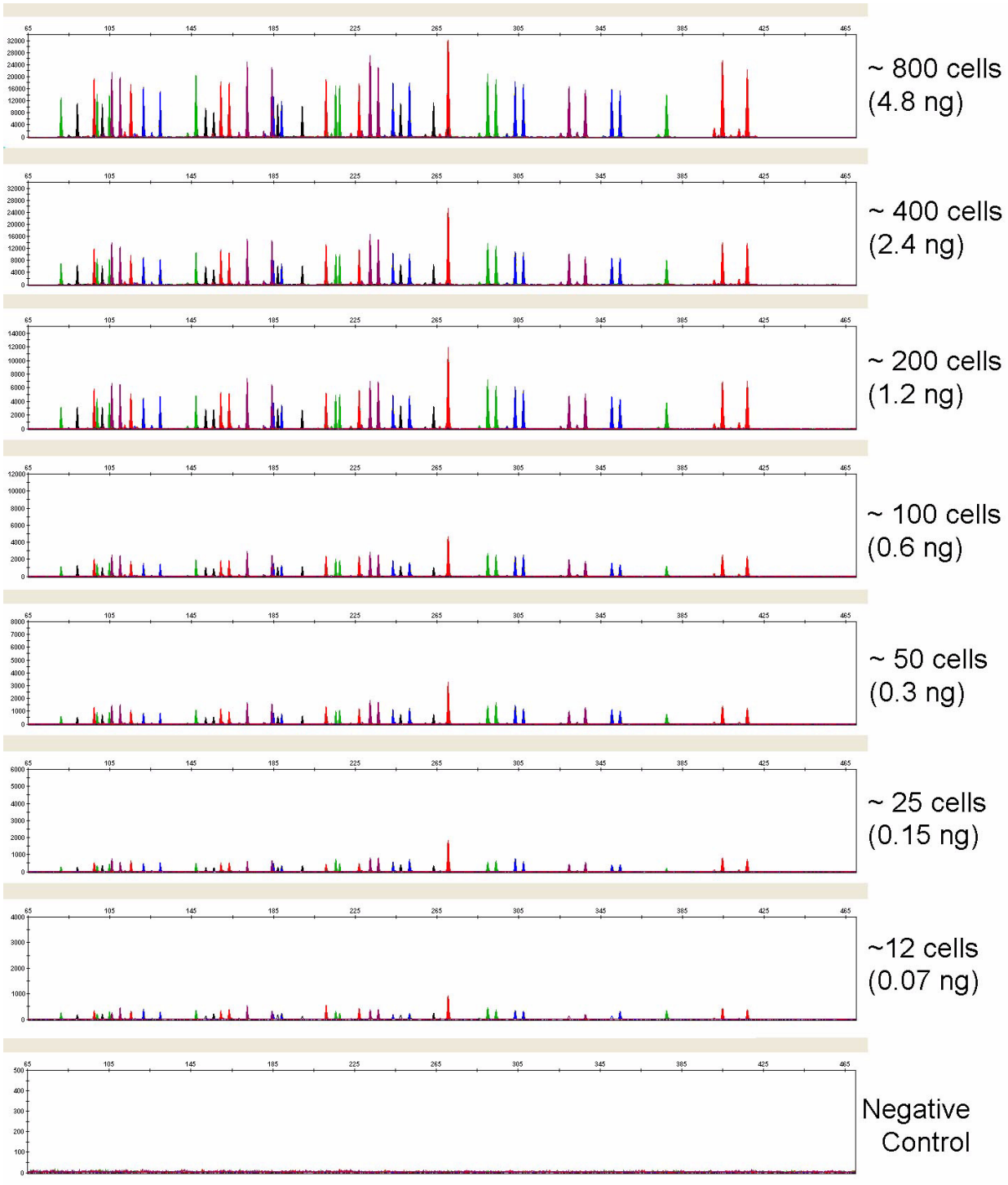


Figure 25 Effect of amplifying varying amounts of white blood cells (WBCs) lysed in Prep-n-Go™ Buffer. Samples were amplified for 25 PCR cycles.

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)

DNA on FTA™ cards

The following aged samples were prepared to examine the sample-on-substrate stability:

- Finger-prick blood that was spotted onto FTA™ Classic Cards stored for 210 days
- Buccal cells that were collected with the EasiCollect™ device, stored for 120 days

Aged FTA™ samples were amplified with the GlobalFiler™ Express kit in a Veriti™ Thermal Cycler, then the PCR products were collected and detected using a 3500xL Genetic Analyzer. The analysis shows that the age of the FTA™ samples did not impact the performance of the GlobalFiler™ Express kit (Figure 26 and Figure 27).

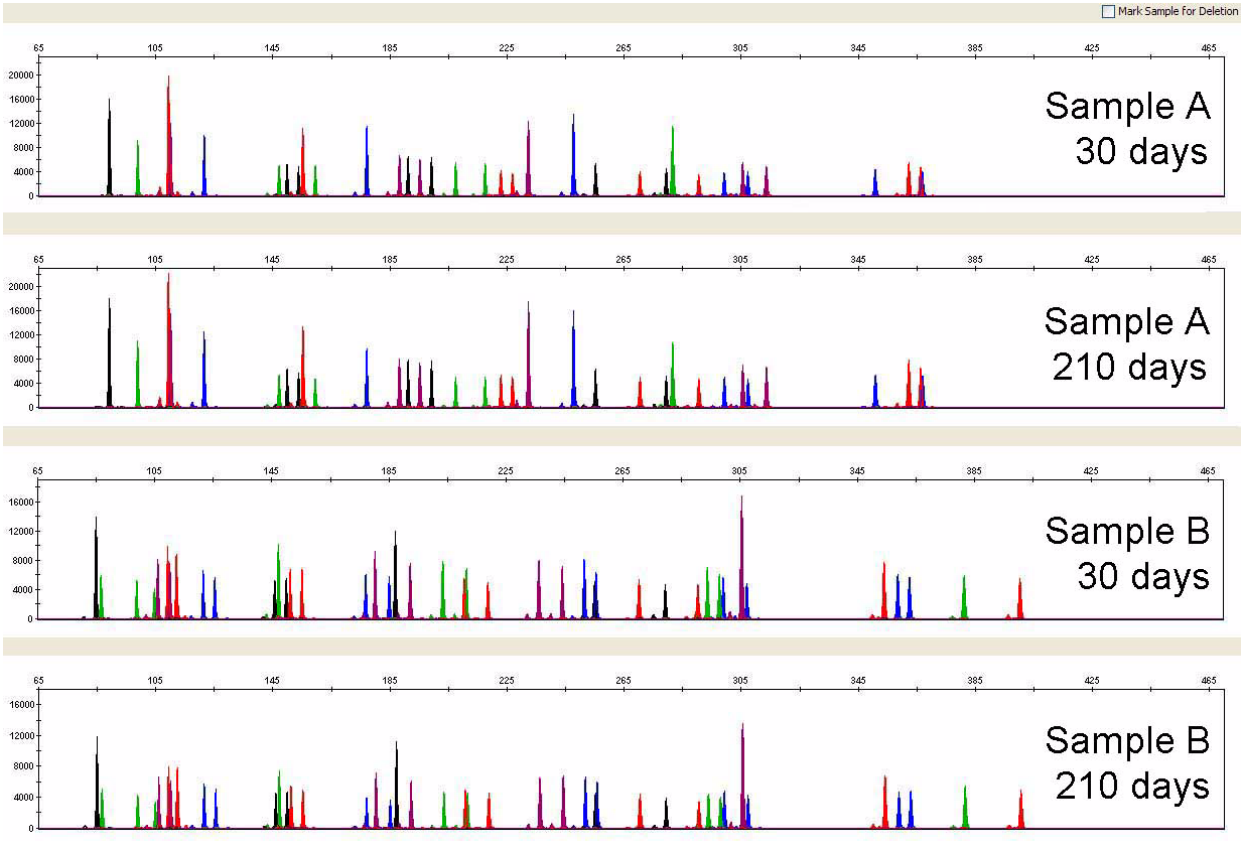


Figure 26 Amplification of blood on FTA™ card stored for various amounts of time at room temperature (Y-axis scale 0 to 19,000 RFU or 0 to 23,000 RFU).

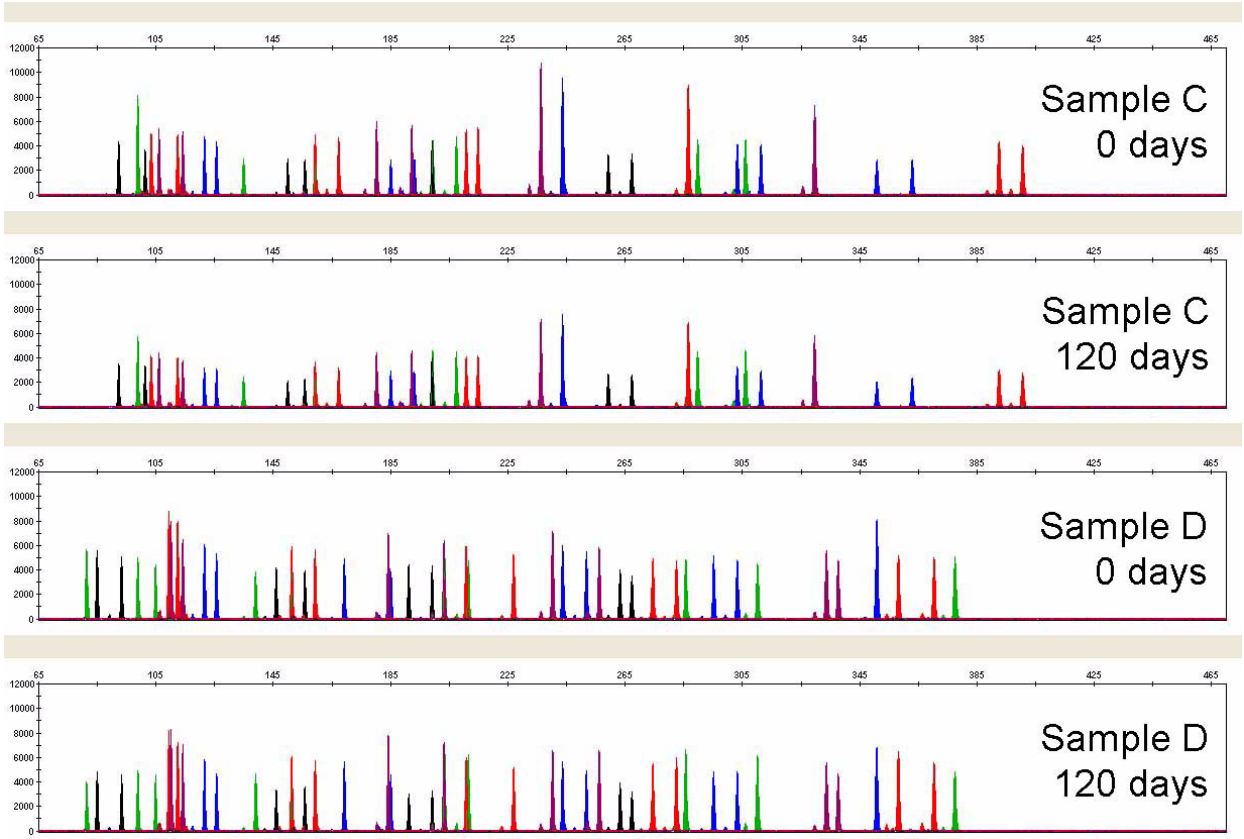


Figure 27 Amplification of buccal cells on Indicating FTA™ card stored for various amounts of time at room temperature (Y-axis scale 0 to 12,000 RFU).

DNA on 4N6FLOQSwabs™ sample collectors

Aged buccal cell samples on 4N6FLOQSwabs™ sample collectors were prepared to verify their sample-on-substrate stability. Buccal swabs were collected from 12 individuals over the course of 4 months. The aged 4N6FLOQSwabs™ samples were lysed in Prep-n-Go™ Buffer and amplified using the GlobalFiler™ Express kit in a Veriti™ Thermal Cycler. The PCR products were run on a 3500xL Genetic Analyzer.

The results from the aged buccal samples collected on 4N6FLOQSwabs™ collectors are shown in Figure 28. The analysis revealed that buccal samples on 4N6FLOQSwabs™ collectors, air-dried immediately after collection and aged up to 4 months at room temperature, produce acceptable profiles when amplified with the GlobalFiler™ Express kit.

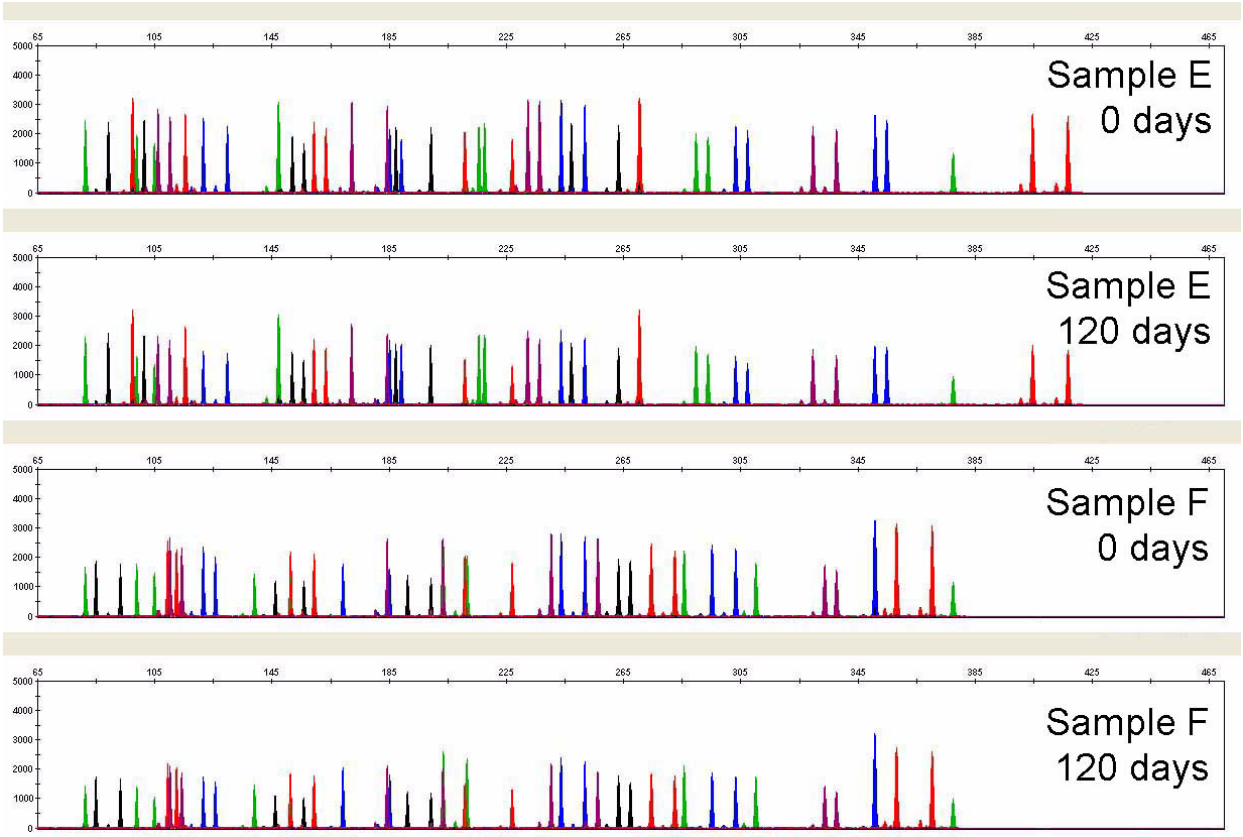


Figure 28 Amplification of buccal cells on 4N6FLOQSwabs™ sample collectors stored for various amounts of time at room temperature and lysed in Prep-n-Go™ Buffer (Y-axis scale 0 to 5,000 RFU).

Population data

SWGDAM guideline 3.7

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

Population data overview

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

- Different from the genotype of the reference sample for a suspect, then the suspect is *excluded* as the donor of the biological evidence that was tested. An exclusion is independent of the frequency of the two genotypes in the population.
- The same as the genotype of the reference sample for a suspect, then the suspect is *included* as a possible source of the evidence sample.

The probability that another, unrelated individual would also match the evidence sample is estimated by the frequency of that genotype in the relevant populations.

Loci in the kit

The GlobalFiler™ Express PCR Amplification Kit contains loci for which extensive population data are available. For additional information on the loci shared between many of the AmpFℓSTR™ kits, see the population data and additional studies section of the *AmpFℓSTR™ NGM SElect™ PCR Amplification Kit Kit User Guide* (Pub. No. 4458841) and the *AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kit User Guide* (Pub. No. 4440211).

Population samples used in these studies

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

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

DNA was extracted with a 6100 Nucleic Acid Prep Station.

The GlobalFiler™ Express PCR Amplification Kit contains loci for which extensive population data are available.

In addition to the alleles that we observed and recorded in our databases, other alleles have been published or reported to us by other laboratories (see the STRBase at **www.cstl.nist.gov/div831/strbase**).

Concordance studies

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

Probability of Identity definition

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

Probability of identity observation

Table 6 shows the Autosomal STR allele frequencies at GlobalFiler™ kit loci by population group.

Table 7 shows the Y-specific allele frequencies by population group for GlobalFiler™ Express PCR Amplification Kit DYS391 and Y indel loci. The Y-specific allele frequencies were not included in the probability of identity calculation.

Table 8 shows the Probability of identity (P_I) values of the GlobalFiler™ Express PCR Amplification Kit loci individually and combined.

Table 6 Autosomal allele frequencies by population group for GlobalFiler™ Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities)

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

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

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

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
23	3.64	3.27	8.16	3.67
24	0.61*	0.98*	3.64	1.9
25	0.61*	0.33*	1.9	1.36
26	*	*	0.29*	0.27*
27	*	*	0.15*	0.14*
D13S317				
5	*	*	*	*
6	*	*	*	*
7	*	*	0.15*	*
8	2.27	29.74	10.93	8.97
9	2.27	12.09	7.14	16.3
10	3.03	13.73	6.85	9.65
11	29.24	25.49	29.01	22.83
12	43.79	14.38	30.76	27.45
13	14.55	3.92	10.64	10.05
14	4.55	0.33*	4.52	4.76
15	0.30*	0.33*	*	*
16	*	*	*	*
17	*	*	*	*
D16S539				
4	*	*	*	*
5	*	*	*	*
6	*	*	*	0.14*
8	3.33	*	1.46	2.04
9	21.67	31.05	12.68	10.19
10	11.52	14.05	4.08	15.76
11	30	20.59	32.22	31.79

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
12	19.09	21.57	30.9	24.18
13	13.03	11.44	16.76	14.4
14	1.36	1.31*	1.75	1.22
15	*	*	0.15*	0.27*
16	*	*	*	*
D18S51				
6	*	*	*	*
7	*	*	*	*
9	*	*	*	0.14*
10	0.15*	*	1.17	0.68
10.2	0.15*	*	*	*
11	0.45*	1.31*	0.87	1.22
12	6.21	5.56	15.01	10.46
13	3.94	17.32	11.95	11.41
13.2	0.30*	*	*	*
14	5.91	22.88	17.64	16.3
14.2	0.45*	*	*	0.14*
15	16.52	16.99	15.31	12.23
15.2	*	*	*	0.14*
16	18.18	12.42	11.95	12.91
17	16.36	6.54	10.79	17.39
18	14.09	4.9	8.31	7.74
19	9.7	5.23	4.08	3.53
20	4.7	1.96	1.31	1.9
20.2	0.15*	*	*	*
21	1.82	1.96	1.02	2.17
22	0.61*	0.98*	0.29*	0.68

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
23	0.30*	0.98*	0.29*	0.54*
24	*	0.65*	*	0.27*
25	*	*	*	0.14*
26	*	0.33*	*	*
27	*	*	*	*
28	*	*	*	*
D19S433				
5.2	*	*	*	*
6	*	*	*	*
7	*	*	*	*
8	*	*	*	*
9	0.30*	0.33*	*	*
10	1.21	*	0.15*	0.41*
10.2	0.15*	*	*	*
11	9.85	*	*	1.63
11.2	0.30*	*	*	0.27*
12	10.45	4.58	7.29	8.42
12.1	*	*	0.15*	*
12.2	3.94	0.33*	0.15*	1.49
13	27.88	28.1	27.26	18.48
13.2	5.3	2.61	1.6	6.93
14	18.94	23.2	35.13	30.71
14.2	5.3	9.48	2.04	4.62
15	6.67	7.52	16.18	13.04
15.2	4.39	20.26	3.5	6.79
16	1.52	0.33*	5.69	4.08
16.2	3.18	2.61	0.29*	2.17

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
17	*	*	0.29*	0.54*
17.2	0.61*	0.65*	0.15*	0.41*
18	*	*	0.15*	*
18.2	*	*	*	*
19.2	*	*	*	*
D1S1656				
8	*	*	*	*
9	0.15*	*	*	0.14*
10	1.36	*	0.29*	0.41*
11	5.3	3.59	6.27	3.94
12	8.48	4.25	15.74	9.38
13	11.06	13.73	7	7.07
14	25	6.21	6.27	11.28
14.3	0.91	*	0.29*	0.27*
15	16.97	20.26	15.31	15.49
15.3	1.82	*	8.75	2.99
16	10	31.05	9.33	15.08
16.1	*	*	*	0.27*
16.3	7.27	0.65*	4.96	5.16
17	2.73	14.05	4.96	6.79
17.1	*	*	0.29*	*
17.3	5.76	3.59	12.68	15.76
18	0.45*	0.33*	0.29*	0.82
18.3	1.82	1.63	5.98	4.48
19	0.15*	*	*	*
19.3	0.61*	0.33*	1.6	0.68
20	*	0.33*	*	*

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
20.3	0.15*	*	*	*
21	*	*	*	*
D21S11				
23.2	*	*	*	*
24	*	*	*	*
24.2	*	*	*	0.27*
25	*	*	*	*
26	0.30*	*	0.58*	0.41*
27	5.91	*	2.62	1.49
28	25.15	4.9	16.76	11.41
28.2	*	0.65*	*	0.14*
29	15.61	26.8	23.76	21.06
29.2	*	*	0.15*	*
29.3	0.15*	*	0.15*	*
30	20.76	30.72	23.18	27.17
30.2	1.67	0.65*	2.77	1.77
31	8.79	9.48	6.85	5.16
31.2	4.55	3.92	8.89	11.14
31.3	*	0.33*	*	*
32	1.36	2.61	2.33	1.36
32.2	7.12	14.38	9.62	12.5
33	0.91	0.98*	*	0.14*
33.2	3.18	4.58	1.9	5.3
34	0.15*	*	*	*
34.2	*	*	0.44*	0.14*
35	3.64	*	*	0.27*
35.2	*	*	*	*

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
36	0.76	*	*	0.14*
37	*	*	*	*
38	*	*	*	0.14*
39	*	*	*	*
D22S1045				
7	*	*	*	*
8	0.61*	*	*	*
9	*	*	*	*
10	4.09	*	0.44*	0.68
11	14.7	15.36	13.85	7.61
12	6.21	0.33*	0.58*	0.95
13	0.30*	0.33*	1.02	1.09
14	7.88	0.33*	3.35	2.04
15	23.33	33.66	36.3	43.48
16	20.3	23.86	36.3	34.65
17	20.45	24.18	7.58	8.42
18	2.12	1.96	0.58*	0.95
19	*	*	*	*
20	*	*	*	0.14*
D2S1338				
10	*	*	*	*
11	*	*	*	*
12	*	*	*	*
13	0.15*	*	0.15*	*
14	*	*	0.15*	*
15	0.30*	*	0.15*	*
16	5.3	1.63	4.08	3.8

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
17	10	14.05	18.37	17.8
18	4.85	13.07	8.31	6.52
19	16.21	16.67	14.14	17.53
20	10.45	8.82	15.74	13.86
21	11.97	2.94	2.92	3.67
22	12.42	5.88	1.75	6.52
23	9.24	18.3	10.06	14.27
24	8.79	11.11	10.2	8.83
25	6.97	5.88	12.1	5.43
26	2.58	*	1.6	1.49
27	0.76	0.33*	0.29*	0.14*
28	*	0.98*	*	0.14*
29	*	0.33*	*	*
D2S441				
8	0.15*	*	*	*
9	*	*	0.58*	0.14*
9.1	*	2.94	*	*
10	9.09	20.59	19.83	30.3
11	35.61	36.27	33.09	31.93
11.3	2.88	2.61	5.1	4.62
12	20.45	20.92	4.08	3.8
12.3	0.15*	*	0.29*	0.41*
13	3.48	6.21	3.35	1.9
14	26.21	9.8	28.86	23.1
15	1.97	0.65*	4.37	3.4
16	*	*	0.44*	0.41*
17	*	*	*	*

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
D3S1358				
8	*	*	*	*
9	0.30*	*	*	0.14*
10	*	*	*	*
11	*	*	0.29*	*
12	0.15*	0.33*	*	0.14*
13	0.61*	*	0.15*	0.41*
14	9.09	2.61	15.16	9.1
15	28.18	49.02	27.26	34.65
15.2	0.30*	*	*	*
16	32.42	21.9	24.34	26.9
17	22.27	19.61	19.68	17.93
18	6.06	6.54	11.66	9.92
19	0.61*	*	1.46	0.82
20	*	*	*	*
21	*	*	*	*
D5S818				
6	*	*	*	*
7	0.30*	1.63	*	5.3
8	6.21	0.33*	0.73	1.49
9	1.97	9.15	5.39	5.03
10	7.27	22.22	5.54	4.35
11	25	28.76	33.82	38.18
12	35.45	24.51	37.61	30.16
13	21.82	12.75	14.87	14.54
14	1.67	0.65*	1.75	0.95
15	0.30*	*	0.29*	*

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
16	*	*	*	*
17	*	*	*	*
18	*	*	*	*
19	*	*	*	*
D7S820				
5	*	*	*	*
6	0.30*	*	*	*
7	0.45*	0.33*	1.31	1.09
8	21.67	16.99	16.47	12.5
9	11.67	7.52	16.62	8.29
9.1	*	0.33*	*	*
10	30.45	20.26	27.26	25.14
10.3	*	*	*	0.14*
11	19.85	31.37	20.99	29.35
11.3	*	*	*	0.14*
12	13.03	20.26	14.58	19.02
13	2.27	2.61	2.33	3.94
13.1	0.15*	*	*	*
14	0.15*	0.33*	0.29*	0.41*
15	*	*	0.15*	*
16	*	*	*	*
D8S1179				
4	*	*	*	*
5	*	*	*	*
6	*	*	*	*
7	*	*	*	*
8	0.30*	*	2.04	0.68

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
9	0.30*	*	1.31	0.27*
10	3.33	9.8	10.5	9.51
11	5.61	9.48	6.71	5.03
12	11.36	13.4	15.16	12.5
13	18.18	24.18	33.24	33.15
14	35.91	15.69	18.8	23.23
15	18.03	22.22	9.04	11.41
16	5.91	4.25	2.77	3.53
17	1.06	0.98*	0.44*	0.68
18	*	*	*	*
19	*	*	*	*
20	*	*	*	*
FGA				
12.2	*	*	*	*
13	*	*	*	*
14	*	*	*	*
15	*	*	*	*
16	*	0.33*	0.15*	*
16.1	0.30*	*	*	*
17	*	0.33*	0.15*	*
18	0.91	3.27	1.02	0.68
18.2	0.61*	*	*	*
19	6.97	4.25	5.69	7.61
19.2	0.45*	*	*	*
20	6.82	3.92	14.87	8.7
20.2	0.30*	*	0.44*	0.27*
21	11.67	13.07	18.22	13.45

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
21.2	0.15*	0.33*	0.29*	*
22	17.27	14.38	19.24	14.4
22.2	0.15*	*	0.87	0.54*
23	17.27	27.12	14.87	12.91
23.2	*	0.65*	0.44*	0.41*
23.3	0.30*	*	*	*
24	18.94	18.3	14.43	15.62
24.2	*	0.33*	*	*
25	9.55	9.8	6.71	13.72
26	4.09	3.27	1.9	7.07
26.2	*	*	*	*
27	2.58	0.65*	0.58*	3.12
28	1.21	*	0.15*	0.95
29	*	*	*	0.41*
30	0.15*	*	*	0.14*
30.2	0.15*	*	*	*
31.2	*	*	*	*
32.2	*	*	*	*
33.2	*	*	*	*
34.2	0.15*	*	*	*
42.2	*	*	*	*
43.2	*	*	*	*
44.2	*	*	*	*
45.2	*	*	*	*
46.2	*	*	*	*
47.2	*	*	*	*
48.2	*	*	*	*

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
50.2	*	*	*	*
51.2	*	*	*	*
SE33				
4.2	*	*	*	*
5.2	0.15*	*	*	*
6.3	*	*	0.15*	*
8	*	*	*	*
9	*	*	*	*
11	*	*	*	*
11.2	0.76	*	*	0.14*
12	0.15*	0.33*	0.44*	0.14*
12.1	*	*	0.15*	*
12.2	0.30*	*	0.15*	0.14*
13	1.36	*	0.87	1.22
13.2	0.45*	*	*	0.14*
14	3.33	*	3.64	1.77
14.2	0.15*	*	*	0.82
14.3	*	*	0.15*	*
15	4.24	1.31*	3.64	4.89
15.2	0.15*	*	0.15*	0.14*
16	6.97	3.59	5.39	5.57
16.2	0.30*	*	*	0.27*
16.3	*	*	*	0.14*
17	7.73	5.23	6.56	8.7
17.2	0.15*	*	*	*
17.3	*	*	0.15*	*
18	10.76	4.9	7.87	10.05

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
18.2	0.15*	*	*	0.27*
19	15	9.48	8.31	8.15
19.2	0.30*	*	0.29*	*
19.3	*	*	0.29*	*
20	9.55	6.86	5.25	4.48
20.2	0.91	0.33*	0.87	0.82
21	5.76	6.21	2.04	3.12
21.2	0.91	1.63	1.17	1.09
22	1.97	2.61	0.58*	1.09
22.2	1.36	2.29	3.35	2.31
23	0.30*	*	*	*
23.2	0.61*	2.61	2.62	2.85
23.3	*	*	*	0.14*
24	0.30*	0.33*	0.15*	0.14*
24.2	1.67	6.54	4.52	2.31
25.2	2.42	7.19	3.79	3.12
26	0.15*	*	*	0.27*
26.2	5.61	7.52	4.52	6.39
27.2	5.91	3.59	6.85	7.07
27.3	*	*	*	0.14*
28.2	3.94	7.84	7.73	6.25
29.2	2.58	8.5	7.87	5.84
30.2	1.21	7.52	4.66	3.8
31	*	*	*	0.14*
31.2	1.06	1.63	2.77	2.31
32	*	*	0.44*	*
32.2	0.76	1.31*	1.6	2.04

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
33	*	*	0.29*	0.41*
33.2	0.45*	0.33*	0.15*	0.54*
34	*	*	0.29*	0.41*
34.2	0.15*	*	*	0.27*
35	*	*	0.15*	*
35.2	*	0.33*	*	*
36	*	*	0.15*	*
37	*	*	*	0.14*
38	*	*	*	*
TH01				
3	*	*	*	*
4	*	*	*	*
5	0.45*	*	0.15*	*
6	15.45	13.07	21.72	27.17
6.1	0.15*	*	*	*
7	37.42	26.14	17.64	32.74
8	20.61	3.59	11.37	8.7
9	16.06	51.63	17.06	12.77
9.3	8.33	4.25	31.2	17.12
10	1.52	1.31*	0.87	1.49
11	*	*	*	*
13.3	*	*	*	*
TPOX				
4	*	*	*	*
5	*	*	*	*
6	8.03	*	0.15*	0.54*
7	2.27	0.98*	*	0.14*

Table 6 Autosomal allele frequencies by population group for GlobalFiler Express PCR Amplification Kit STR loci. (*=Alleles not detected or not detected in significant quantities) *(continued)*

Allele	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
8	35.91	49.35	50.15	47.83
9	19.09	13.07	12.97	8.02
10	9.55	3.59	4.66	6.11
11	21.67	29.74	28.28	26.36
12	3.33	3.27	3.79	10.73
13	0.15*	*	*	0.14*
14	*	*	*	0.14*
15	*	*	*	*
16	*	*	*	*
vWA				
10	*	*	*	*
11	0.45*	*	*	0.14*
12	*	*	*	0.27*
13	0.91	*	0.15*	0.14*
14	7.27	23.53	8.75	6.52
15	20.91	1.63	12.24	9.78
16	27.58	15.36	22.3	30.57
17	19.85	29.74	27.41	27.17
17.3	*	*	*	0.14*
18	13.79	19.61	17.78	18.07
19	6.52	9.15	10.06	6.39
20	1.97	0.98*	1.31	0.82
21	0.61*	*	*	*
22	*	*	*	*
23	0.15*	*	*	*
24	*	*	*	*
25	*	*	*	*

Table 7 Y-specific frequencies by population group for GlobalFiler™ Express PCR Amplification Kit DYS391 and Y indel loci. (*=Alleles not detected or not detected in significant quantities)

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

Table 8 Probability of identity (P_I) values for the GlobalFiler™ Express PCR Amplification Kit STR loci

Locus	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
CSF1PO	0.0850	0.1317	0.1333	0.1353
D10S1248	0.0693	0.1045	0.0943	0.1131
D12S391	0.0377	0.0664	0.0231	0.0318
D13S317	0.1451	0.0817	0.0761	0.0564
D16S539	0.0727	0.0915	0.1043	0.0809
D18S51	0.0322	0.0402	0.0311	0.0281
D19S433	0.0388	0.0663	0.0862	0.0484
D1S1656	0.0340	0.0564	0.0223	0.0247
D21S11	0.0453	0.0671	0.0520	0.0487
D22S1045	0.0559	0.1073	0.1309	0.1604
D2S1338	0.0225	0.0337	0.0316	0.0316

Table 8 Probability of identity (PI) values for the GlobalFiler Express PCR Amplification Kit STR loci *(continued)*

Locus	African American (n = 330)	Asian (n = 153)	U.S. Caucasian (n = 343)	U.S. Hispanic (n = 368)
D2S441	0.1030	0.0961	0.0976	0.1079
D3S1358	0.0984	0.1689	0.0749	0.0949
D5S818	0.0968	0.0883	0.1341	0.1122
D7S820	0.0784	0.0875	0.0680	0.0790
D8S1179	0.0762	0.0527	0.0631	0.0661
FGA	0.0322	0.0555	0.0384	0.0282
SE33	0.0118	0.0139	0.0085	0.0081
TH01	0.0949	0.1750	0.0801	0.0902
TPOX	0.0833	0.1788	0.1757	0.1456
vWA	0.0622	0.0840	0.0650	0.0926
Combined	6.18×10^{-27}	3.34×10^{-24}	3.71×10^{-26}	3.09×10^{-26}

Probability of paternity exclusion observation

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

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

The average observed heterozygosity across the 21 autosomal STR loci was 0.815 in the African American population, 0.779 in the Asian population, 0.804 in the U.S. Caucasian population, and 0.789 in the Hispanic population. The most heterozygous locus was SE33 (mean observed heterozygosity across all populations of 0.948), and the least heterozygous STR locus was TPOX (mean observed heterozygosity across all populations of 0.652). The cumulative match probability (including the Y chromosome loci) was 2.17×10^{-27} for African American, 2.26×10^{-25} for Asian, 5.27×10^{-27} for Caucasian, and 5.0×10^{-27} for Hispanics.

Table 9 Allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), Match probability (MP), and p-value of STR loci

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

Table 9 Allele frequencies, observed heterozygosity (Ho), expected heterozygosity (He), Match probability (MP), and p-value of STR loci *(continued)*

Marker	African American				Asian				U.S. Caucasian				U.S. Hispanic			
	Ho	He	MP	p-value	Ho	He	MP	p-value	Ho	He	MP	p-value	Ho	He	MP	p-value
D10S1248	0.792	0.789	0.075	0.823	0.757	0.764	0.091	0.928	0.785	0.769	0.090	0.630	0.691	0.724	0.124	0.336
D1S1656	0.921	0.863	0.033	0.351	0.757	0.818	0.056	0.043	0.912	0.899	0.019	0.550	0.871	0.896	0.020	0.048
D12S391	0.861	0.864	0.032	0.190	0.771	0.808	0.063	0.650	0.904	0.896	0.020	0.450	0.842	0.874	0.028	0.071
D2S1338	0.911	0.894	0.020	0.763	0.871	0.872	0.030	0.356	0.880	0.878	0.027	0.230	0.906	0.877	0.027	0.929

The following table shows the Probability of paternity exclusion (PE) values of the GlobalFiler™ Express PCR Amplification Kit STR loci individually and combined.

The PE value is the probability, averaged over all possible mother-child pairs, that a random alleged father will be excluded from paternity after DNA typing using the GlobalFiler™ Express PCR Amplification Kit STR loci (Chakraborty, Stivers, and Zhong, 1996).

Table 10 Probability of paternity exclusion values for the GlobalFiler™ Express PCR Amplification Kit STR loci

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

A

Troubleshooting

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

Observation	Possible cause	Recommended action
Faint no signal Control and samples all loci	The sample punch location was not optimal.	For blood samples on treated paper, punch in the center of the blood stain.
		For buccal samples on treated paper, punch in the center of the buccal transfer or punch in the optimal location you have previously determined.
		For buccal samples collected with the Bode Buccal DNA Collector™ device, punch from near the tip of the collector.
	Insufficient volume of swab lysate was added to the reaction.	Ensure the swab heads are incubated for 20 minutes in 400 µL of Prep-n-Go™ Buffer.
	Proper low-TE buffer not used for treated paper substrates.	Prepare low-TE buffer. See “Prepare low-TE buffer” on page 20.
Positive signal from DNA Control 007 but partial or no signal from DNA test samples	The test sample was diluted in the wrong buffer (for example, a TE buffer with an incorrect EDTA concentration).	Redilute DNA using low-TE buffer (with 0.1 mM EDTA).
	The sample punch location was not optimal.	For blood samples on treated paper, punch in the center of the blood stain.
		For buccal samples on treated paper, punch in the center of the buccal transfer or punch in the optimal location you have previously determined.
		For buccal samples collected with the Bode Buccal DNA Collector™ device, punch from near the tip of the collector.
	Insufficient lysis of the swab head occurred.	Ensure the swab heads are incubated for 20 minutes in 400 µL of Prep-n-Go™ Buffer.
	Proper low-TE buffer not used for treated paper substrates.	Prepare low-TE buffer.
More than two alleles present at a locus	Exogenous DNA is present in the sample.	Use appropriate techniques to avoid introducing foreign DNA during laboratory handling.
	Stutter product (–1 repeat unit position) was amplified.	See Chapter 5, “Experiments and results”
	Triallelic patterns occur.	Confirm the triallelic pattern per the laboratory's guidelines.
	Incomplete 3´ A base addition (n–1 nt position) occured.	Include the final extension step of 60°C for 10 minutes in the PCR.
		Include the final extension step of 60°C for 10 minutes in the PCR.
	The signal exceeds the dynamic range of the instrument and is causing signal "pull-up" into adjacent channels.	Ensure the cycle number is optimized. Use fewer PCR cycles or interpret the off-scale data according to your laboratory procedure.

Observation	Possible cause	Recommended action
More than two alleles present at a locus *(continued)*	Poor spectral separation occurred.	Perform a spectral calibration.
		Confirm that Filter Set J6 modules are installed and used for analysis.
	The double-stranded DNA was not completely denatured.	Use the recommended amount of Hi-Di™ Formamide and heat the sample plate at 95°C for 3 minutes.
	Contamination was carried over from the disc punching tool.	Clean the disc punching tool thoroughly. If necessary, include a blank punch step in between the sample punches.
Poor peak height balance	Incorrect thermal cycler conditions were used.	Use correct thermal cycler conditions.
Some but not all loci visible on electropherogram of DNA Test Samples	The punched disc you used was too large.	Use a 1.2 mm disc.
	Insufficient lysis of the swab head occurred.	Ensure the swab heads are incubated for 20 minutes in 400 µL of Prep-n-Go™ Buffer.
	The PCR reaction volume you used is lower than the volume required for the amplification.	Use the correct PCR reaction volume: 15 µL
STR profiles contain many off-scale alleles	The PCR cycle number used was too high.	Perform a sensitivity experiment to determine the optimal PCR cycle number based on the sample type.
	Blood samples: Too much liquid blood was spotted onto the paper substrate.	Spot <100 µL of liquid blood per sample area.
Data collected on the 3730 instrument with POP-7™ polymer fails sizing	The 60-bp size-standard peak is occasionally obscured by the primer peak.	Reinject samples that fail to recognize the 60 base-pair peak.
		Use the 80 to 460 bp size-standard definition after performing appropriate validation studies (as a general rule, the 60 base-pair peak is not required for accurate fragment sizing using the 3rd Order Least Squares sizing method).
		For more information, see the *GeneMapper™ ID-X Software v1.4 New Features and Installation Procedures User Bulletin* (Pub. No. 4477684), "Known issues: 3730 DNA Analyzer sizing failures".
Data collected on the 3730 instrument with POP-7™ polymer: the D2S441 and D1S1656 markers in some allelic ladder samples fail base-pair spacing quality assessment	Data was analyzed using the **Light** setting for Smoothing.	Use the **None** setting for Smoothing after performing appropriate validation studies.
		For more information, see the *GeneMapper™ ID-X Software v1.4 New Features and Installation Procedures User Bulletin* (Pub. No. 4477684), "Known issues: 3730 DNA Analyzer sizing failures".

B Materials required but not supplied

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

STR kit required materials

Item	Source
GlobalFiler™ Express PCR Amplification Kit, 200-reaction kit	4476609
GlobalFiler™ Express PCR Amplification Kit, 1,000-reaction kit	4474665
GeneScan™ 600 LIZ™ Size Standard v2.0, 2 × 200 µL **IMPORTANT!** Do not use GeneScan™ 350 ROX™, GeneScan™ 500 ROX™, or GeneScan™ 500 LIZ™ Size Standards with this kit.	4408399
Hi-Di™ Formamide, 25-mL	4311320

Sample preparation required materials

Treated paper substrate

Item	Source
Collection system: NUCLEIC-CARD™ system or Whatman FTA™	
NUCLEIC-CARD™ Sample Collection Device	A32607
NUCLEIC-CARD™ matrix, 1 spot	4474001
NUCLEIC-CARD™ COLOR matrix, 1 spot	4473974

(continued)

Item	Source
Whatman™ FTA™ Classic Cards	MLS
Whatman™ EasiCollect™ system	MLS
Sample preparation:	
Prep-n-Go™ Buffer (for use with untreated paper substrates)	4467079
Low-TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0)	Teknova T0223
Punch tool:	
Harris Micro-Punch™ tool, 1.2-mm	MLS
BSD600-Duet Semi-Automated Dried Sample Punch Instrument with a 1.2-mm punch head	Contact your local sales office.
BSD1000-GenePunch Automated Dried Sample Punch Instrument with a 1.2-mm punch head	

Untreated paper substrate

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

Swab substrate

Item	Source
Collection system	
4N6FLOQSwabs™, regular tip	4473979
Sample preparation:	
Prep-n-Go™ Buffer (for use with buccal swab substrates)	4471406

(continued)

Item	Source
Heated lysis protocol only: 1.5 mL tube format or 96-well deep-well plate format	
1.5 mL tube format	
1.5 mL tubes	MLS
Oven	VWR™ Scientific dry heat block or equivalent
96-well deep-well plate format	
PrepFiler™ 96-Well Processing Plates	A47010
Robbins Scientific™ Model 400 Hybridization Incubator or equivalent	MLS
Agilent™ Benchtop Rack for 200 µL Tubes/V Bottom Plates (metal) or equivalent **IMPORTANT!** Do not use a plastic plate adaptor.	Agilent Technologies 410094

Thermal cycler required materials

Veriti™ Thermal Cycler

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

GeneAmp™ PCR System 9700

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

Genetic analyzer required materials

SeqStudio™ Genetic Analyzer

Item	Source
SeqStudio™ Data Collection Software v1.2	A46168
SeqStudio™ Genetic Analyzer Cartridge v2	A41331
SeqStudio™ Genetic Analyzer Cathode Buffer Container	A33401
Reservoir Septa (for SeqStudio™ Cathode Buffer Container)	A35640
SeqStudio™ Integrated Capillary Protector	A31923
MicroAmp™ Optical 96-Well Reaction Plate	4316813
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4326659
MicroAmp™ Optical 8-Tube Strip, 0.2 mL	4316567
Septa for SeqStudio™ Genetic Analyzer, 96 well	A36541
Septa for SeqStudio™ Genetic Analyzer, 8 strip	A36543
DS-36 Matrix Standard Kit (Dye Set J6)	4425042

3500 Series Genetic Analyzer

Item	Source
3500 Series HID Data Collection Software v4.0.1	A46085
3500 Series Data Collection Software 3.1	4405187[1], A26287
3500 Series Data Collection Software 3	4405186[1]
3500 Series Data Collection Software 2	4475183[1]
HID Updater 3500 Data Collection Software v2	4480670
Anode buffer container (ABC)	4393927
Cathode buffer container (CBC)	4408256
POP-4™ Polymer (960 samples) for 3500/3500xL Genetic Analyzers	4393710
POP-4™ Polymer (384 samples) for 3500/3500xL Genetic Analyzers	4393715
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
Conditioning reagent	4393718
8-Capillary array, 36 cm for 3500 Genetic Analyzers	4404683

(continued)

Item	Source
24-Capillary array, 36 cm for 3500xL Genetic Analyzers	4404687
96-well retainer & base set (Standard) 3500/3500xL Genetic Analyzers	4410228
8-Tube retainer & base set (Standard) for 3500/3500xL Genetic Analyzers	4410231
8-Strip Septa for 3500/3500xL Genetic Analyzers	4410701
96-Well Septa for 3500/3500xL Genetic Analyzers	4412614
Septa Cathode Buffer Container, 3500 series	4410715

[1] Contact your Thermo Fisher Scientific HID representative.

3130 Series Genetic Analyzer

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

3730 Series Genetic Analyzer

Item	Source
3730xl Data Collection Software 5	4475133
3730/3730 *xl* Data Collection Software-v4	4475154
3130/3730 Data Collection Software v4 6-Dye Module v1	4480670
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3730 DNA Analyzer 48-Capillary Array, 36-cm	4331247

(continued)

Item	Source
POP-7™ Polymer for 3730/3730*xl* DNA Analyzers	4335611
Running Buffer, 10×	4335613
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
MicroAmp™ Optical 96-Well Reaction Plate	N8010560
250-µL Glass Syringe (array-fill syringe)	4304470
5.0-mL Glass Syringe (polymer-reserve syringe)	628-3731

Analysis software required materials

GeneMapper™ *ID-X* Software

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

Miscellaneous required materials

Plates and tubes

Item	Source
MicroAmp™ 96-Well Tray	N8010541
MicroAmp™ Reaction Tube with Cap, 0.2 mL	N8010540
MicroAmp™ 8-Tube Strip, 0.2 mL	N8010580
MicroAmp™ Optical 8-Cap Strips	4323032
MicroAmp™ 96-Well Tray/Retainer Set	403081
MicroAmp™ 96-Well Base	N8010531

(continued)

Item	Source
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Adhesive Film	4311971
MicroAmp™ Optical 96-Well Reaction Plate	N8010560

Laboratory supplies

Item	Source
Various procedures	
Aerosol resistant pipette tips	MLS[1]
Microcentrifuge tubes	MLS
Pipettors	MLS
Tape, labeling	MLS
Tube, 50-mL Falcon™	MLS
Tube decapper, autoclavable	MLS
Deionized water, PCR grade	MLS
Vortex	MLS

[1] Major laboratory supplier

C

Plate layouts

Example PCR plate layout

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

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

Example electrophoresis plate layout

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

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

Cycle 1 Cycle 2 Cycle 3

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 this kit for:

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

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

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

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

PCR setup work area materials

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

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

- Tube decapper, autoclavable
- Vortex

Amplified DNA work area

IMPORTANT! Place the thermal cyclers in the Amplified DNA Work Area.

Use only the validated thermal cyclers listed in “Instrument and software compatibility” on page 15.

Safety

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

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document title	Pub. No.
STR kits	
GlobalFiler™ Express PCR Amplification Kit—PCR and CE Quick Reference	4480794
GlobalFiler™ Express PCR Amplification Kit—PCR Setup—Swab Substrate Quick Reference	4477601
GlobalFiler™ Express PCR Amplification Kit—PCR Setup—Treated Paper Substrate Quick Reference	4480904
GlobalFiler™ Express PCR Amplification Kit—PCR Setup—Untreated Paper Substrate Quick Reference	4480795
Thermal cyclers	
Veriti™ Thermal Cycler User Guide	4375799
GeneAmp™ PCR System 9700 Base Module User Manual	4303481
SeqStudio™ Genetic Analyzer	
SeqStudio™ Genetic Analyzer Instrument and Software User Guide	MAN0018646
SeqStudio™ Genetic Analyzer for HID Instrument and Software v1.2.1 User Bulletin—New Features and Developmental Validation	100086084
3500 Series Genetic Analyzer	
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v1 User Guide	4401661
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v2 User Guide	4476988
HID Updater 3500 Data Collection Software v2.0 User Bulletin	NA
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 3 User Guide	100025036
3500 Series Data Collection Software v3 User Bulletin: New Features and HID Validation Summary	MAN0010812
3500 Series Data Collection Software v3.1 User Bulletin: New Features and HID Validation Summary	MAN0014110
3500 Series Data Collection Software 4 User Bulletin: New Features and Developmental Validation	100075298

(continued)

Document title	Pub. No.
3130 Series Genetic Analyzer	
3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide	4352716
3130/3130xl Genetic Analyzers Using Data Collection Software v3.0 User Bulletin	4363787
3130/3130xl Genetic Analyzers Getting Started Guide	4352715
3130/3130xl Genetic Analyzers Quick Reference Card	4362825
3130/3130xl Genetic Analyzers AB Navigator Software Administrator Guide	4359472
3730 Series Genetic Analyzer	
3730/3730xl Genetic Analyzer Getting Started Guide	4359476
3730xl Data Collection Software 5 for HID User Bulletin: New Features and Developmental Validation	MAN0019461
GeneMapper™ *ID-X* Software all versions	
GeneMapper™ ID-X Software Bin Overlap User Bulletin	100029546
GeneMapper™ *ID-X* Software v1.3	
GeneMapper™ ID-X Software v1.3 Verification Experiments and Installation Procedures User Bulletin	4470483
GeneMapper™ *ID-X* Software v1.4	
GeneMapper™ ID-X Software v1.4 New Features and Installation Procedures User Bulletin	4477684
GeneMapper™ *ID-X* Software v1.5	
GeneMapper™ ID-X Software v1.5 New Features and Verification User Bulletin	100031708
GeneMapper™ ID-X Software v1.5 Getting Started Guide— Basic Features	100031701
GeneMapper™ ID-X Software v1.5 Quick Reference— Basic Features	100031702
GeneMapper™ ID-X Software v1.5 Getting Started Guide— Mixture Analysis Tool	100031704
GeneMapper™ ID-X Software v1.5 Quick Reference— Mixture Analysis Tool	100031705
GeneMapper™ ID-X Software v1.5 Installation Guide	100031706
GeneMapper™ ID-X Software v1.5 Administrator Guide	100031703
GeneMapper™ ID-X Software v1.5 Reference Guide	100031707
GeneMapper™ *ID-X* Software v1.6	
GeneMapper™ ID-X Software v1.6 New Features and Software Verification User Bulletin	100073905

Customer and technical support

For support:

- **In North America**—Send an email to **HIDTechSupport@thermofisher.com**, or call **888-821-4443 option 1**.
- **Outside North America**—Contact your local support office.

For the latest services and support information for all locations, go to **thermofisher.com/support** to obtain the following information.

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

References

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