

# VeriFiler™ Express PCR Amplification Kit

## USER GUIDE

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Revision	Date	Description
E	8 April 2021	Add "Performance of direct amplification using a 10- $\mu$ L PCR reaction volume" on page 116.
D	18 January 2021	Add the SeqStudio™ Genetic Analyzer. Consolidate sample preparation for electrophoresis; it is the same for all instruments.
C	12 September 2017	<ul style="list-style-type: none"><li>• Add 4N6FLOQSwabs™ as a validated collection method.</li><li>• Add DNA-dependent artifact VIC110-120 (extra peaks in the electropherogram).</li></ul>
B	14 November 2016	Add representative electropherograms for several types of substrate.
A	04 October 2016	New document

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

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## Product description

### Kit overview

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

The kit is part of a fully integrated and validated forensic DNA workflow backed by best-in-class global training, service, and support. This kit is optimized for paternity and single-source samples.

The kit amplifies the following loci within the read region of 75–465 nt:

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

The VeriFiler™ Express kit delivers a 25-locus multiplex with the highest discrimination power of any Thermo Fisher Scientific Human Identification Kit. The highly optimized buffer formulation allows completion of amplification in ~45 minutes.

The VeriFiler™ Express 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 VeriFiler™ Express 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: COPAN NUCLEIC-CARD™ system or Whatman FTA™ cards
- Untreated paper: Bode Buccal DNA Collector™ or 903 paper
- Swab: COPAN 4N6FLOQSwabs™ reference collection devices or cotton swabs

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

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## About the primers

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

The VeriFiler™ Express kit includes the same primers as the GlobalFiler™ Express PCR Amplification Kit with the following primer additions and modifications:

- Addition of new primers for Penta D, Penta E, and D6S1043.
- Addition of 1 new SNP-specific primer for D19S433 loci to address a SNP 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 25 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	

(continued)

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

## Loci amplified by the kit

Table 1 VeriFiler™ Express 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
Penta E	15q26.2	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26	7, 12	
D2S441	2p14	8, 9, 10, 11, 11.3, 12, 13, 14, 15, 16, 17	NED™	14, 15
D19S433	19q12	6, 7, 8, 9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, 19.2		14, 15
TH01	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3		7, 9.3
FGA	4q28	13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2		24, 26
D22S1045	22q12.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	TAZ™	11, 16

Table 1 VeriFiler Express kit loci and alleles (continued)

Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	DNA Control 007
D5S818	5q21-31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18	TAZ™	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
D6S1043	6q16.1	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25		12, 14
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
Penta D	21q22.3	2.2, 3.2, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17		11, 12

## Standards and controls that are required

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

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

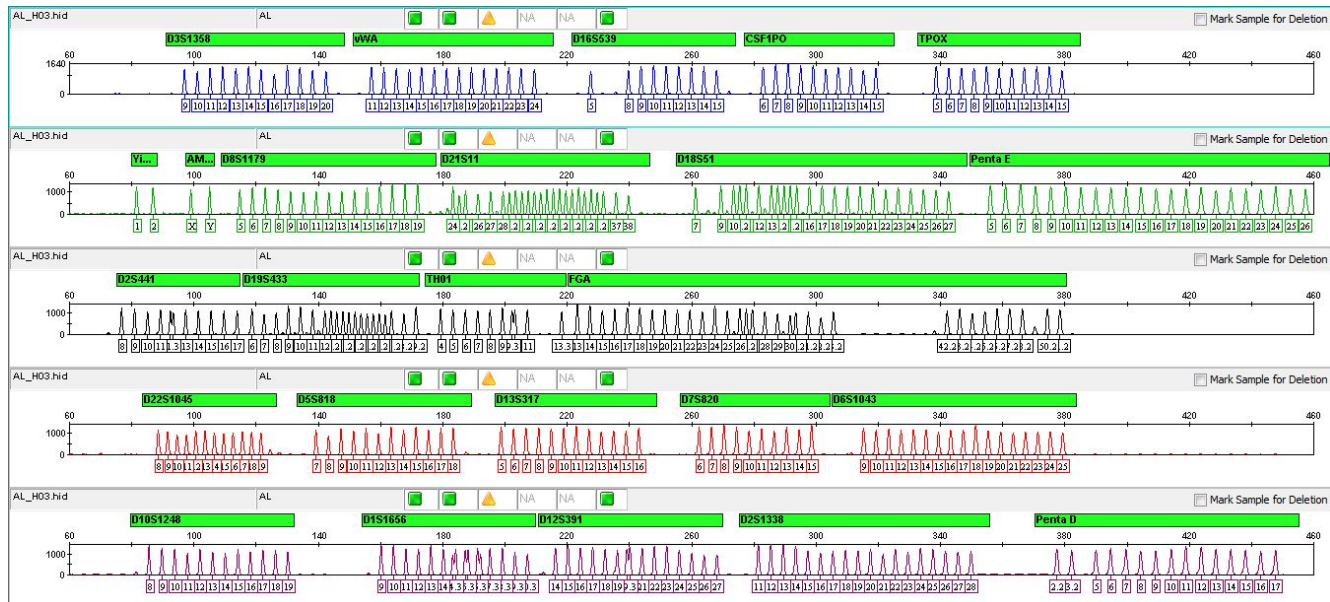


Figure 1 GeneMapper™ ID-X Software plot of the VeriFiler™ Express Allelic Ladder

## DNA Control 007 profile

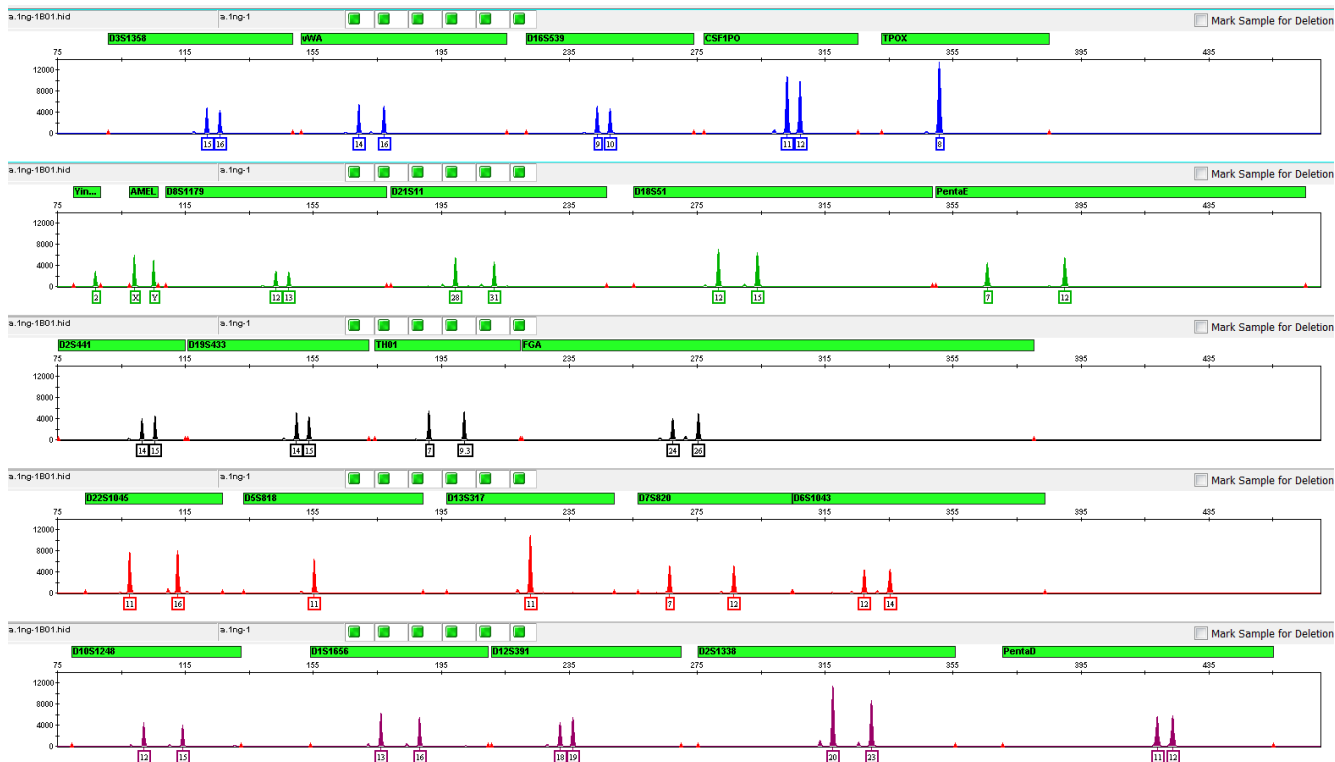


Figure 2 DNA Control 007 (2 ng) amplified at 27 PCR cycles with the VeriFiler™ Express kit and analyzed on an Applied Biosystems™ 3500xL Genetic Analyzer (Y-axis scale 0 to 14,000 RFU).

## Contents and storage

Table 2 VeriFiler™ Express PCR Amplification Kit (Cat. No. A32014; 200 reactions)

Contents	Amount	Storage
<p>VeriFiler™ Express Master Mix</p> <p>Contains MgCl<sub>2</sub>, dATP, dGTP, dCTP, and dTTP, bovine serum albumin, enzyme, and 0.05% sodium azide in buffer and salt.</p>	2 tubes, 1.0 mL	<p>–25°C to –15°C on receipt.</p> <p>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).</p>
<p>VeriFiler™ Express Primer Set</p> <p>Contains locus-specific 6-FAM™, VIC™, NED™, TAZ™, and SID™ dye-labeled and unlabeled primers in buffer. The primers amplify the STR loci D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, Penta E, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, D6S1043, D10S1248, D1S1656, D12S391, D2S1338, Penta D, and the sex-determining markers, Y indel and Amelogenin.</p>	2 tubes, 1.0 mL	<p>–25°C to –15°C on receipt.</p> <p>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).</p> <p>Store protected from light.</p>

Table 2 VeriFiler Express PCR Amplification Kit (Cat. No. A32014; 200 reactions) (continued)

Contents	Amount	Storage
<p>VeriFiler™ Express Allelic Ladder</p> <p>Contains the following amplified alleles:</p> <ul style="list-style-type: none"> <li>• 6-FAM™ dye (blue): D3S1358 9–20; vWA 11–24; D16S539 5, 8–15; CSF1PO 6–15; TPOX 5–15.</li> <li>• VIC™ dye (green): Y indel 1, 2; Amelogenin X, Y; D8S1179 5-19; D21S11 24, 24.2, 25–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38; D18S51 7, 9, 10, 10.2, 11–13, 13.2, 14, 14.2, 15–27; DYS391 7–13; Penta E 5–26.</li> <li>• NED™ dye (yellow): D2S441 8–11, 11.3, 12–17; D19S433 6–12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, 19.2; TH01 4–9, 9.3, 10, 11, 13.3; FGA 13–26, 26.2, 27–30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2.</li> <li>• TAZ™ dye (red): D22S1045 8–19; D5S818 7–18; D13S317 5–16; D7S820 6–15; D6S1043 9–25.</li> <li>• SID™ dye (purple): D10S1248 8–19; D1S1656 9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18.3, 19.3, 20.3; D12S391 14–19, 19.3, 20–27; D2S1338 11–28; Penta D 2.2, 3.2, 5–17.</li> </ul>	1 tube, 0.05 mL	<p>–25°C to –15°C on receipt.</p> <p>2°C to 8°C after first use, up to the expiration date stated on the kit.</p> <p>Store protected from light.</p> <p><b>IMPORTANT!</b> The allelic ladder contains PCR products. Do not amplify. To avoid contamination, store the allelic ladder separate from the other kit components and unamplified DNA.</p>
<p>DNA Control 007</p> <p>Contains 2.0 ng/μL human male genomic DNA in 0.05% sodium azide and buffer.</p> <p>The profile of this male DNA is D3S1358 15,16; vWA 14, 16; D16S539 9, 10; CSF1PO 11,12; TPOX 8; D8S1179 12, 13; D21S11 28, 31; D18S51 12, 15; Penta E 7, 12; D2S441 14,15; D19S433 14,15; TH01 7, 9.3; FGA 24, 26; D22S1045 11, 16; D5S818 11; D13S317 11; D7S820 7,12; D6S1043 12, 14; D10S1248 12, 15; D1S1656 13, 16; D12S391 18, 19; D2S1338 20, 23; Penta D 11, 12.</p>	1 tube, 0.05 mL	<p>–25°C to –15°C on receipt.</p> <p>2°C to 8°C after first use, up to the expiration date stated on the kit.</p>

## Required materials not supplied

See Appendix B, “Required materials not supplied”.

## Instrument and software compatibility

Catalog numbers that appear as links open the web pages for those products.

Instrument type	Validated models
Thermal cyclers	<ul style="list-style-type: none"> <li>• ProFlex™ 96-well PCR System (Cat. No. <a href="#">4484075</a>)</li> <li>• GeneAmp™ PCR System 9700, 96-Well Silver (Cat. No. N8050001)</li> <li>• GeneAmp™ PCR System 9700, 96-Well Gold-Plated (Cat. No. 4314878)</li> </ul> <p><b>IMPORTANT!</b> VeriFiler™ Express kit is NOT validated for use with:</p> <ul style="list-style-type: none"> <li>• Veriti™ 96-Well Thermal Cycler (Cat. No. 4479071)</li> <li>• Veriti™ Fast 96-Well Thermal Cycler (Cat. No. 4375305)</li> <li>• GeneAmp™ PCR System 9700, 96-Well Aluminum (Cat. No. 4314879)</li> </ul>
Genetic analyzers <sup>[1]</sup>	<p>SeqStudio™ Genetic Analyzer</p> <ul style="list-style-type: none"> <li>• SeqStudio™ Data Collection Software v1.2</li> <li>• SeqStudio™ Data Collection Software v1.2.1</li> </ul> <hr/> <p>3500/3500xL Genetic Analyzer</p> <ul style="list-style-type: none"> <li>• 3500 Series Data Collection Software 1 (Windows™ Vista operating system) and HID Updater 3500 Data Collection Software v2 (Cat. No. 4480670)</li> <li>• 3500 Series Data Collection Software 2 (Windows™ 7 operating system) and HID Updater 3500 Data Collection Software v2 (Cat. No. 4480670)</li> <li>• 3500 Series Data Collection Software 3 (Windows™ 7 operating system)</li> <li>• 3500 Series Data Collection Software 3.1 (Windows™ 7 operating system)</li> <li>• 3500 Series Data Collection Software 4 (Windows™ 10 operating system)</li> <li>• 3500 Series HID Data Collection Software v4.0.1 (Windows™ 10 operating system)</li> </ul> <hr/> <p>3130/3130xL Genetic Analyzer</p> <ul style="list-style-type: none"> <li>• 3130 Series Data Collection Software 4 (Windows™ 7 operating system)</li> <li>• 3130/3730 Data Collection 4 6-Dye Module v1</li> </ul>

(continued)

Instrument type	Validated models
Genetic analyzers <sup>[1]</sup>	3730/3730xl DNA Analyzer <ul style="list-style-type: none"> <li>• 3730 Series Data Collection Software 4 (Windows™ 7 operating system)</li> <li>• 3730 Series Data Collection Software 4 6-Dye Module v1</li> <li>• 3730xl Data Collection Software 5 (Windows™ 10 operating system)</li> </ul> <p><b>Note:</b> For information on using the 3730xl DNA Analyzer, see the <i>3730xl Data Collection Software 5 for HID User Bulletin: New Features and Developmental Validation</i> (Pub. No. MAN0019461).</p>
Analysis software	GeneMapper™ ID-X Software v1.4 or later Windows™ XP, Windows™ 7, or Windows™ 10 operating system

<sup>[1]</sup> We conducted validation studies using the SeqStudio™, 3130xl, 3500, 3500xL, and 3730xl instruments. For validation information on the SeqStudio™ instrument, see the *SeqStudio™ Genetic Analyzer for HID Instrument and Software v1.2.1 User Bulletin—New Features and Developmental Validation* (Pub. No. 100086084). 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 reactions: treated paper substrate” on page 21 or

“Prepare the 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 VeriFiler™ Express kit



Amplify with the GeneAmp™ PCR System 9700 or ProFlex™ 96-well PCR System



### 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 34 or

“Set up the 3730/3730xl instruments for electrophoresis (before first use of the kit)” on page 36



“Prepare samples for electrophoresis” on page 39



### Analyze data

“Set up the GeneMapper™ ID-X Software for analysis (before first use of the kit)” on page 43

“Create an analysis method” on page 48

“Create a size standard definition file” on page 56

“Analyze and edit sample files with GeneMapper™ ID-X Software” on page 58

“Examine or edit a project” on page 59

### Perform PCR on swab substrates

“Prepare the reactions: swab substrate” on page 26



Lyse in Prep-n-Go™ Buffer



Process with VeriFiler™ Express kit





# Perform PCR

■ Optimize PCR cycle number (before first use of the kit) .....	18
■ Before you begin .....	19
■ Treated paper substrates: prepare the amplification kit reactions .....	20
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■ Swab substrates: prepare the amplification kit reactions .....	24
■ Perform PCR .....	28

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

Before using the VeriFiler™ Express 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 Optimize PCR cycle number

- Use 19 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. Additional samples may be used for a more comprehensive sensitivity experiment. Examples of PCR and electrophoresis plate layouts are provided on page “Example PCR plate layout” on page 129.
- To maximize result quality, prepare and amplify Plate 1, then repeat for Plates 2, 3, and 4. Do not prepare all 4 plates before amplification.
- To minimize the effect of instrument-to-instrument variation, use the same thermal cycler to amplify all 4 plates.

### Select samples and prepare plates

1. Select 19 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 4 identical PCR plates (see page “Example PCR plate layout” on page 129 for a suggested plate layout).

- 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, 28 cycles	25, 26, 27, 28 cycles	N/A
Buccal	25, 26, 27, 28 cycles	25, 26, 27, 28 cycles	25, 26, 27, 28 cycles

## Determine optimum PCR conditions

- Run the PCR products on the appropriate CE platform using the recommended protocol that is described in Chapter 3, “Perform electrophoresis”.
- 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 (before first use of the kit)

Thaw the Master Mix and Primer Set.

---

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

---

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

---

## 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 25- $\mu$ L reaction mix into the wells of the reaction plate before adding the punches.  
Alternatively, dispense 3  $\mu$ L 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 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 (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 ...	Add...	
Negative control	1.2 mm blank disc	
Test samples	1.2 mm sample disc	
Positive control <b>IMPORTANT!</b> 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	10.0 µL
Primer Set	10.0 µL
Low-TE buffer	5.0 µL

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

**IMPORTANT!** This kit is optimized for a 25-µL PCR reaction 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. See page 116 for performance testing results using a 10-µL PCR reaction volume.

4. Vortex the reaction mix for 3 seconds, then centrifuge briefly.
5. Dispense 25 µL of the reaction mix into each reaction well of a MicroAmp™ Optical 96-Well Reaction Plate.

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

---

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

---

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

---

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

---

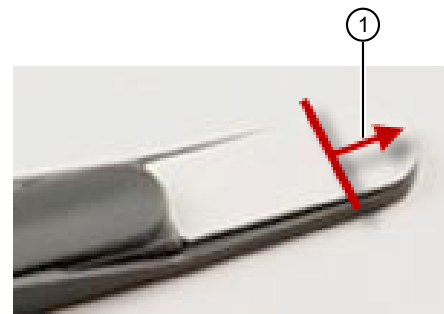
**IMPORTANT!** This kit is not validated for use with the GeneAmp™ PCR System 9700 with the aluminum 96-well block. Use of this thermal cycling platform may adversely affect performance of this kit.

---

## 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 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 (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	5 µL of Prep-n-Go™ Buffer	
Test samples	5 µL of Prep-n-Go™ Buffer	
Positive control	For 25 and 26 cycles	2 µL of Prep-n-Go™ Buffer
	For 27 cycles	3 µL of Prep-n-Go™ Buffer
	For 28 cycles	4 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 <b>IMPORTANT!</b> 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	10.0 µL
Primer Set	10.0 µL

---

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

---

**IMPORTANT!** This kit is optimized for a 25- $\mu$ L PCR reaction 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. See page 116 for performance testing results using a 10- $\mu$ L PCR reaction volume.

---

6. Vortex the reaction mix for 3 seconds, then centrifuge briefly.
7. Dispense 20  $\mu$ L of the reaction mix into each reaction well of a MicroAmp™ Optical 96-Well Reaction Plate.  
The final volume in each well is 25  $\mu$ L (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 of challenging or aged samples.

1. Preheat the heat block to 90°C, or preheat the oven with a metal plate adaptor to 99°C.
2. Add 400 µL of Prep-n-Go™ Buffer (for buccal swabs, Cat. No. 4471406) to 1.5-mL tubes or to 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.
4. Let the tubes or plate stand for 20 minutes in the preheated heat block or oven to lyse the sample.
5. After 20 minutes, remove the tubes or plate from the heat block or oven, then let the lysate stand at room temperature for ≥15 minutes to cool the lysate (for accurate pipetting).
6. Transfer the sample lysate to tubes or plates for storage. Discard the 1.5-mL tubes or PrepFiler™ 96-Well Processing Plate containing the swab heads.

---

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

---

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

## Prepare the reactions: swab substrate

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

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

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

Reaction component	Volume per reaction
Master Mix	10.0 $\mu$ L
Primer Set	10.0 $\mu$ L
Low-TE buffer	3.0 $\mu$ L

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

**IMPORTANT!** This kit is optimized for a 25- $\mu$ L PCR reaction 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. See page 116 for performance testing results using a 10- $\mu$ L PCR reaction volume.

3. Vortex the reaction mix for 3 seconds, then centrifuge briefly.
4. Dispense 23  $\mu$ L of the reaction mix into each reaction well of a MicroAmp™ Optical 96-Well Reaction Plate.  
The final volume in each well is 25  $\mu$ L (reaction mix plus Prep-n-Go™ Buffer or sample lysate).
5. Add samples to the MicroAmp™ Optical 96-Well Reaction Plate:

To these wells ...	Add...	
Test samples	2 $\mu$ L of sample lysate	
Positive control	For 25 and 26 cycles	3 $\mu$ L of Control DNA 007
	For 27 cycles	2 $\mu$ L of Control DNA 007
	For 28 cycles	1 $\mu$ L 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.

- 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.

---

- Vortex the reaction mix at medium speed for 3 seconds.
- Centrifuge the plate at 3,000 rpm for about 20 seconds in a tabletop centrifuge with plate holders.
- 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 or the Veriti™ Thermal Cyclers. Use of these thermal cycling platforms may adversely affect performance of this kit.

---

## Store the sample lysate

- Cap the sample lysate storage tubes or seal the sample lysate storage plate with MicroAmp™ Clear Adhesive Film.
- Store the sample lysate as needed.

Storage time	Storage temperature
<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 validated 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.

Initial incubation step	Optimum cycle number <sup>[1]</sup>			Final extension	Final hold
	Denature	Anneal	Extend		
HOLD	CYCLE			HOLD	HOLD
95°C, 1 minute	94°C, 3 seconds	59°C, 16 seconds	65°C, 29 seconds	60°C, 5 minutes	4°C, up to 24 hours <sup>[2]</sup>

<sup>[1]</sup> See “Optimize PCR cycle number (before first use of the kit)” on page 18.

<sup>[2]</sup> 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 ProFlex™ 96-well PCR System does not require a compression pad.

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

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

**IMPORTANT!** Protect the amplified DNA from light.

# 3

## Perform electrophoresis

- Allelic ladder requirements for electrophoresis ..... 29
- Materials required for electrophoresis ..... 30
- Set up the SeqStudio™ instruments for electrophoresis (before first use of the kit) ..... 30
- Set up the 3500/3500xL instruments for electrophoresis (before first use of the kit) ..... 32
- Set up the 3130/3130xl instruments for electrophoresis (before first use of the kit) ..... 34
- Set up the 3730/3730xl instruments for electrophoresis (before first use of the kit) ..... 36
- Prepare samples for electrophoresis ..... 39

### 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
3500xL	1 per injection	24 samples	23 samples + 1 allelic ladder
3130	1 per 4 injections	4 samples	15 samples + 1 allelic ladder
3130xl	1 per injection	16 samples	15 samples + 1 allelic ladder
3730/3730xl, 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, “Required materials 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 136.

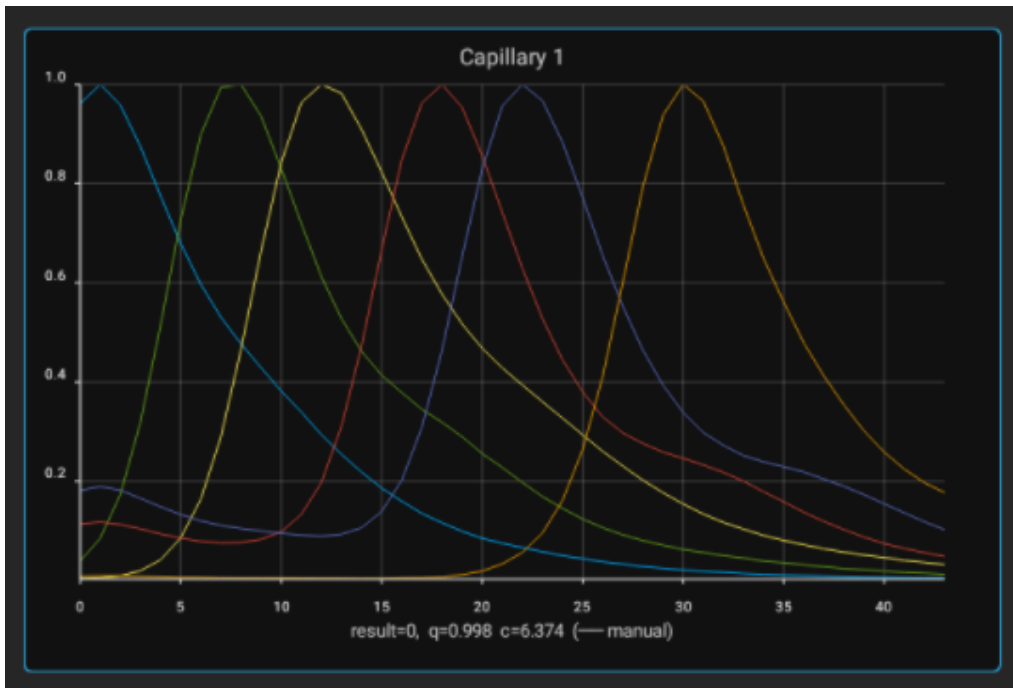
Genetic Analyzer	Data Collection Software	Additional software	Run modules and conditions
SeqStudio™	SeqStudio™ Data Collection Software v1.2.1	None	<ul style="list-style-type: none"> <li>• Run Module: HIDAnalysis</li> <li>• Injection Conditions: 1.2 kV/10 sec</li> <li>• Run Conditions: 11 kV/1,120 sec</li> <li>• Dye Set J6</li> <li>• Kit: VeriFiler™ Express (to enable marker-to-marker pull-up reduction feature)</li> </ul>

## 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 136.

**Note:** We conducted original validation studies for the kit using the 3130xL, 3500, or 3500xL configurations. Subsequent validation studies for the kit were performed using the 3730xL 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: <ul style="list-style-type: none"> <li>• Run Module: HID36_POP4(xl)</li> <li>• Injection Conditions: 1.2 kV/15 sec (24 sec for xl)</li> <li>• Run Conditions: 13 kV/1,550 sec</li> <li>• Dye Set J6 or J6-OSR</li> </ul>
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.
3500 3500xL	Windows™ 7	v2	HID Updater 3500 DC v2 (Cat. No. 4480670)	All assays use the following conditions:
3500 3500xL	Windows™ Vista	v1	HID Updater 3500 DC v2 (Cat. No. 4480670)	<ul style="list-style-type: none"> <li>• Run module: HID36_POP4(xl)</li> <li>• Injection conditions: 1.2 kV/15 sec (24 sec for xl)</li> <li>• Run conditions: 13 kV/1,550 sec</li> <li>• Dye Set J6</li> </ul>



## 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 VeriFiler™ Express 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.

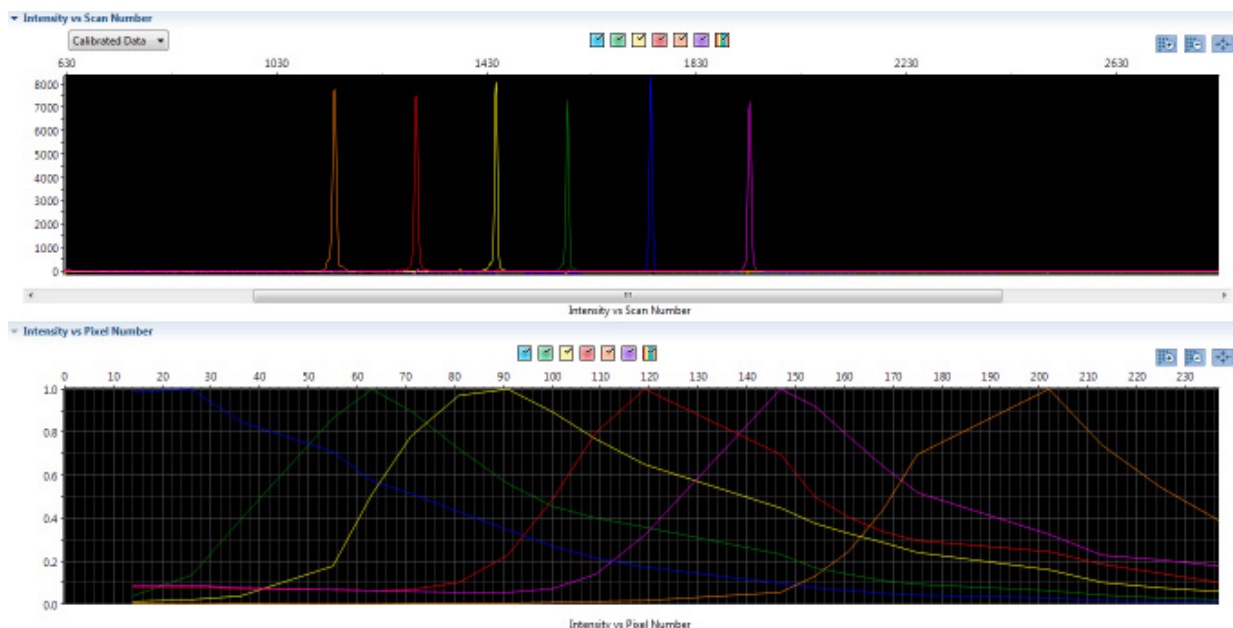
1. Shut down the 3500/3500xL Data Collection Software.
2. Download the updater from [www.thermofisher.com/us/en/home/technical-resources/software-downloads/3500-Series-Genetic-Analyzers-for-Human-Identification.html](http://www.thermofisher.com/us/en/home/technical-resources/software-downloads/3500-Series-Genetic-Analyzers-for-Human-Identification.html).
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.

## 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 3130/3130xl 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 136.

**Note:** We conducted original validation studies for the VeriFiler™ Express kit using the 3130xl, 3500, or 3500xL configurations. Subsequent validation studies for the kit were performed using the 3730xl/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 <sup>[1]</sup>	3130/3730 DC v4 6-Dye Module v1	<ul style="list-style-type: none"> <li>HIDFragmentAnalysis36_POP4_1 Injection conditions: 3 kV/5 sec</li> <li>Run conditions: 15 kV/1500 sec</li> <li>Dye Set J6</li> </ul>
3130 xl				<ul style="list-style-type: none"> <li>HIDFragmentAnalysis36_POP4_1 Injection conditions: 3 kV/10 sec</li> <li>Run conditions: 15 kV/1500 sec</li> <li>Dye Set J6</li> </ul>

<sup>[1]</sup> 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.

3xxx Series Data Collection Software 4 Software Activation

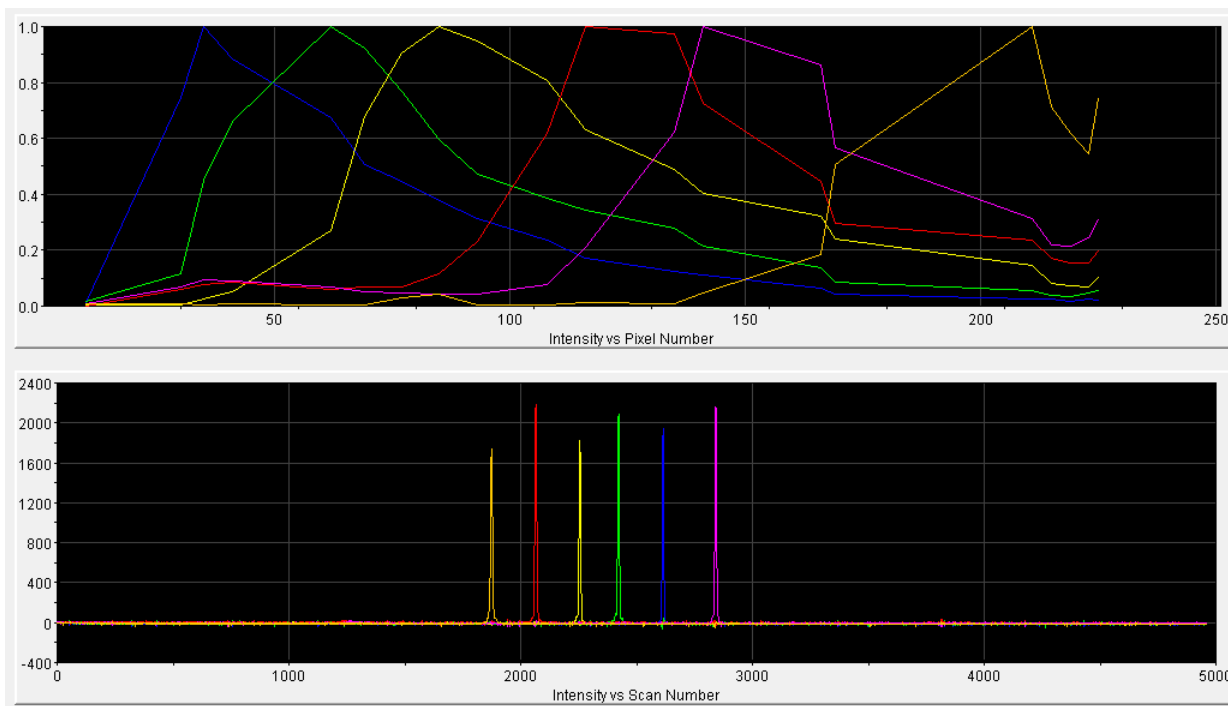
1. Request license file for Computer ID:  
d4bed9a7d416 d4bed9a7d417  
*This ID is unique to this computer and cannot be used to obtain a license file for another computer.*
  - a. Enter the license key (from CD or email):
  - b. Enter your email address:  
john.doe@thermofisher.com
  - c. Is this computer currently connected to the internet?
2. Retrieve the license file from email, then save it to the desktop of this computer.
3. Find the license file:
4. Click

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 3730/3730xl 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 136.

Genetic Analyzer	Operating System	Data Collection Software	Additional software	Run modules and conditions
3730xl	Windows™ 10	Data Collection Software v5	None	<ul style="list-style-type: none"> <li>GeneMapper36_POP7_1 Injection conditions: 2 kV/10 sec</li> <li>Run conditions<sup>[1]</sup>: 7 kV/2,600 sec</li> <li>Dye Set J6</li> </ul>
3730	Windows™ 7	Data Collection Software v4 <sup>[2]</sup>	3130/3730 DC v4 6-Dye Module v1	

<sup>[1]</sup> To account for migration variation (particularly in larger, more complex markers such as Penta D), use a 7 kV run voltage with a customized bin set. For information on customizing the run conditions, see the *3730xl DNA Analyzer with 3730xl Data Collection Software 5 User Guide* (Pub. No. 100077621).

<sup>[2]</sup> 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.

3xxx Series Data Collection Software 4 Software Activation

1. Request license file for Computer ID:  
  
*This ID is unique to this computer and cannot be used to obtain a license file for another computer.*
  - a. Enter the license key (from CD or email):
  - b. Enter your email address:
  - c. Is this computer currently connected to the internet?
2. Retrieve the license file from email, then save it to the desktop of this computer.
3. Find the license file:
4. Click

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.**

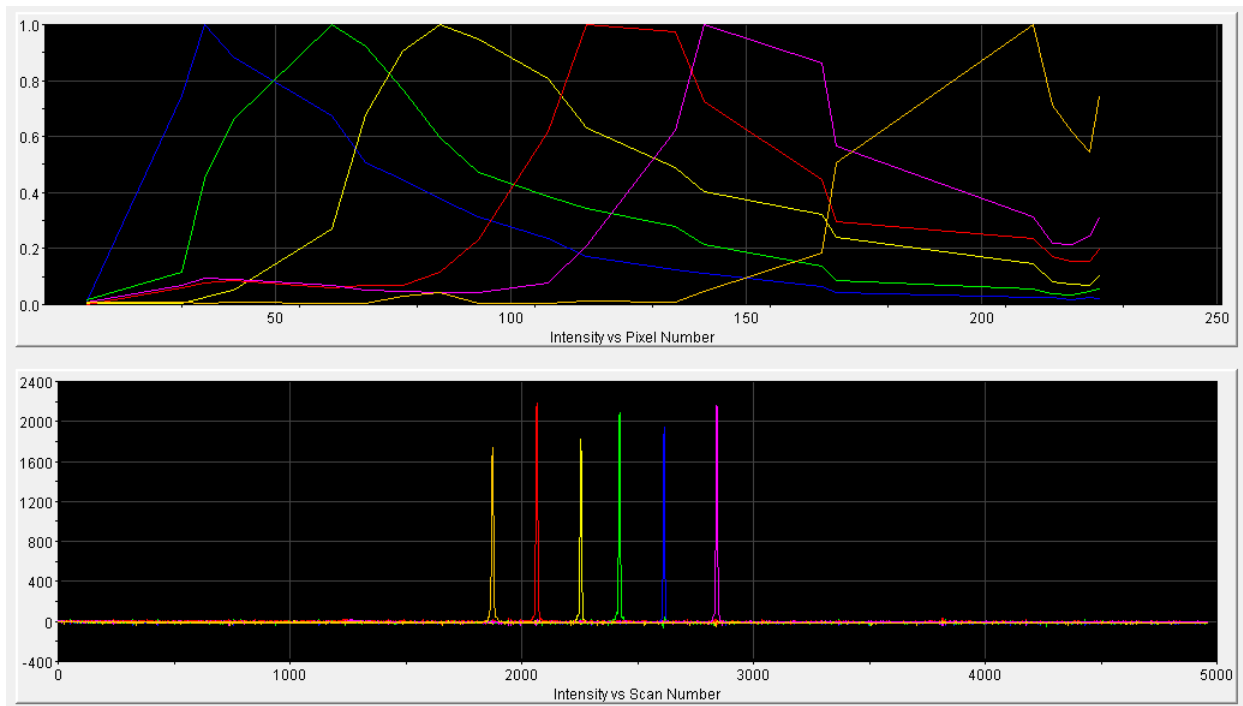
A message is displayed when the license is installed and validated.

**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.





# Analyze data with GeneMapper™ ID-X Software

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■ Allelic ladder requirements for data analysis .....	41
■ File names and versions used in this section .....	42
■ Set up the GeneMapper™ ID-X Software for analysis (before first use of the kit) .....	43
■ Create an analysis method .....	48
■ Create a size standard definition file if needed .....	55
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■ Examine or edit a project .....	59
■ For more information on using the GeneMapper™ ID-X Software .....	59

## 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 VeriFiler™ Express 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](https://www.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 43



“(If needed) Download newer versions of panel, bin, and stutter files” on page 44



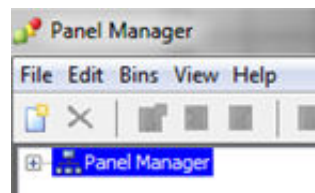
“Import panels, bins, and marker stutter” on page 44



“(Optional) Define custom table or plot settings” on page 47

## 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](http://www.thermofisher.com/GMIDXsoftware).
2. If the file versions listed are newer than the versions on your computer, download the file **VeriFilerExpress 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.

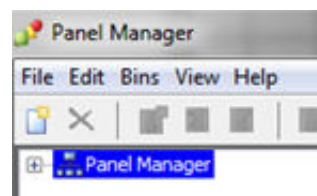
---

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:

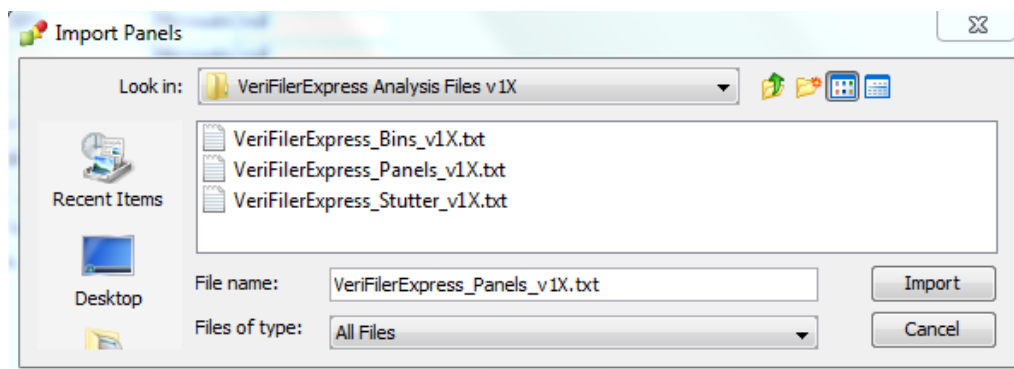


- a. Select **Panel Manager**, then select **File ▶ Import Panels** to open the **Import Panels** dialog box.
  - b. Navigate to, then open the VeriFilerExpress Analysis Files folder that you unzipped in the previous procedure.
4. Select VeriFilerExpress\_Panels.txt, then click **Import**.

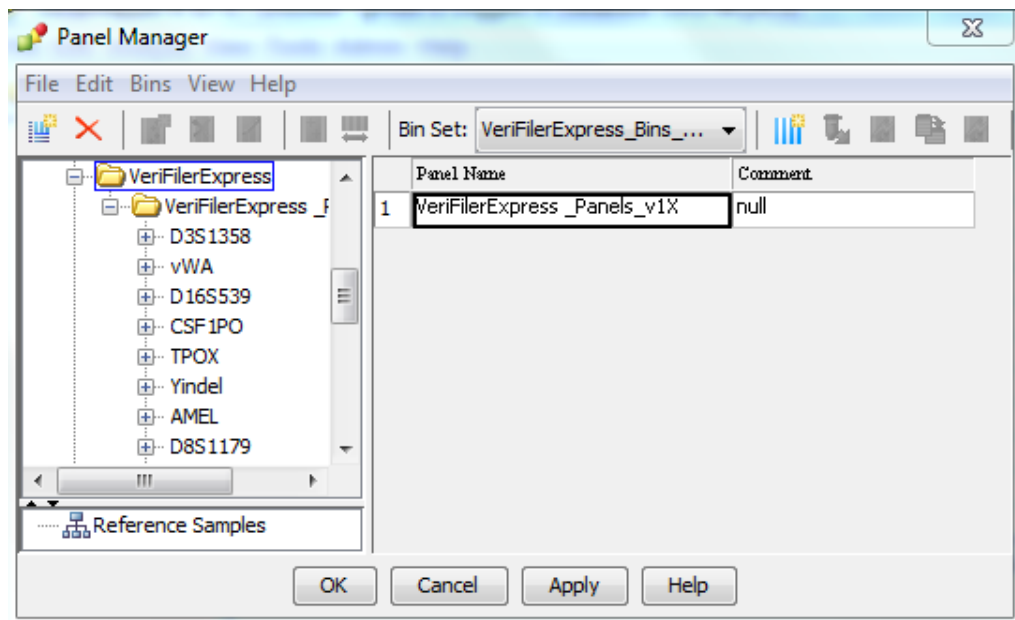
---

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

---

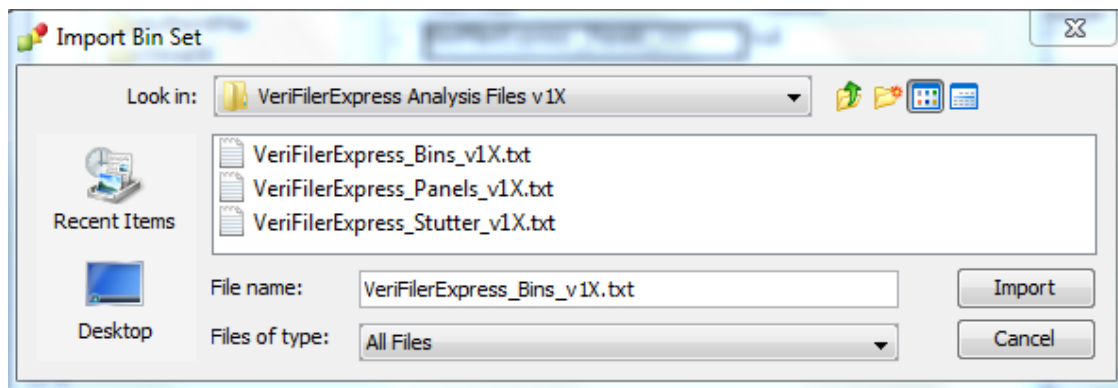


5. Import the bins file:
  - a. Select the VeriFilerExpress\_Panels folder in the navigation pane.



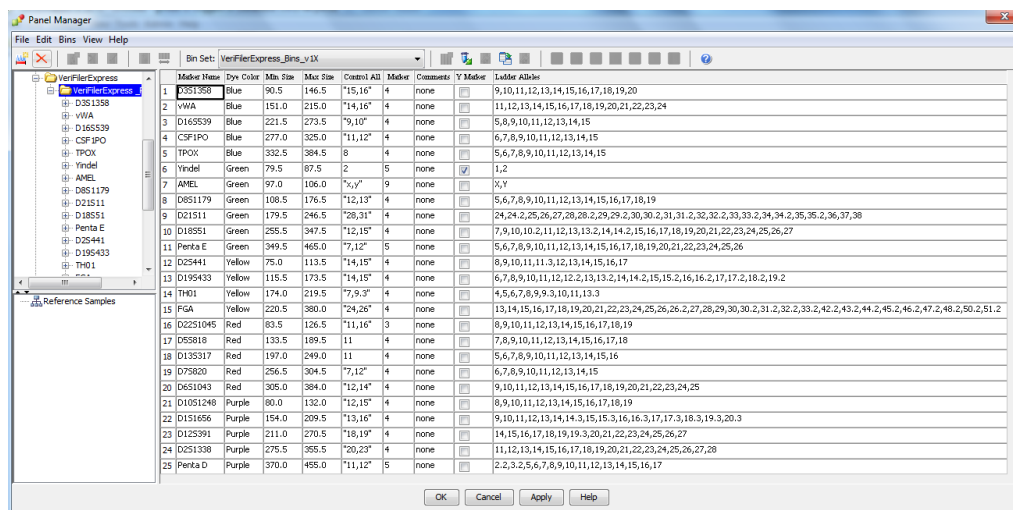
- b. Select **File** ► **Import Bin Set** to open the **Import Bin Set** dialog box.
- c. Navigate to, then open the VeriFilerExpress Analysis Files folder.
- d. Select VeriFilerExpress\_Bins.txt, then click **Import**.

**Note:** Importing this file associates the bin set with the panels in the VeriFilerExpress\_Panels folder.



6. (Optional) View the imported panels and bins in the navigation pane: Double-click the VeriFilerExpress\_Panels folder.

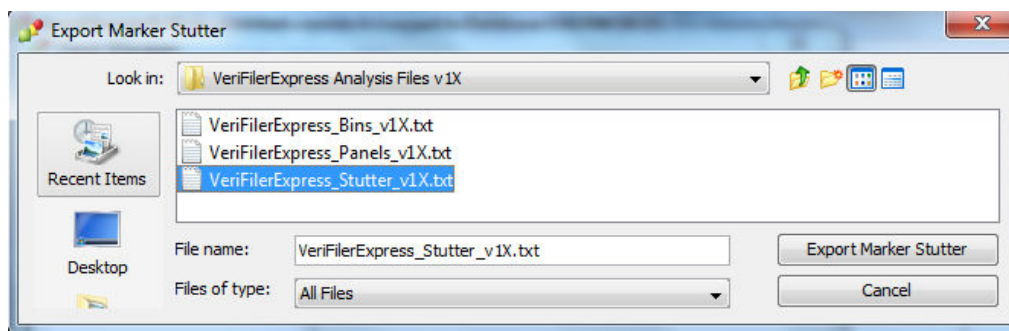
The panel information is displayed in the right pane and the markers are displayed below it.



7. Import the stutter file:

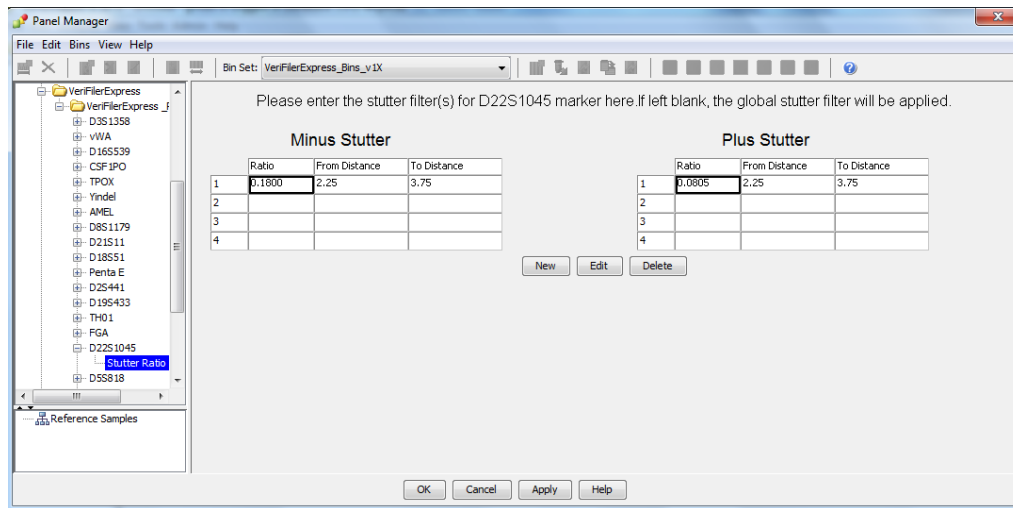
- Select the VeriFilerExpress\_Panels folder in the navigation panel.
- Select **File** ► **Import Marker Stutter** to open the **Import Marker Stutter** dialog box.
- Navigate to, then open the VeriFilerExpress Analysis Files folder.
- Select VeriFilerExpress\_Stutter.txt, then click **Import**.

**Note:** Importing this file associates the marker stutter ratio with the bin set in the VeriFilerExpress\_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:
- Double-click the VeriFilerExpress\_Panels folder to display the folder.
  - 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

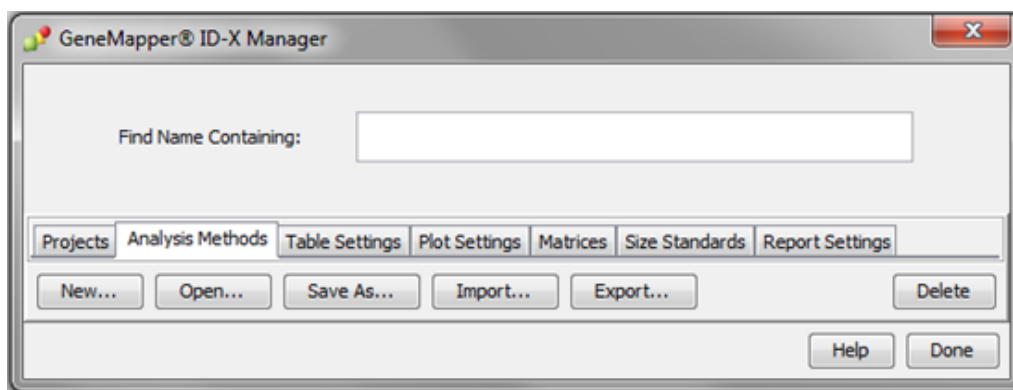
### Create an analysis method

---

**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.

---

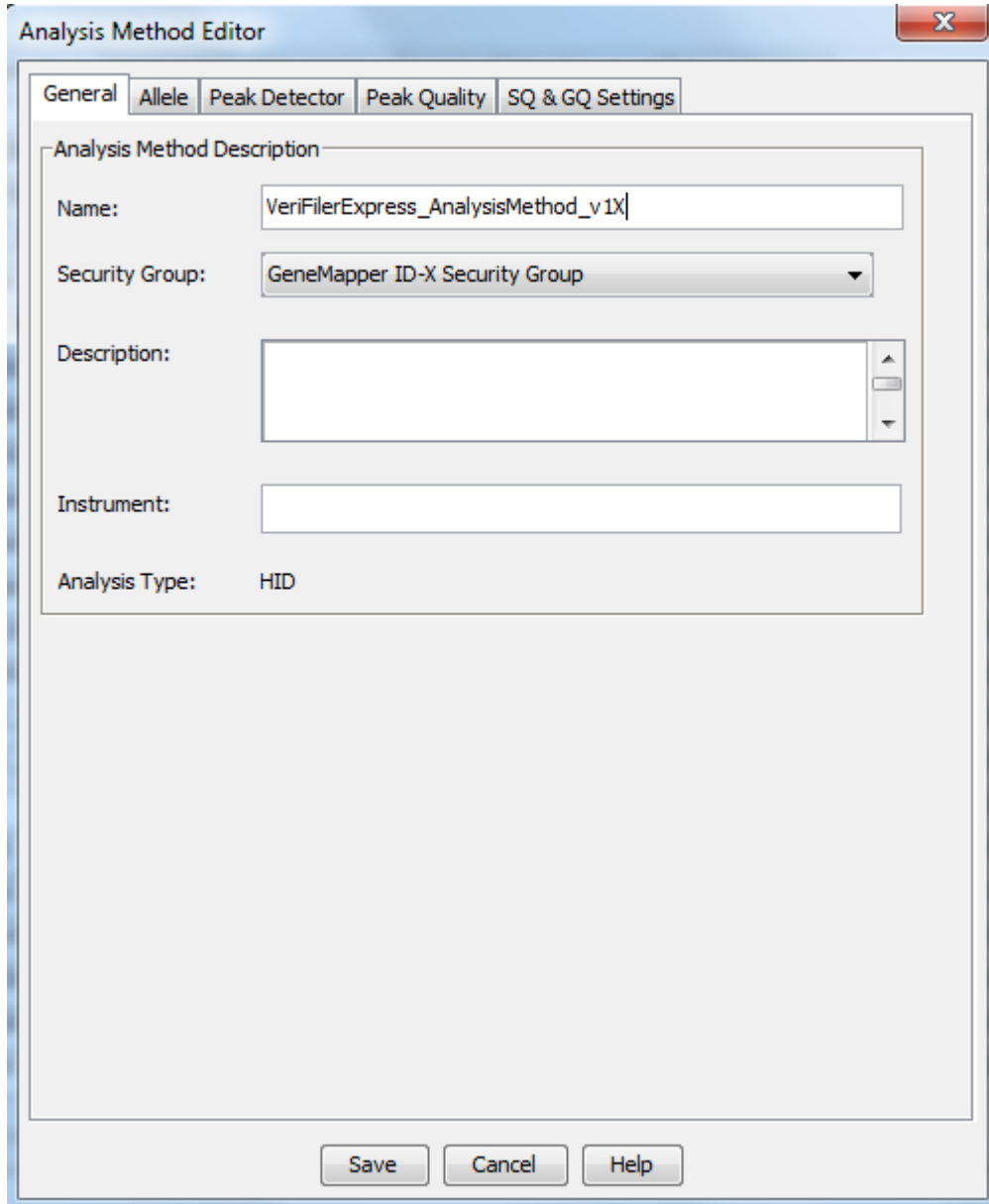
4. After you enter the settings on all tabs, click **Save**.



## Enter Analysis Method settings

### Enter General tab settings

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



The screenshot shows the 'Analysis Method Editor' dialog box with the 'General' tab selected. The 'Analysis Method Description' section contains the following fields:

- Name:** VeriFilerExpress\_AnalysisMethod\_v1X
- Security Group:** GeneMapper ID-X Security Group
- Description:** (Empty text area)
- Instrument:** (Empty text field)
- Analysis Type:** HID

At the bottom of the dialog are three buttons: Save, Cancel, and Help.

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 **VeriFilerExpress\_Bins\_v1X** bin set.

Analysis Method Editor

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

Bin Set: VeriFilerExpress\_Bins\_v1X

Use marker-specific stutter ratio and distance if available

Marker Repeat Type:		Tri	Tetra	Penta	Hexa
Global Cut-off Value		0.2	0.2	0.2	0.0
MinusA Ratio		0.0	0.0	0.0	0.0
MinusA Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0
Global Minus Stutter Ratio		0.0	0.0	0.0	0.0
Global Minus Stutter Distance	From	0.0	3.25	0.0	0.0
	To	0.0	4.75	0.0	0.0
Global Plus Stutter Ratio		0.0	0.0	0.0	0.0
Global Plus Stutter Distance	From	0.0	0.0	0.0	0.0
	To	0.0	0.0	0.0	0.0

Amelogenin Cutoff: 0.0

Range Filter... Factory Defaults

Save Cancel Help

Figure 3 Settings used in developmental validation of the kit

2. (Optional) To apply the stutter ratios contained in the **VeriFilerExpress\_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**.

---

**Note:** For paternity and database applications, we recommended using a **20% Global Cut-off Value** for the Tri, Tetra, and Penta loci as shown in Figure 3.

---

4. Enter the appropriate filter settings.

## Enter Peak Detector tab settings

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

Enter the appropriate values:

The screenshot shows the 'Analysis Method Editor' dialog box with the 'Peak Detector' tab selected. The 'Peak Detection Algorithm' is set to 'Advanced'. The 'Ranges' section includes 'Analysis' (Full Range) and 'Sizing' (All Sizes) dropdowns, with 'Start Pt: 3400', 'Stop Pt: 9000', 'Start Size: 60', and 'Stop Size: 500'. The 'Smoothing and Baseline' section has 'Smoothing' set to 'Light' and 'Baseline Window' set to 33 pts. The 'Size Calling Method' section has '3rd Order Least Squares' selected. The 'Peak Detection' section includes 'Peak Amplitude Thresholds' (B, R, G, P, Y, O), 'Min. Peak Half Width: 2 pts', 'Polynomial Degree: 3', 'Peak Window Size: 13 pts', and 'Slope Threshold' (Peak Start: 0.0, Peak End: 0.0). The 'Normalization' section has 'Use Normalization, if applicable' unchecked. A 'Factory Defaults' button is located at the bottom right of the dialog. At the bottom of the dialog are 'Save As', 'Save', 'Cancel', and 'Help' buttons.

Figure 4 Settings used in developmental validation of the kit

**Note:** When using the 3730/3730xl DNA Analyzer with POP-7™ polymer only; a **Smoothing** setting of **None** and the **Peak Window Size** of **9** pts, 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.

## 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:

The screenshot shows the 'Analysis Method Editor' window with the 'Peak Quality' tab selected. The settings are as follows:

Section	Parameter	Value
Min/Max Peak Height (LPH/MPH)	Homozygous min peak height	[Empty]
	Heterozygous min peak height	[Empty]
	Max Peak Height (MPH)	[Empty]
Peak Height Ratio (PHR)	Min peak height ratio	[Empty]
Broad Peak (BD)	Max peak width (basepairs)	1.5
Allele Number (AN)	Max expected alleles: For autosomal markers & AMEL	2
	For Y markers	1
Allelic Ladder Spike	Spike Detection	Enable
	Cut-off Value	0.2
Sample Spike Detection	Spike Detection	Enable

Buttons at the bottom: Save As, Save, Cancel, Help, and a Factory Defaults button.

## 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:

The screenshot shows the 'Analysis Method Editor' dialog box with the 'SQ & GQ Settings' tab selected. The dialog contains several sections for configuring quality weights and ranges.

Quality weights are between 0 and 1.

**Sample and Control GQ Weighting**

Broad Peak (BD)	0.8	Allele Number (AN)	1.0
Out of Bin Allele (BIN)	0.8	Low Peak Height (LPH)	0.3
Overlap (OVL)	0.8	Max Peak Height (MPH)	0.3
Marker Spike (SPK)	0.3	Off-scale (OS)	0.8
AMEL Cross Check (ACC)	0.0	Peak Height Ratio (PHR)	0.3

Control Concordance (CC) Weight = 1.0 (Only applicable to controls)

**SQ Weighting**

Broad Peak (BD)	0.5
-----------------	-----

**Allelic Ladder GQ Weighting**

Spike (SSPK/SPK)	1	Off-scale (OS)	1
------------------	---	----------------	---

**SQ & GQ Ranges**

	Pass Range:	Low Quality Range:
Sizing Quality:	From 0.75 to 1.0	From 0.0 to 0.25
Genotype Quality:	From 0.75 to 1.0	From 0.0 to 0.25

Buttons: Save, Cancel, Help, Reset Defaults

**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 Y indel marker.

## Create a size standard definition file if needed

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

### About the GS600\_LIZ\_ (60–460) size standard definition file

The GS600\_LIZ\_(60–460) size standard definition that is provided with GeneMapper™ ID-X Software and used with the Local Southern size calling method contains the following peaks: 60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, and 460.

This size standard definition has been validated for use with this kit on the genetic analyzers listed in “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 56.

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

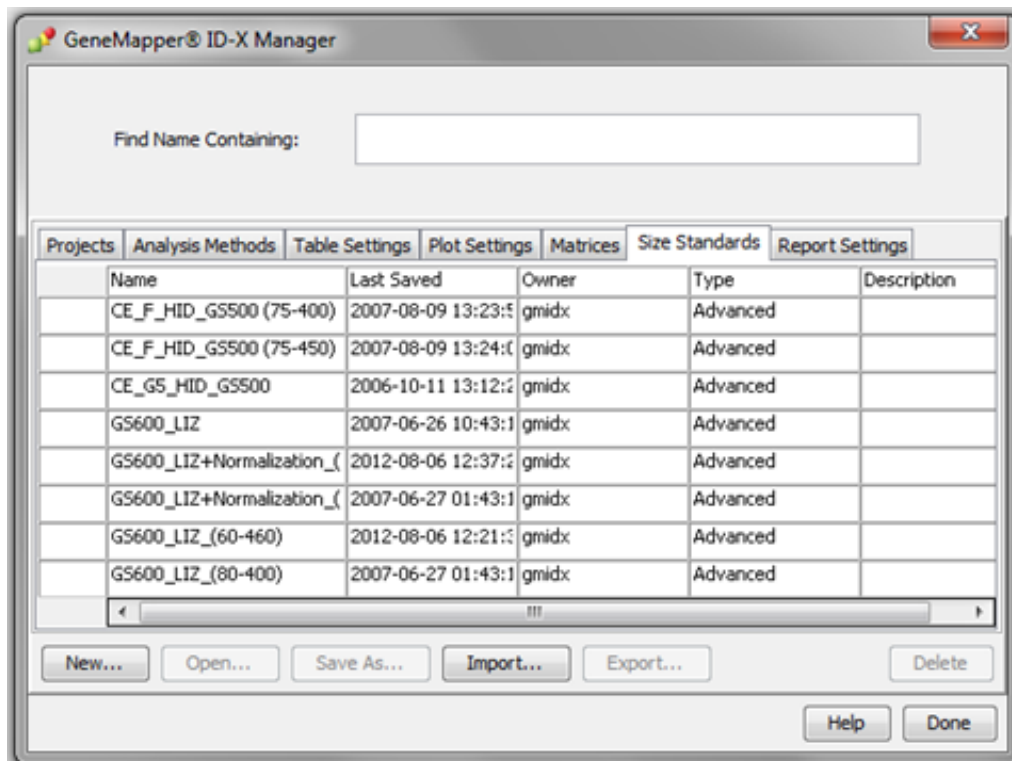
The 60 bp size-standard peak may occasionally be obscured by the primer peak. The issue can be addressed by either of the following steps:

- 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

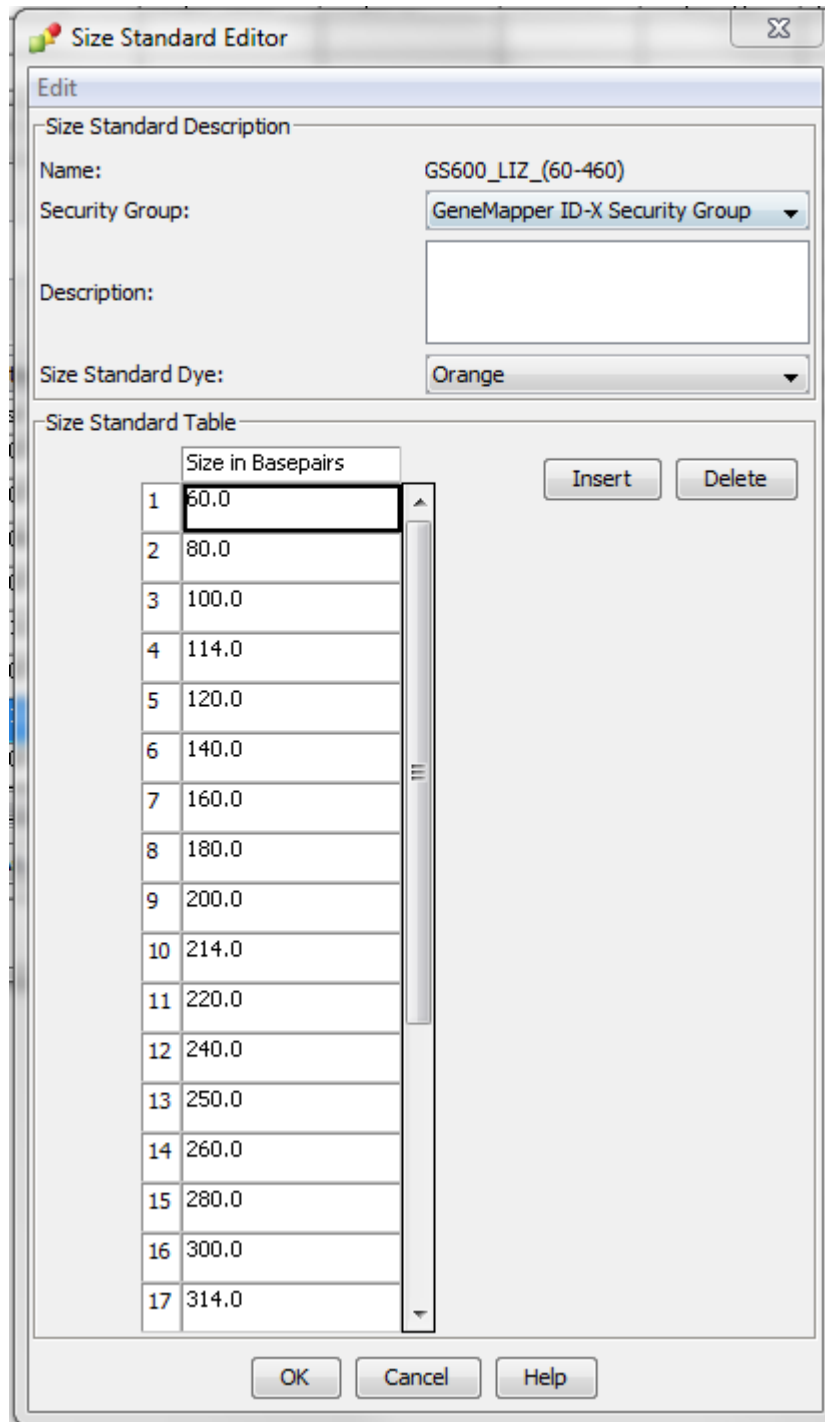
1. Select **Tools** ▶ **GeneMapper ID-X Manager** to open the **GeneMapper ID-X Manager**.
2. Click the **Size Standards** tab, then click **New**.



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
<b>Sample Type</b>	Select the sample type.
<b>Analysis Method</b>	Select <b>VeriFilerExpress_AnalysisMethod</b> (or the name of the analysis method you created).
<b>Panel</b>	Select <b>VeriFiler_Express</b> .
<b>Size Standard</b>	Use a size range of 60–460 bp for <b>Local Southern</b> size calling method or a size range of 80–460 bp for <b>3rd Order Least Squares</b> size-calling method. <sup>[1]</sup>

<sup>[1]</sup> The VeriFiler™ Express 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 VeriFiler™ Express 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.

The screenshot shows the GeneMapper ID-X software interface with the **Analysis Summary** tab selected. The interface includes a menu bar (File, Edit, Analysis, View, Tools, Admin, Help) and a toolbar. The main content area displays the following information:

- Analysis Summary**: A dropdown menu for "Select run folder to display:" set to "All".
- Sample Status**: A table showing the total number of samples in various states.
 

Sample Status	Total # of Samples
Unanalyzed	0
Analyzed	0
Analysis Setting Changed	36
- Allelic Ladder Quality per run folder (based on SQ and CGQ only)**: A table showing quality metrics for two run folders.
 

Run Folder	Total # of Analyzed Ladders	All	Some	None
Run 2014-04-19-09-44-42-917	1	1	0	0
Run 2014-04-19-09-44-42-927	1	1	0	0
- Control Quality per project (based on sample PQVs: SQS, SSPK, MIX, OMR, SQ, CGQ)**: A table showing control quality metrics.
 

Control Type	Total # of Samples	All thresholds met	One or more thresholds not met
Positive Control	0	0	0
Custom Control	0	0	0
Negative Control	0	0	0
Total	0	0	0
- Sample Quality per project (based on sample PQVs: SQS, SSPK, MIX, OMR, SQ, CGQ)**: A table showing sample quality metrics.
 

Samples	Total # of Samples	All thresholds met	One or more thresholds not met
Samples	25	21	4

The status bar at the bottom indicates "Analysis Completed."

## 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 136 for a list of available documents.



# Experiments and results

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## 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 (SWGDM, 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.

---

**Note:** All results were analyzed on a 3500xL Genetic Analyzer in accordance with the recommended analysis settings that are outlined in Chapter 4, “Analyze data with GeneMapper™ ID-X Software”.

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## 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 GeneAmp™ PCR System 9700 96-Well thermal cycler or the ProFlex™ PCR System according to the protocol described in the Perform PCR chapter.

Unless otherwise indicated, the data in this chapter are from the re-validation of the NGM Detect™ kit after redesigning the IQCL and TH01 markers. The performance of the updated kit formulation assay is fully comparable to that of the original kit. See the *Technical Note: Updated NGM Detect™ PCR Amplification Kit: Validation and Comparative Study* for studies directly related to soil specificity and a direct comparison of the NGM Detect™ kit original formulation to the updated formulation.

### SWGAM 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.” (SWGAM, December 2012)

### SWGAM 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.” (SWGAM, December 2012)

## PCR components

We examined the concentration of each component of the kit and established that the concentration of each component was within the range where data indicated that the amplification met the required performance criteria for specificity, sensitivity, and reproducibility. For example, blood and buccal samples on treated-paper substrates were amplified in the presence of varying concentrations of magnesium chloride, and the results were analyzed on a 3500xL Genetic Analyzer (Figure 5). The performance of the multiplex is most robust within  $\pm 20\%$  of the optimal magnesium chloride concentration.

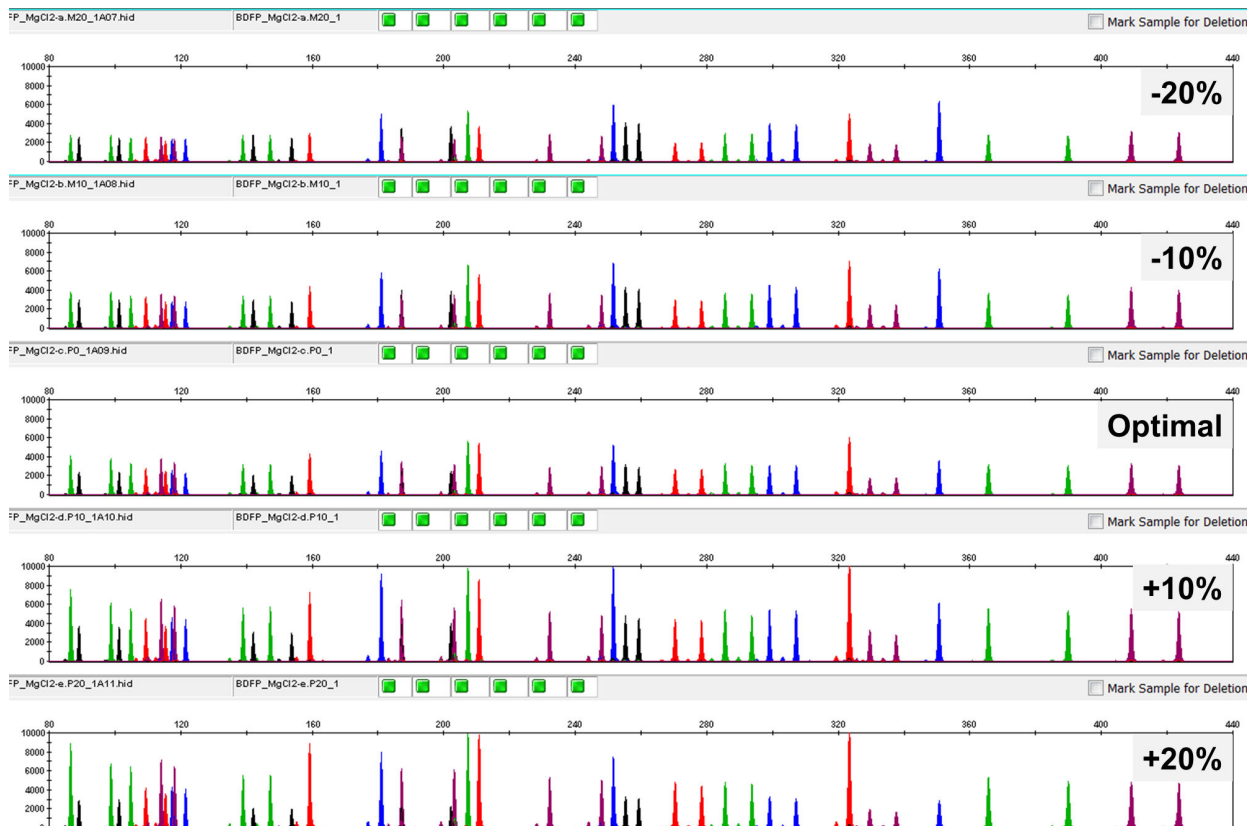
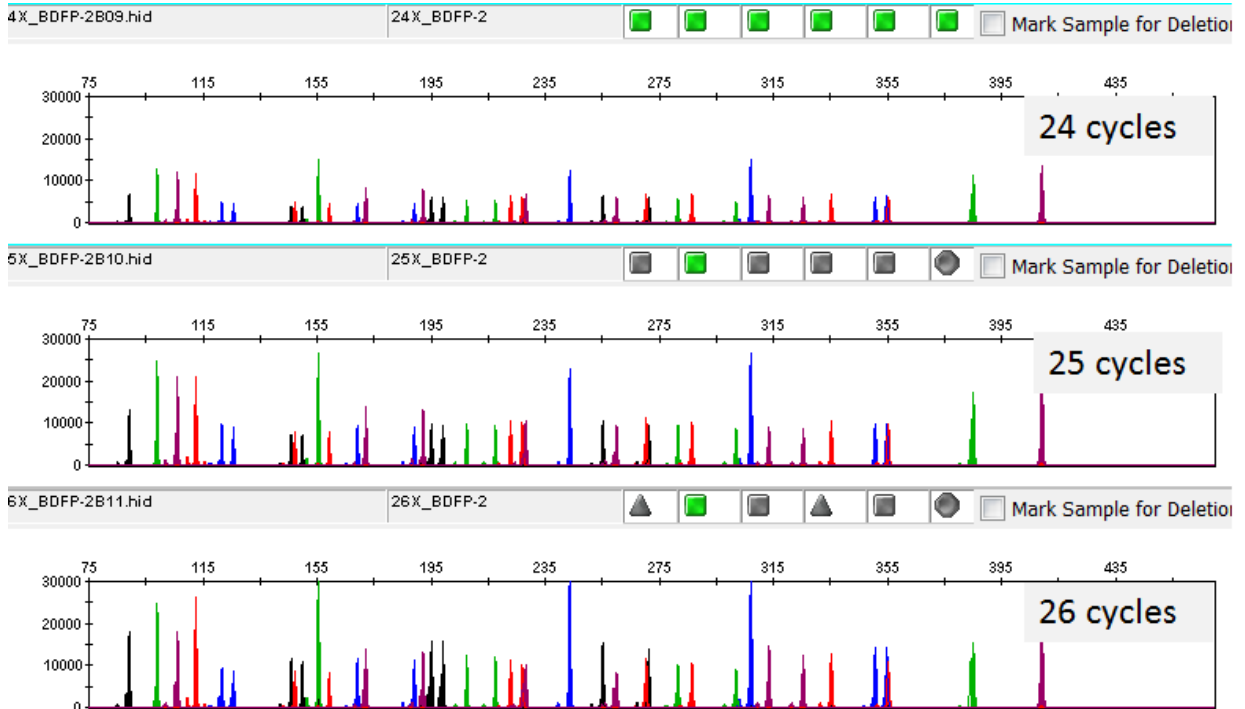


Figure 5 Blood sample on FTA™ card with the VeriFiler™ Express kit in the presence of varying concentrations of magnesium chloride and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 28,000 RFU).

## PCR cycle number

Reactions were amplified for 24, 25, and 26 cycles on the GeneAmp™ PCR System 9700 using a blood sample on treated paper. As expected, the amount of PCR product increased with the number of cycles. A full profile was generated for all numbers of thermal PCR cycles (24–26) and off-scale data were collected for several allele peaks at 26 cycles (Figure 6).

The data demonstrates that for this workflow 25 PCR cycles optimizes signal peak height and minimizes artifactual or other undesirable peaks.



**Figure 6** Representative VeriFiler™ Express kit profiles obtained from amplification of blood sample on untreated paper using 24, 25, and 26 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, and 60°C were tested using a GeneAmp™ PCR System 9700 (Figure 7). The PCR products were analyzed using a 3500xL Genetic Analyzer.

All of the tested annealing temperatures, 58 to 60°C produced robust profiles. The optimal combination of specificity, sensitivity, and resistance to PCR inhibition was observed at 59°C. Thermal cycler temperature is critical to assay performance; therefore, routine, regularly scheduled thermal cycler calibration is strongly recommended.

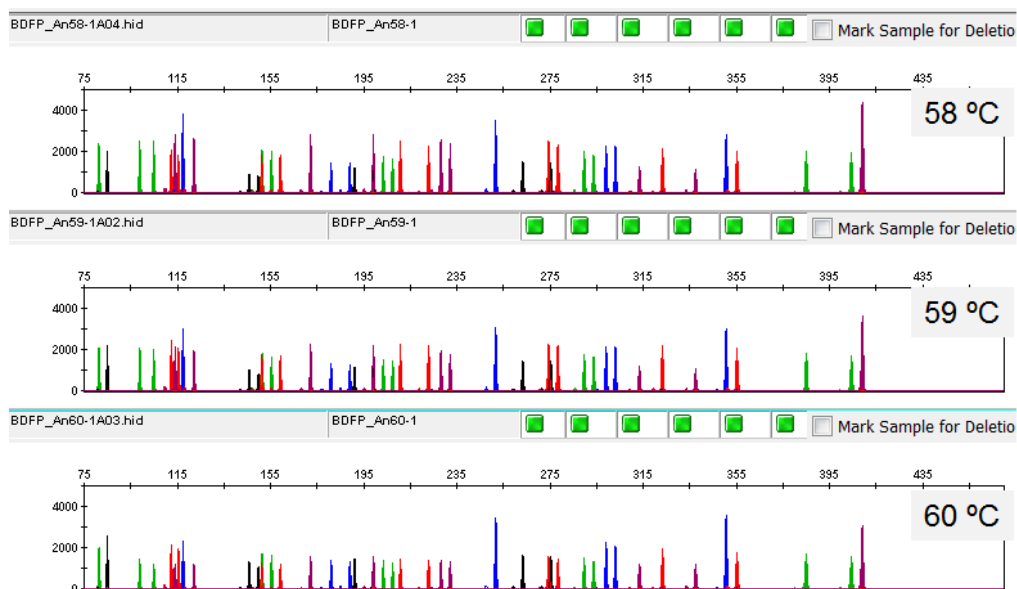


Figure 7 Electropherograms obtained from amplification of a blood sample on an FTA™ card at annealing temperatures of 58, 59, and 60°C, analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 5,000 RFU).



# Representative data for common sample and substrate types

For all the representative electropherograms shown (Figure 8 through Figure 12), peak heights at the D22S1045 marker may be elevated as compared to the other markers in the TAZ™ dye channel. This result is typical to the assay and has no impact on the ability to generate a full STR profile.

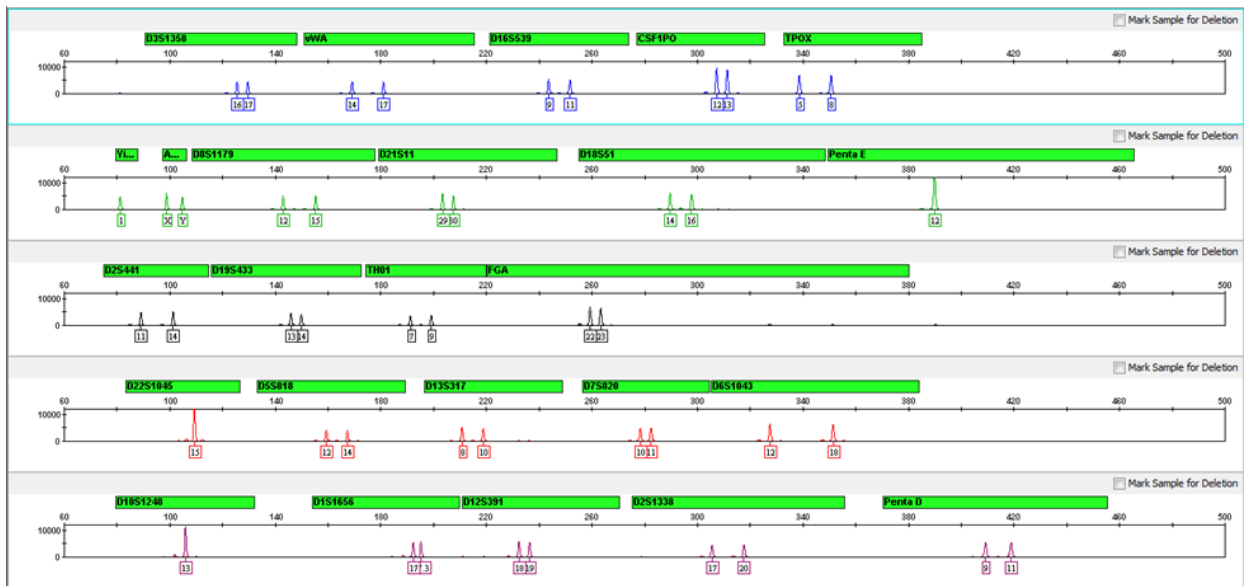


Figure 8 Electropherogram of a blood sample on an FTA™ card amplified with the VeriFiler™ Express kit at a customer test site and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 12,000 RFU).

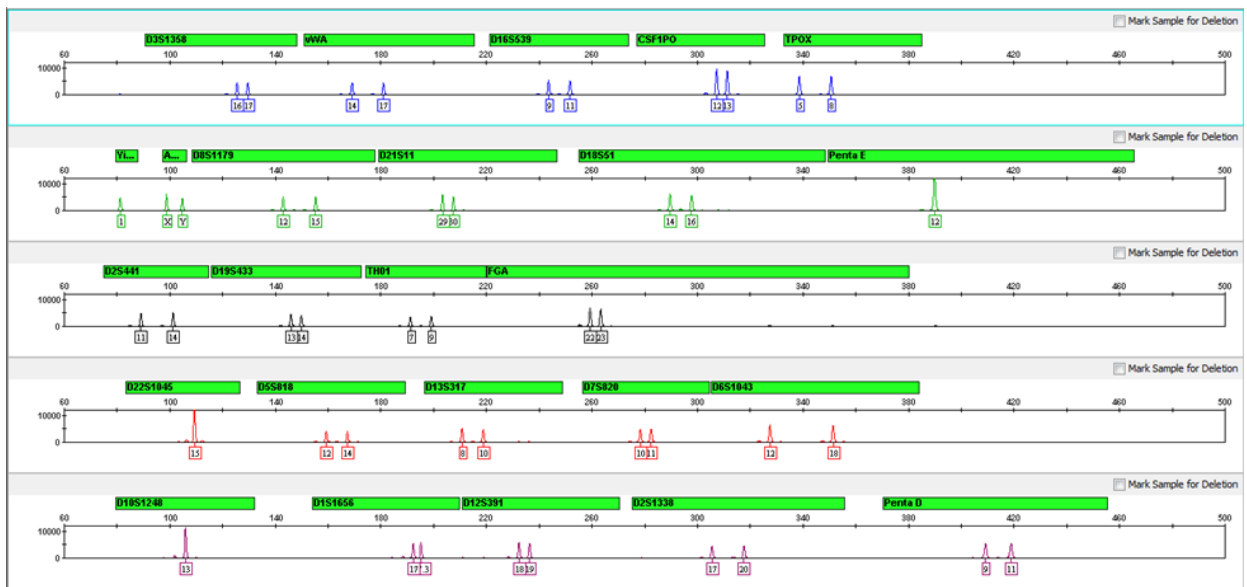


Figure 9 Electropherogram of a blood sample on a NUCLEIC-CARD™ Collection Device amplified with the VeriFiler™ Express kit at a customer test site and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 25,000 RFU).

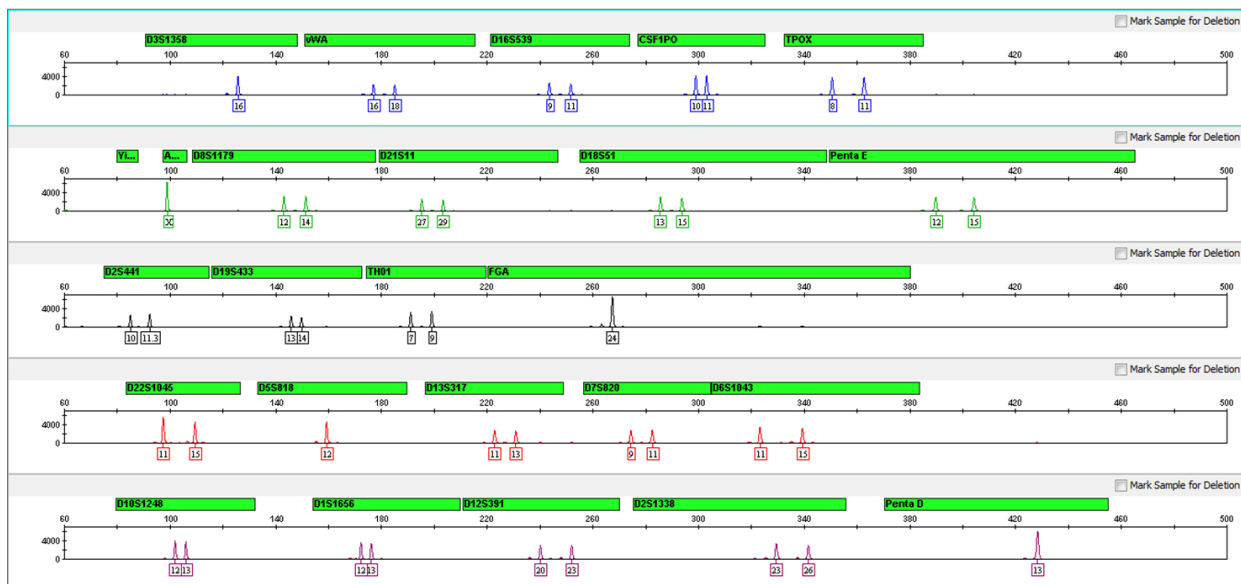


Figure 10 Electropherogram of a buccal sample on an FTA™ card amplified with the VeriFiler™ Express kit at a customer test site and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 7,000 RFU).

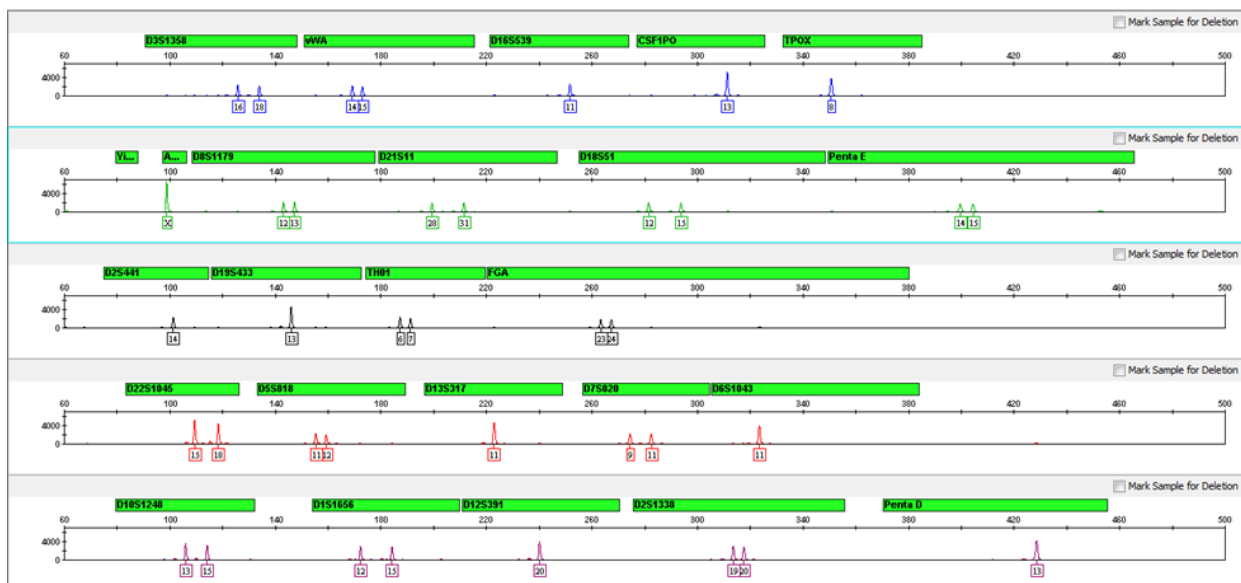


Figure 11 Electropherogram of a buccal sample on a NUCLEIC-CARD™ Collection Device amplified with the VeriFiler™ Express kit at a customer test site and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 7,000 RFU).

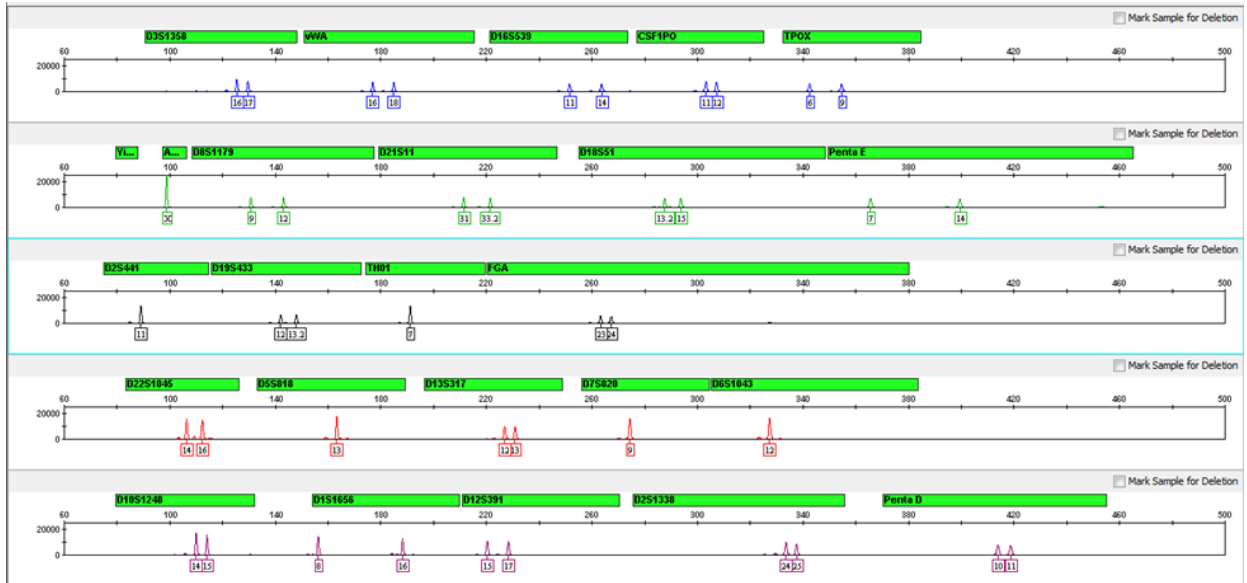


Figure 12 Electropherogram of a buccal sample on a cotton swab (with lysate) extracted using Prep-n-Go™ Buffer, and amplified with the VeriFiler™ Express kit at a customer test site and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0 to 25,000 RFU).

## Accuracy, precision, and reproducibility

### SWGDM 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.” (SWGDM, 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 three figures show the size differences that are typically observed between sample alleles and allelic ladder alleles on the 3130xL, and 3500xL Genetic Analyzers with POP-4™ Polymer and the 3730xL Genetic Analyzer with POP-7™ Polymer. The X-axis in the following figures represents the nominal nucleotide sizes for the VeriFiler™ Express Allelic Ladder. The dashed lines parallel to the X-axis represent the  $\pm 0.25$ -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  $\pm 0.5$  nt from a corresponding allele in the Allelic Ladder, irrespective of the capillary electrophoresis platforms.

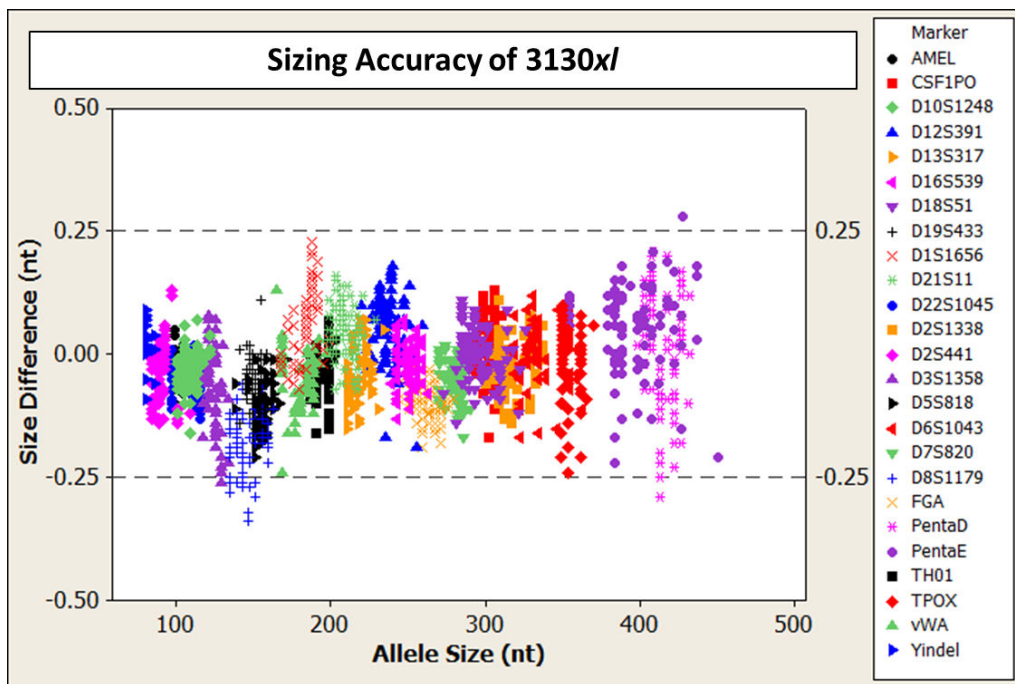


Figure 13 Allele Size vs. Allelic Ladder Sizing for 84 samples analyzed on a 3130xI Genetic Analyzer. Size and ladder sizing for the VeriFiler™ Express kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.

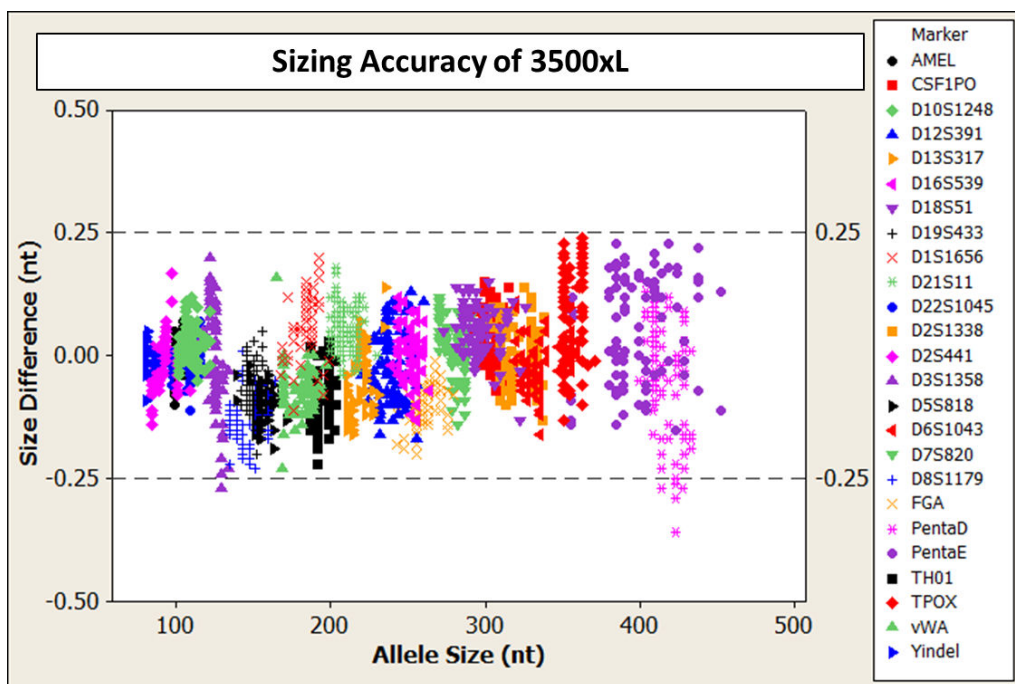


Figure 14 Allele Size vs. Allelic Ladder Sizing for 84 samples analyzed on a 3500xL Genetic Analyzer. Size and ladder sizing for the VeriFiler™ Express kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.

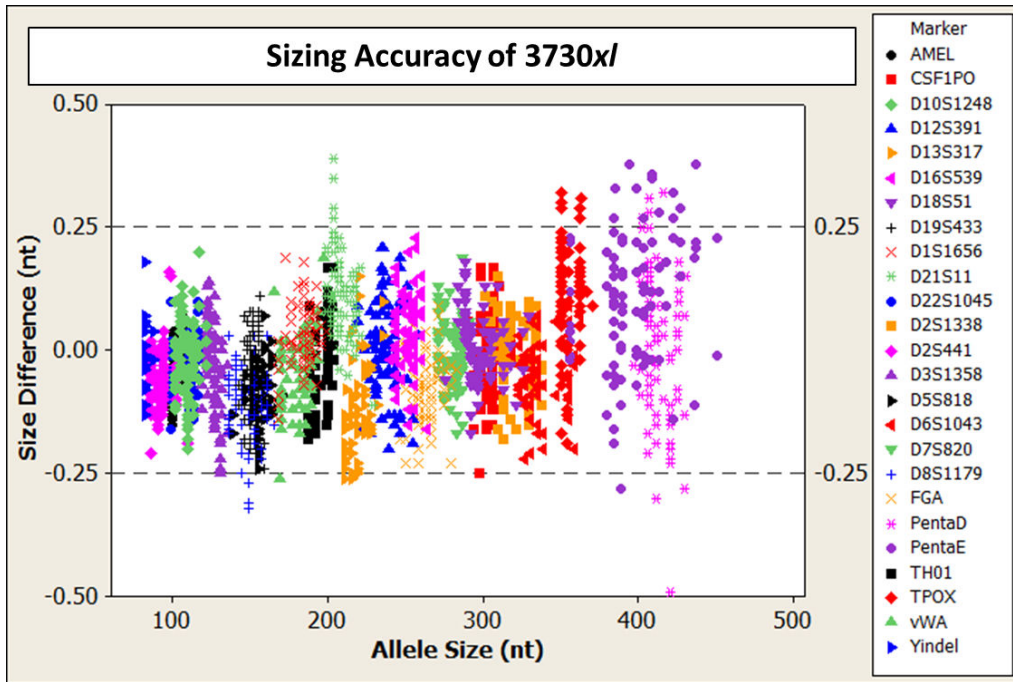


Figure 15 Allele Size vs. Allelic Ladder Sizing for 84 samples analyzed on an 3730xl Genetic Analyzer. Size and ladder sizing for the VeriFiler™ Express kit were calculated using the GeneScan™ 600 LIZ™ Size Standard v2.0.

## Precision and size window description

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

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

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

## Precision and size window observation

Figure 16 lists typical precision results obtained from multiple runs of the VeriFiler™ 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	# Injections	Sizing method
3130xl	16/injection	5	3rd Order Least Square, 60–460 bp
3500	8/injection	12	3rd Order Least Square, 60–460 bp
3500xL	24/injection	4	3rd Order Least Square, 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 (Figure 16). 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  $\pm 0.5$ -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  $\pm 0.5$ -nt window because of measurement error is relatively rare when the standard deviation in sizing is approximately 0.15 nt or less (Smith, 1995).

---

**Note:** The read region for the VeriFiler™ Express kit is 75–465 nt.

---

For sample alleles that do not size within a  $\pm 0.5$ -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.

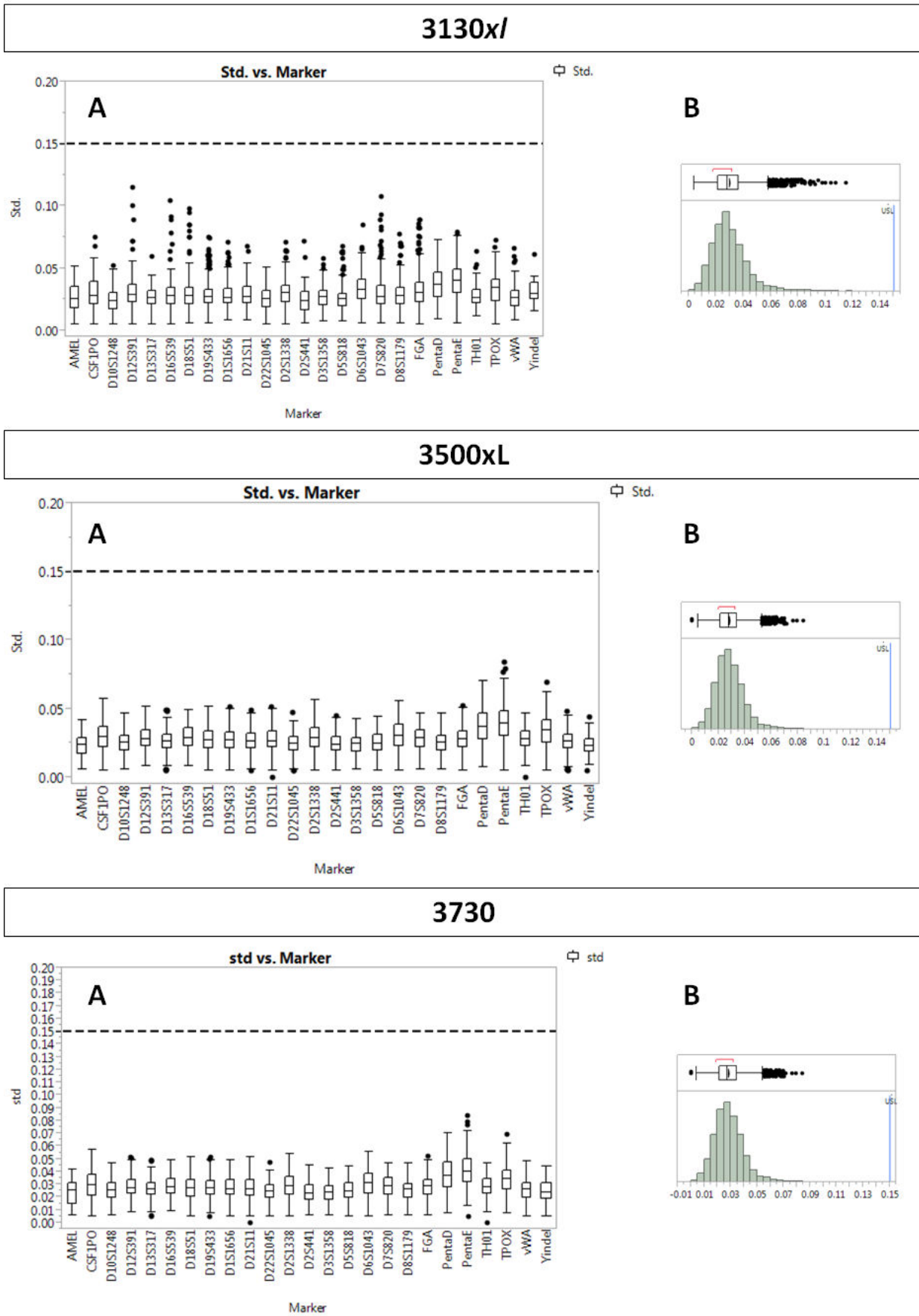
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**IMPORTANT!** To minimize the variation in sizing between runs and to ensure accurate genotyping, follow the guidelines in “Allelic ladder requirements for data analysis” on page 41 and use allelic ladders obtained from the same run as samples to analyze the samples.

---

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

In the following figure, the sizes for all the alleles across multiple injections of the VeriFiler™ Express Allelic Ladder were calculated. Each box represents the 25th through the 75th percentile of the observed values, with the center line indicating the median. Standard error bars indicate the range of the data, with presumed outliers (points that are different from the mean by more than twice the pooled standard deviation) shown as black dots. Panel B shows the distribution of the standard deviations for all the markers combined for each CE instrument platform.





# 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

The level of stutter in this kit is normal and as expected for STR chemistries that are designed to overcome inhibitors and obtain robust performance with single source reference samples. Note the following:

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 stutter observed in the population study with the VeriFiler™ Express kit is shown in Figure 17 through Figure 23 and listed in Table 3.

The stutter filter settings that are derived from this data are listed in “Stutter percentage filter settings provided with GeneMapper™ ID-X Software” on page 81.

Workflow	Number of samples
Treated paper	49 blood samples on FTA™ Classic Cards
Untreated paper	425 blood samples on FTA™ Bloodstain Cards

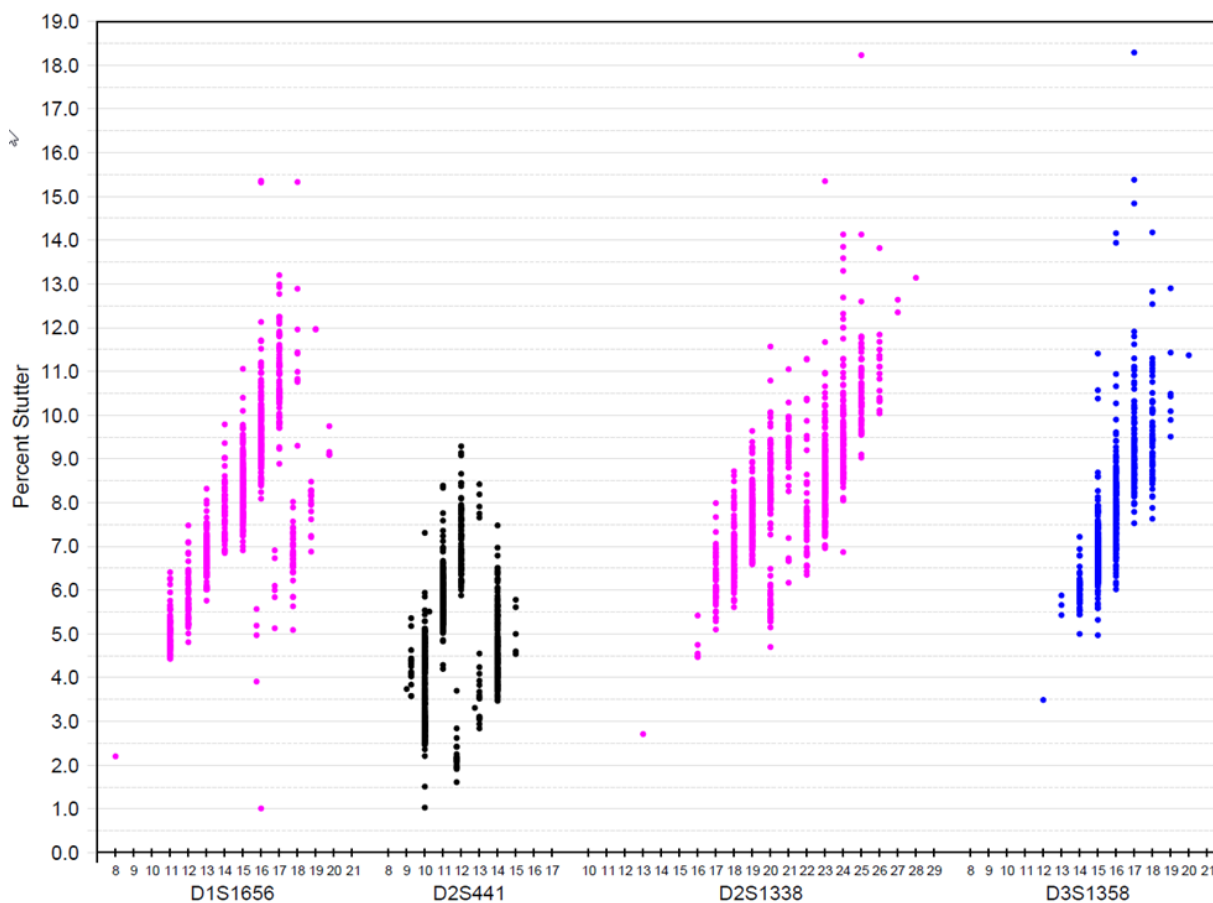


Figure 17 Stutter percentages for D1S1656, D2S441, D2S1338, and D3S1358 loci (Blue=FAM™ dye, black=NED™ dye, purple=SID™ dye)

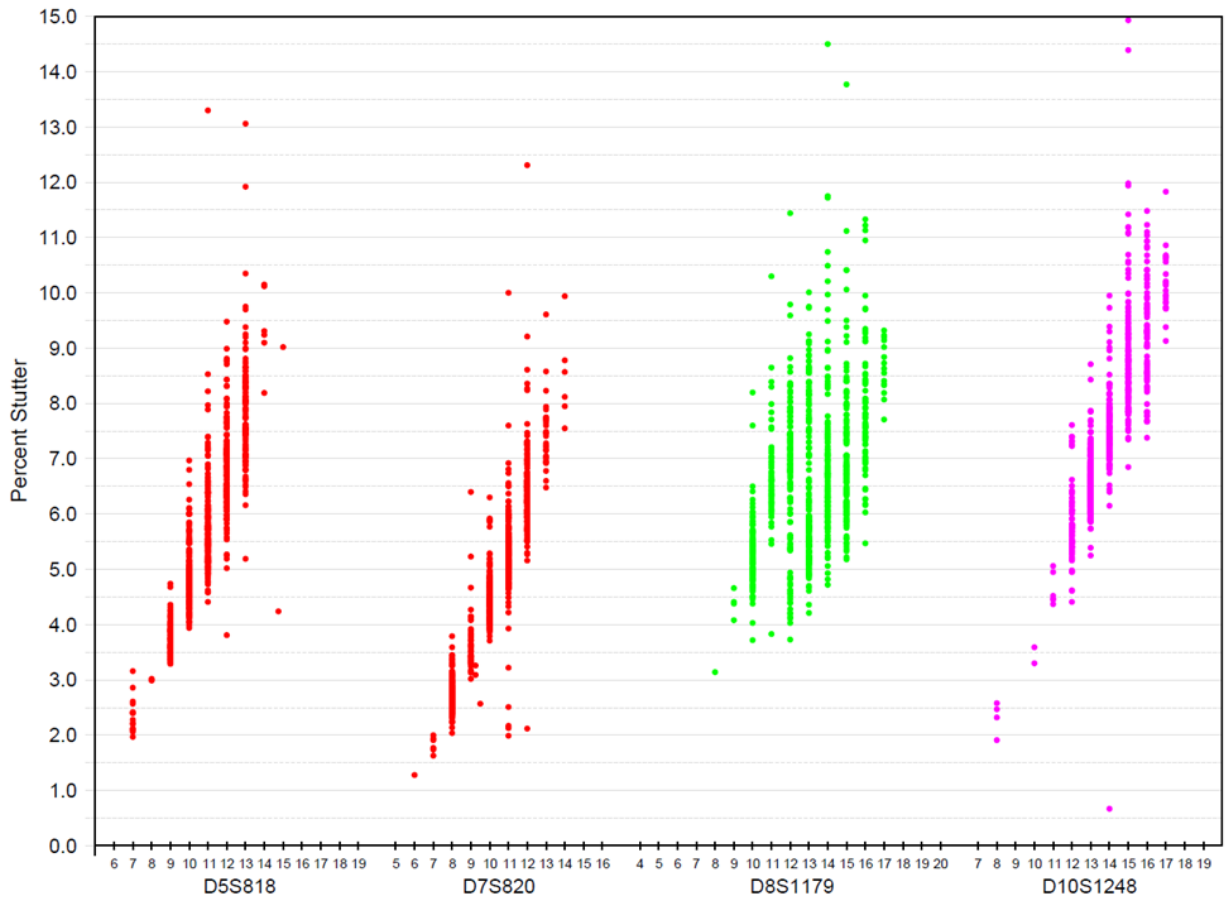


Figure 18 Stutter percentages for D5S818, D7S820, D8S1179, and D10S1248 (Green=VIC™ dye, red=TAZ™ dye, purple=SID™ dye)

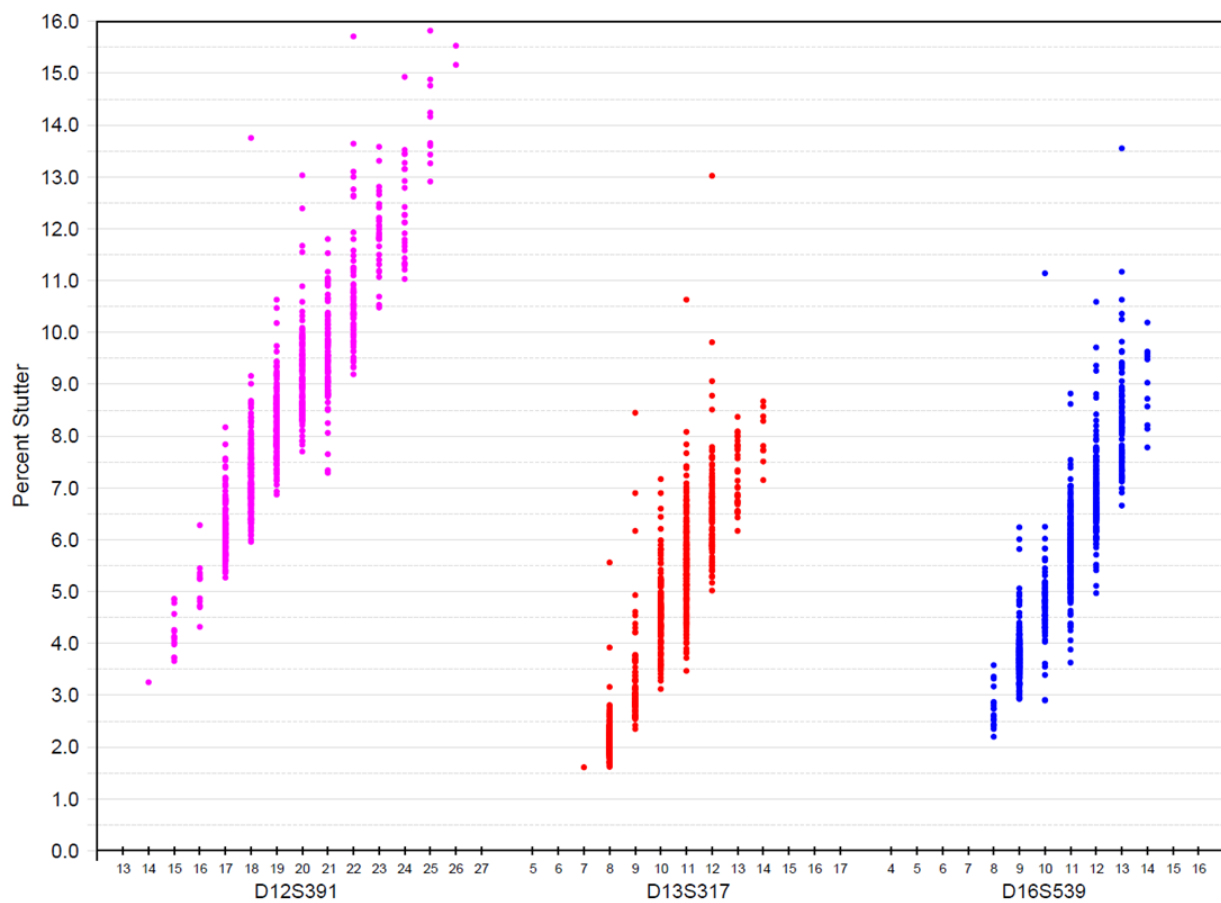


Figure 19 Stutter percentages for D12S391, D13S317, and D16S539 loci (Blue=FAM™ dye, red=TAZ™ dye, purple=SID™ dye)

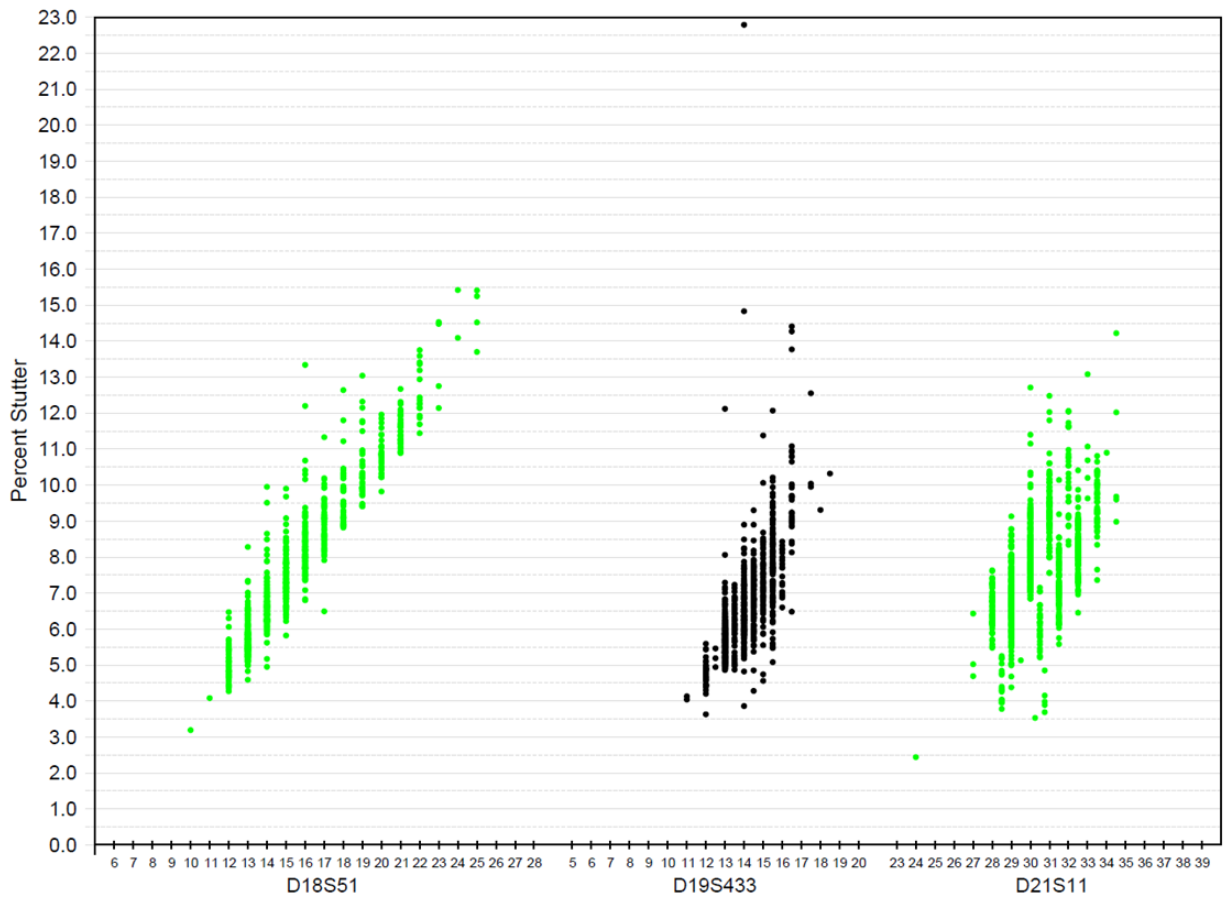


Figure 20 Stutter percentages for D18S51, D19S433, and D21S11 loci (Green=VIC™ dye, black=NED™ dye)

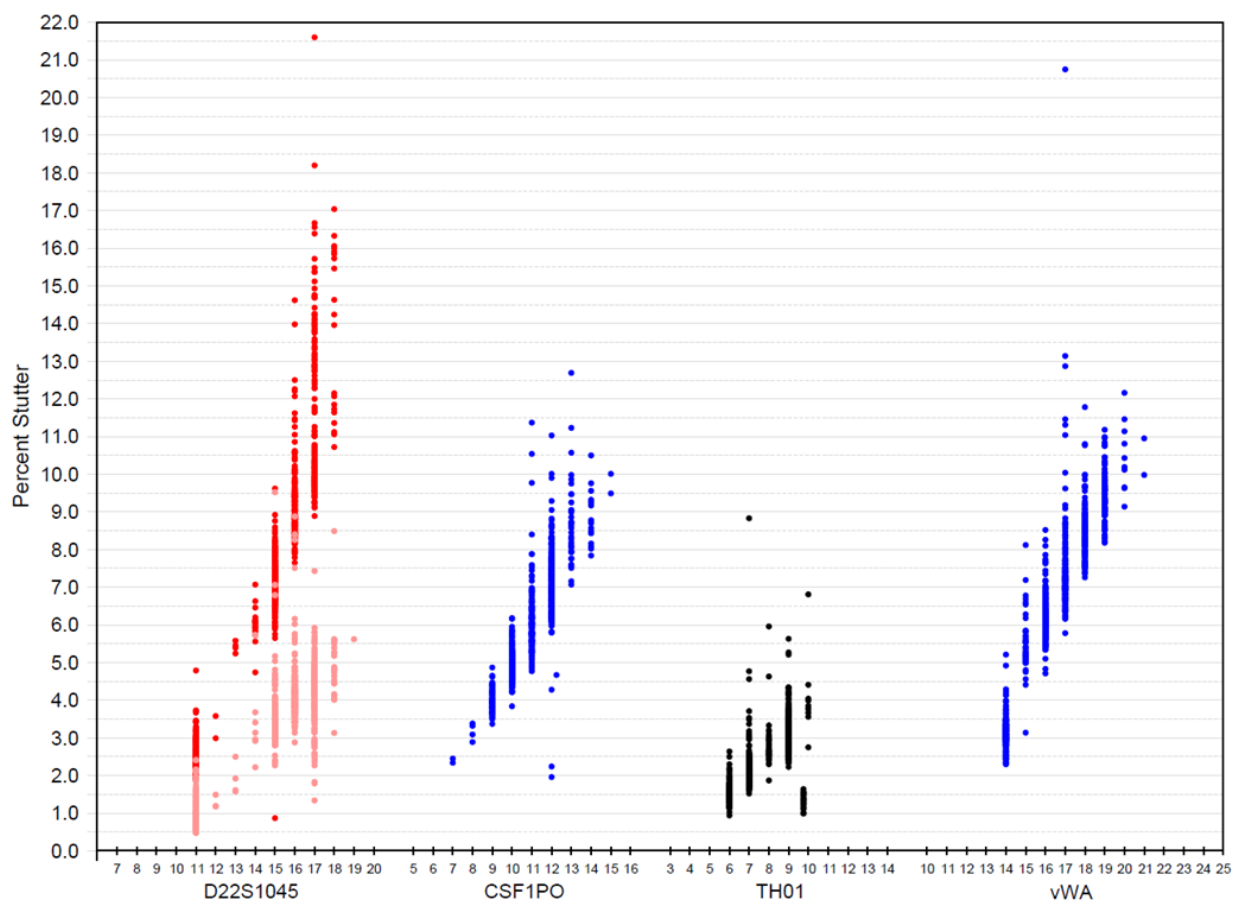


Figure 21 Stutter percentages for D22S1045, CSF1PO, TH01, and vWA loci (Blue=FAM™ dye, green=VIC™ dye, black=NED™ dye, red=TAZ™ dye). Red and orange data points associated with D22S1045 locus indicate minus and plus stutter, respectively.

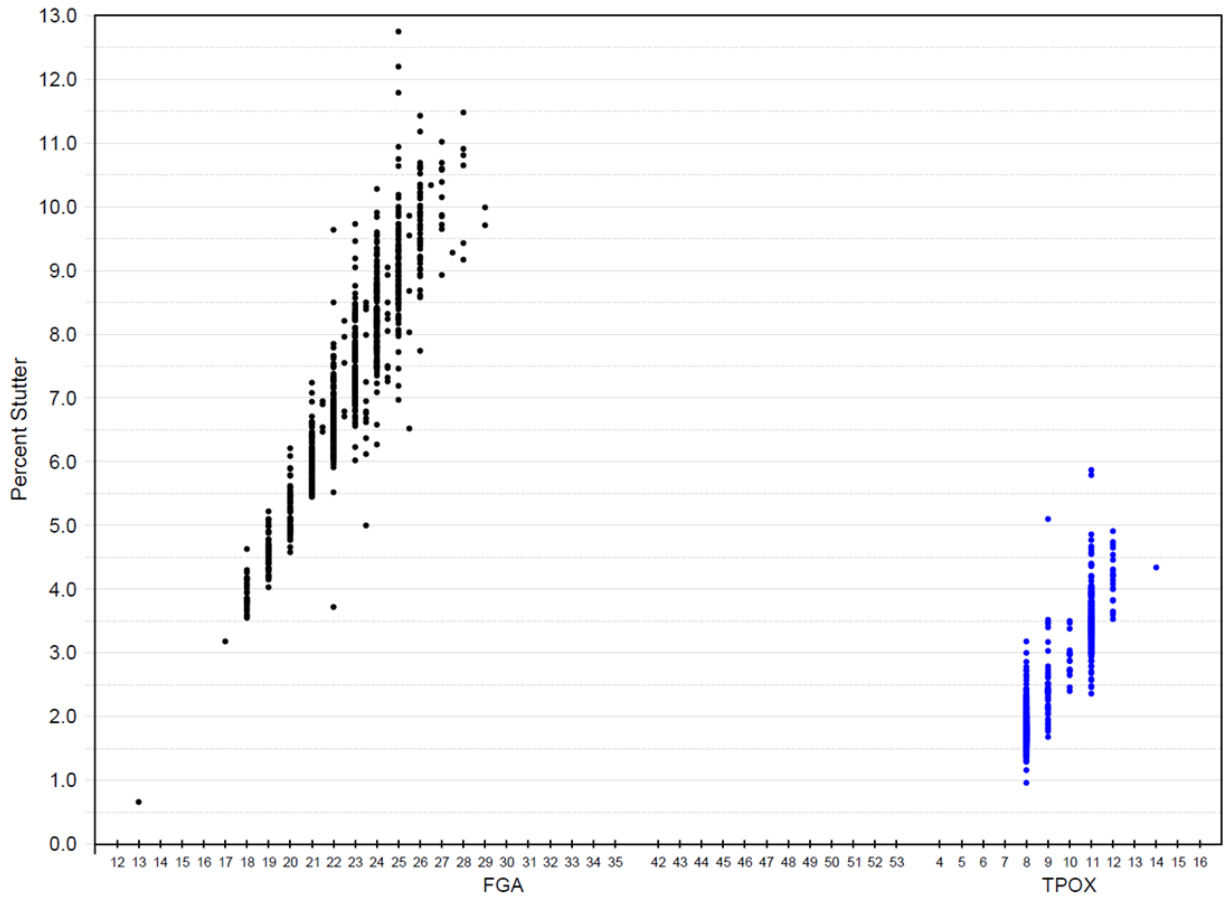


Figure 22 Stutter percentages for FGA and TPOX loci (Blue=FAM™ dye, black=NED™ dye)

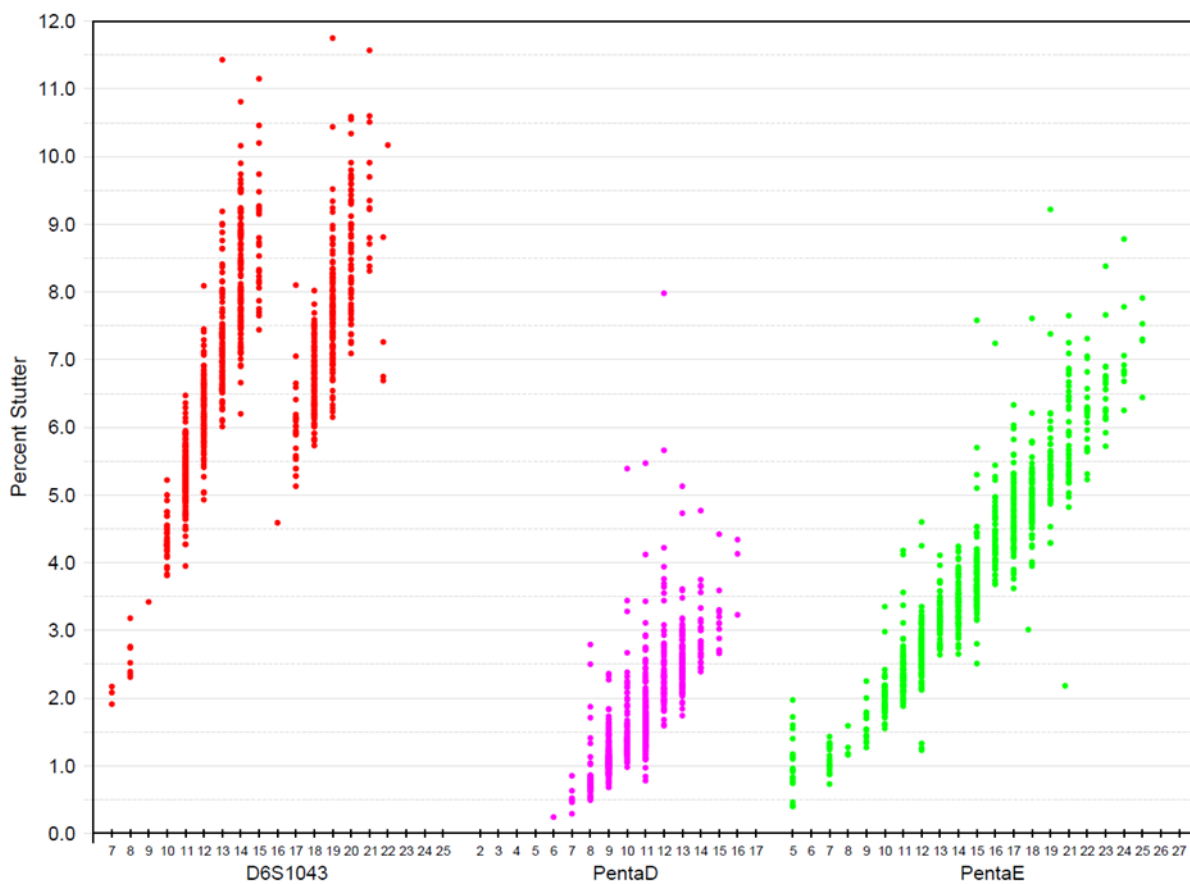


Figure 23 Stutter percentages for D6S1043, Penta D, and Penta E loci (Red=TAZ™ dye, purple=SID™ dye, green=VIC™ dye)



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

The settings in Table 3 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 3** Marker-specific stutter filter percentages for VeriFiler™ Express kit loci

Locus [1]	% Stutter
CSF1PO	10.54
D10S1248	12.27
D12S391	14.44
D13S317	10.10
D16S539	10.76
D18S51	14.15
D19S433	10.77
D1S1656	13.62
D21S11	12.24
D22S1045	8.05
D22S1045 (+3 nt)	18.0
D2S1338	13.29
D2S441	9.40
D3S1358	12.16
D5S818	10.53
D7S820	9.39
D8S1179	10.85
FGA	12.27
Penta D	4.09
Penta E	8.31
TH01	4.93

**Table 3** Marker-specific stutter filter percentages for VeriFiler Express kit loci (continued)

Locus [1]	% Stutter
TPOX	5.20
vWA	13.18

[1] These percentages are used as stutter filters in VeriFilerExpress\_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 VeriFiler™ Express 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 60°C for 5 minutes .

This final extension step gives the DNA polymerase additional time to complete +A addition to all double-stranded PCR products. Figure 24 shows examples of incomplete and normal +A addition. Final extension incubation for longer than the recommended time can result in double +A addition, in which two nontemplate adenosine residues are added to the PCR product. Double +A addition can cause "shoulders" on the right side of main allele peaks, and is therefore to be avoided.

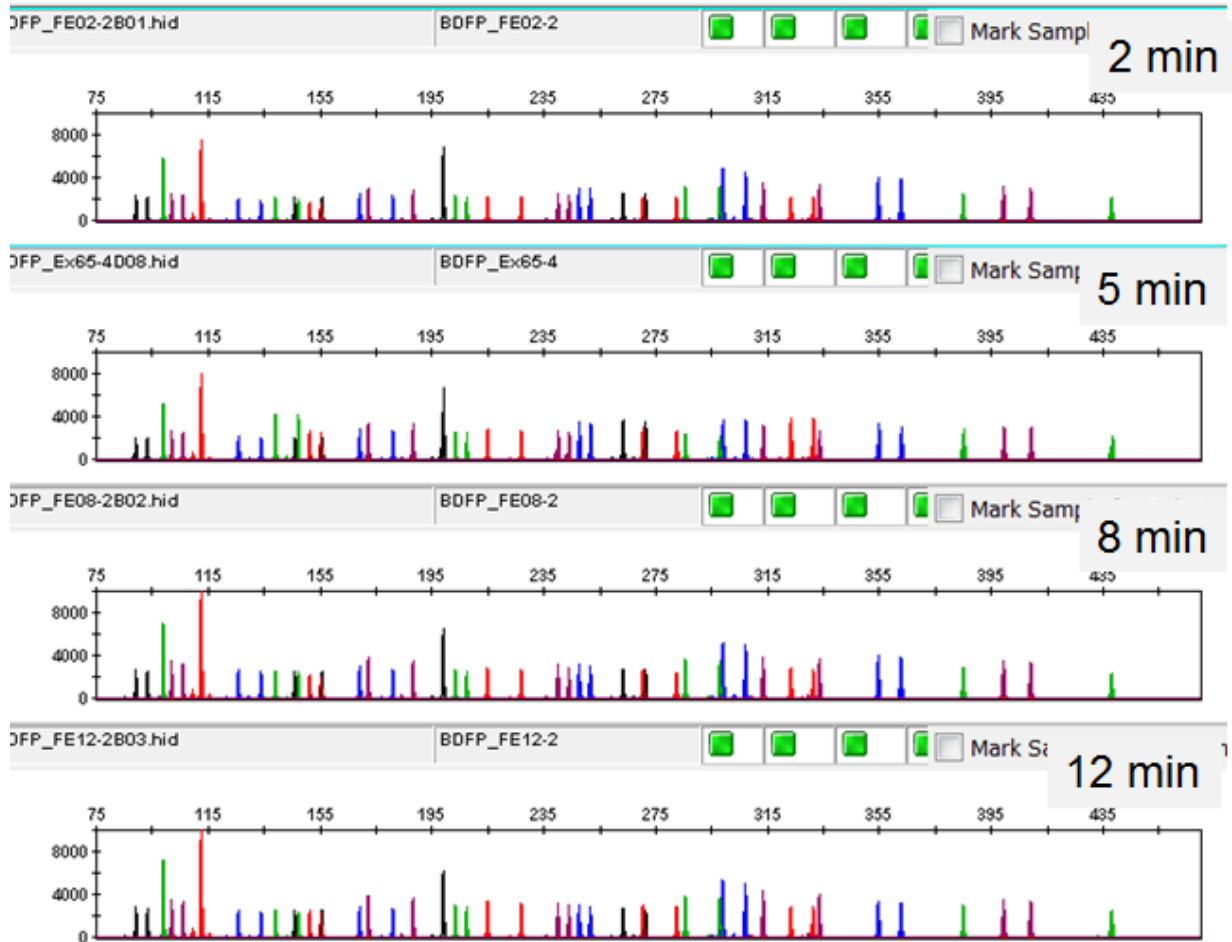


Figure 24 Omitting the final extension step results in shoudering on main allele peaks due to incomplete A nucleotide addition. Examples shown are the smaller amplicons of FAM™, NED™, and SID™ dye channel data from a 3500xL Genetic Analyzer using the VeriFiler™ Express kit.

Due to improved PCR buffer chemistry, the lack of +A addition is generally less of an issue with the VeriFiler™ Express kit than with earlier generation kits. However, "shoudering" 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 VeriFiler™ Express kit. Kit electropherograms are free of reproducible dye artifacts in the kit read region of 75–465 nt for commonly used analytical thresholds. Figure 25 shows the low baseline-level fluorescence that is observed in a typical negative control PCR.

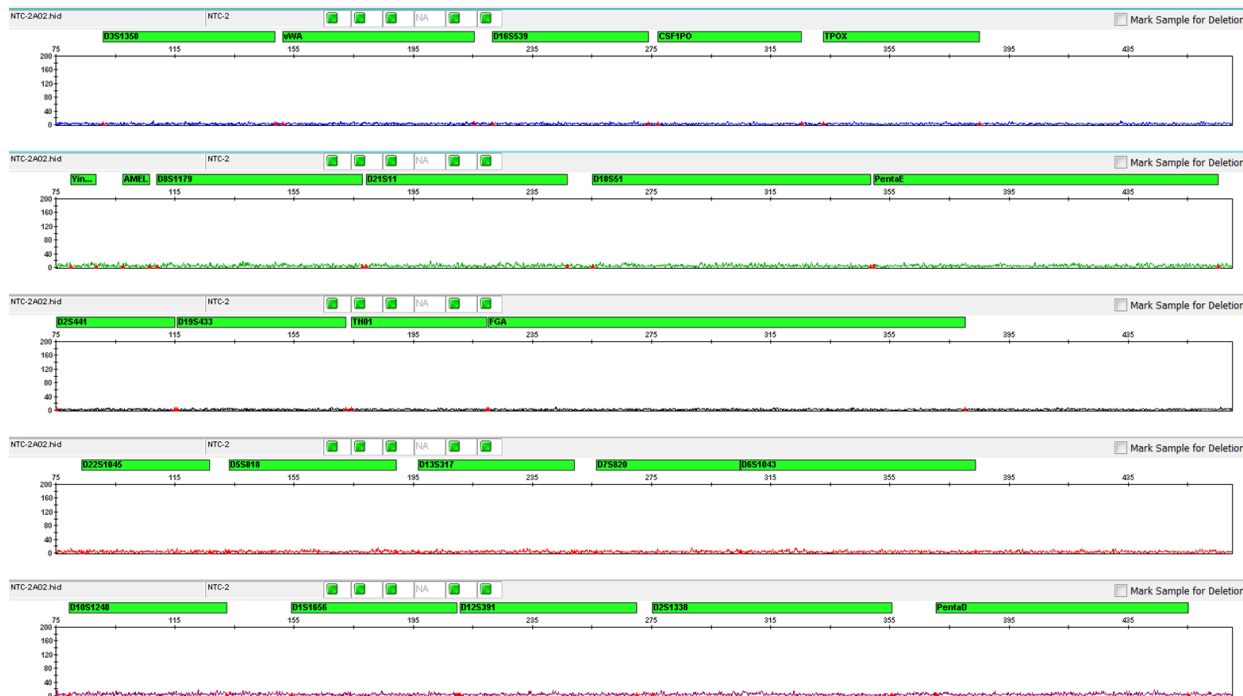


Figure 25 Examples of fluorescence background in data produced on a 3500xL Genetic Analyzer (Y-axis scale 0 to 200 RFU).

Additional reproducible DNA-dependent artifacts have been characterized and documented in Table 4. It is important to consider noise and other amplification-related artifacts when interpreting data.

Table 4 DNA-dependent artifacts observed with the VeriFiler™ Express kit

Artifact	Color	Size	Type
TAZ60–80	Red	60–80 nt	PCR artifact
SID130	Purple	130–131 nt	PCR artifact
SID202	Purple	202–203 nt	PCR artifact
SID402–411	Purple	402–411 nt	PCR artifact
VIC110–120	Green	110–120 nt	Sample-independent artifact intrinsic to the assay

# Characterization of loci

## SWGDM guideline 3.1

“The basic characteristics of a genetic marker should be determined and documented.” (SWGDM, December 2012)

### Loci in this kit

This section describes basic characteristics of the 23 autosomal STR loci, one Y indel locus, and the sex-determining marker (Amelogenin), which are amplified with the VeriFiler™ Express 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 104-nt and 110-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 locus, and Penta D and Penta E, pentanucleotide STR loci (Bacher and Schumm, 1998), 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 VeriFiler™ Express 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 VeriFiler™ Express 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 VeriFiler™ Express 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 <sup>[1]</sup>	Chromosome Nuclear Coordinates <sup>[1]</sup> (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			

<sup>[1]</sup> STR locus mapping data was obtained from the NCBI Map Viewer ([http://www.ncbi.nlm.nih.gov/projects/mapview/map\\_search.cgi?taxid=9606](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

### SWGDM 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.” (SWGDM, December 2012)

## Nonhuman studies

The VeriFiler™ Express 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 VeriFiler™ Express kit kit:

- Primates: chimpanzee, gorilla, and orangutan (1.0 ng each)
- Non-primates: cow, chicken, dog, horse, mouse, pig, rabbit, rat, sheep (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 VeriFiler™ Express kit with any of these non-human species.

“Nonhuman studies” on page 87 shows example electropherogram results from the species specificity tests. The chimpanzee, gorilla, and orangutan DNA samples produced partial profiles in the 75–465 nucleotide region.

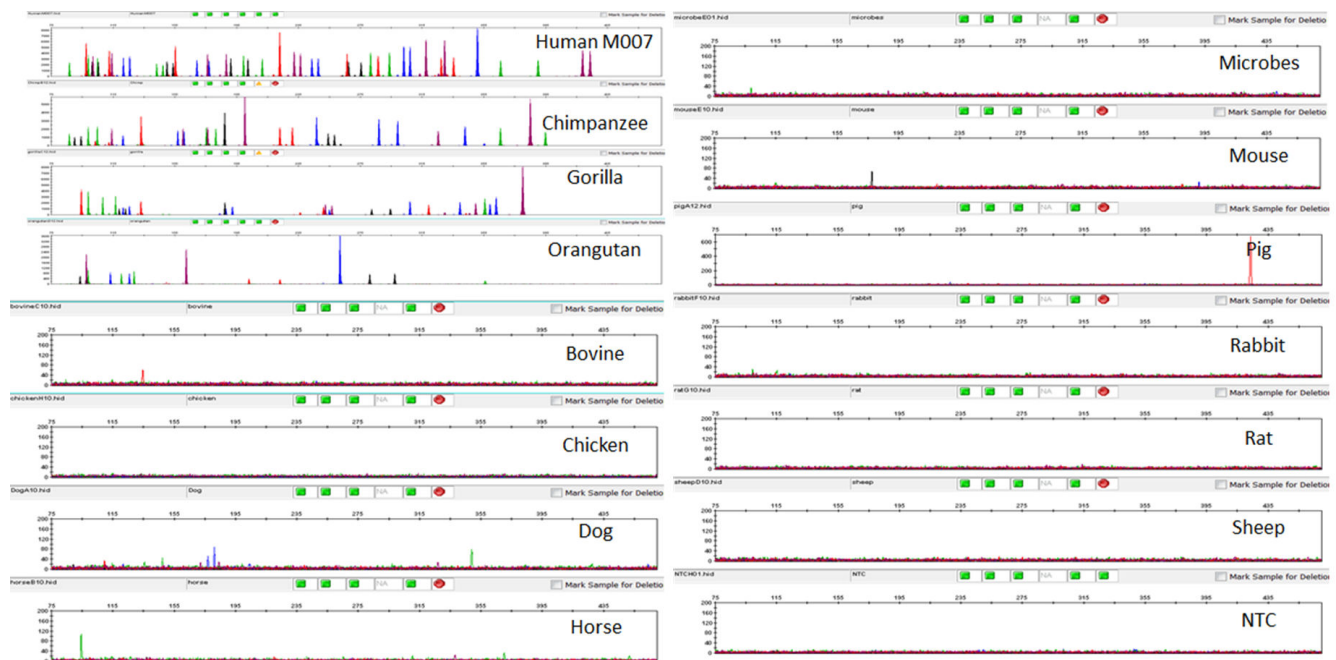


Figure 26 Representative electropherograms for some species tested in a species specificity study. Data produced on a 3500xL Genetic Analyzer.

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.

## Sensitivity

### SWGDM 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.” (SWGDM, December 2012)

### Sample collection factors that can affect DNA quantity

The VeriFiler™ Express kit has been optimized at 25 µL PCR reaction volume to overcome the PCR inhibition expected when amplifying:

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

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

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

It is essential to optimize the PCR conditions for types of blood samples and spotting protocol. See “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 VeriFiler™ 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.



# Population data

## SWGAM guideline 3.7

“The distribution of genetic markers in populations should be determined in relevant population groups.” (SWGAM, 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 this kit

This section describes basic characteristics of the 23 autosomal STR loci, one Y indel locus, and the sex-determining marker (Amelogenin), which are amplified with the VeriFiler™ Express kit. Most of these loci have been extensively characterized by other laboratories.

## Population samples used in these studies

Population data for loci contained in the VeriFiler™ Express PCR Amplification Kit was obtained from data published by (Hill *et. al.*, 2013). See the original data at [www.cstl.nist.gov/biotech/strbase/NISTpop.htm](http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm). Ethnicities of sample donors were:

- African-American—342 samples
- Asian—97 samples
- Caucasian—361 samples
- Hispanic—236 samples

The VeriFiler™ Express kit contains loci for which extensive population data are available.

## Concordance studies

The primer sequences used in the VeriFiler™ Express PCR Amplification Kit and GlobalFiler™ Express kit are identical except for the D6S1043, Penta E, and Penta D loci which are not shared between the two kits.

We compared allele calls between the VeriFiler™ Express PCR Amplification Kit and the PowerPlex™ 21 System. Genotype data from 200 blood samples on FTA™ Classic Cards showed 99.6% concordance between the two kits. Null alleles were found with the PowerPlex™ 21 System at the D5S818 and Penta E loci. These alleles were accurately genotyped by the VeriFiler™ Express PCR Amplification Kit.

## Probability of Identity definition

The  $P_1$  value is the probability that two individuals selected at random will have an identical genotype (Sensabaugh, 1982).

## Probability of identity

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

Table 7 shows the Probability of identity ( $P_1$ ) values of the VeriFiler™ Express kit loci individually and combined.

**Table 6 Autosomal allele frequencies by population group for VeriFiler™ Express kit Kit STR loci. (\*=Alleles not detected)**

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
<b>CSF1PO</b>				
5.0	*	*	*	*
6.0	*	*	*	*
7.0	0.0556	0.0206	*	0.0127
7.3	*	*	*	*
8.0	0.0556	*	0.0055	0.0042
8.1	*	*	*	*
9.0	0.0395	0.0670	0.0139	0.0233
9.1	*	*	*	*
10.0	0.2500	0.2010	0.2202	0.2373
10.1	*	*	*	*
10.2	*	*	*	*
10.3	*	*	*	*
11.0	0.2485	0.2165	0.3089	0.2797

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
11.1	*	*	*	*
11.2	*	*	*	*
11.3	*	*	*	*
12.0	0.2953	0.3866	0.3601	0.3750
12.1	*	*	*	*
12.3	*	*	*	*
13.0	0.0468	0.0876	0.0817	0.0593
14.0	0.0088	0.0155	0.0097	0.0064
15.0	*	0.0052	*	0.0021
16.0	*	*	*	*
<b>D10S1248</b>				
7.0	*	*	*	*
8.0	0.0029	*	*	*
9.0	0.0029	*	*	*
10.0	0.0073	0.0052	*	*
11.0	0.0351	*	0.0014	0.0042
12.0	0.1301	0.0876	0.0319	0.0424
13.0	0.2339	0.3196	0.3075	0.2733
14.0	0.2763	0.2526	0.2978	0.3390
15.0	0.1974	0.2062	0.1967	0.2119
16.0	0.0877	0.0979	0.1330	0.0996
17.0	0.0249	0.0309	0.0277	0.0254
18.0	0.0015	*	0.0014	0.0021
19.0	*	*	0.0028	0.0021
<b>D12S391</b>				
13.0	*	*	*	*
14.0	0.0015	*	*	*

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
15.0	0.0775	0.0412	0.0319	0.0445
16.0	0.0673	0.0103	0.0222	0.0424
17.0	0.1667	0.0825	0.1274	0.0763
17.1	0.0044	*	*	*
17.3	0.0044	*	0.0208	0.0169
18.0	0.2529	0.2629	0.1717	0.1780
18.1	0.0015	*	*	*
18.2	*	*	*	*
18.3	0.0044	*	0.0249	0.0127
19.0	0.1477	0.1753	0.1247	0.1886
19.1	0.0088	*	*	0.0021
19.3	0.0044	0.0052	0.0042	0.0064
20.0	0.1038	0.1959	0.1108	0.1547
20.1	0.0029	*	*	*
20.3	*	*	*	*
21.0	0.0643	0.0979	0.1288	0.1123
21.1	*	*	*	*
22.0	0.0365	0.0567	0.0956	0.0678
22.3	*	*	*	*
23.0	0.0292	0.0258	0.0693	0.0572
24.0	0.0132	0.0103	0.0471	0.0169
24.3	*	0.0052	*	*
25.0	0.0088	0.0155	0.0166	0.0064
26.0	*	0.0103	0.0028	0.0064
27.0	*	0.0052	0.0014	0.0064
<b>D13S317</b>				
5.0	*	*	*	*

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
6.0	*	*	*	*
7.0	*	*	*	*
7.1	*	*	*	*
8.0	0.0278	0.2216	0.1205	0.1102
8.1	*	*	*	*
9.0	0.0336	0.1443	0.0776	0.1653
10.0	0.0307	0.1031	0.0471	0.0996
11.0	0.3099	0.2680	0.3255	0.2182
11.3	*	*	*	*
12.0	0.4181	0.2062	0.2687	0.2352
12.3	*	*	*	*
13.0	0.1404	0.0567	0.1163	0.1059
13.3	*	*	*	*
14.0	0.0395	*	0.0429	0.0614
15.0	*	*	0.0014	0.0042
16.0	*	*	*	*
17.0	*	*	*	*
<b>D16S539</b>				
4.0	*	*	*	*
5.0	0.0015	*	*	*
6.0	*	*	*	*
7.0	*	*	*	*
8.0	0.0322	*	0.0180	0.0191
8.3	*	*	*	*
9.0	0.1827	0.3557	0.1066	0.1398
9.3	*	*	*	*
10.0	0.1170	0.1649	0.0568	0.1504

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
11.0	0.3143	0.1907	0.3144	0.2648
11.3	*	*	*	*
12.0	0.2047	0.1753	0.3144	0.2775
12.1	*	*	*	*
12.2	*	*	*	*
13.0	0.1228	0.0979	0.1634	0.1335
13.3	*	*	*	*
14.0	0.0249	0.0155	0.0263	0.0127
15.0	*	*	*	0.0021
16.0	*	*	*	*
<b>D18S51</b>				
6.0	*	*	*	*
7.0	*	*	*	*
8.0	*	*	*	*
9.0	0.0029	*	*	*
9.2	*	*	*	*
10.0	0.0044	*	0.0083	0.0021
10.2	*	*	*	*
11.0	0.0015	*	0.0097	0.0148
11.2	*	*	*	*
12.0	0.0760	0.0361	0.1136	0.1144
12.2	*	*	*	*
12.3	*	*	*	*
13.0	0.0409	0.2165	0.1233	0.1229
13.1	*	*	*	*
13.2	0.0044	*	*	*
13.3	*	*	*	*

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
14.0	0.0716	0.2371	0.1343	0.1610
14.2	*	*	0.0014	0.0021
15.0	0.1652	0.1804	0.1704	0.1589
15.2	0.0015	*	*	*
15.3	*	*	*	*
16.0	0.1711	0.1289	0.1468	0.1250
16.1	*	*	*	*
16.2	*	*	0.0014	*
17.0	0.1520	0.0670	0.1385	0.1250
17.2	*	*	*	*
17.3	*	*	*	*
18.0	0.1213	0.0309	0.0776	0.0784
18.1	*	*	*	*
18.2	*	*	*	*
19.0	0.0994	0.0412	0.0402	0.0466
19.2	*	*	*	*
20.0	0.0629	0.0258	0.0180	0.0275
20.2	*	*	*	*
21.0	0.0102	0.0103	0.0097	0.0085
21.1	*	*	*	*
21.2	0.0015	*	*	*
22.0	0.0073	0.0155	0.0069	0.0106
22.2	*	*	*	*
23.0	0.0044	0.0052	*	*
23.2	*	*	*	*
24.0	0.0015	*	*	0.0021
25.0	*	*	*	*

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
26.0	*	*	*	*
27.0	*	*	*	*
28.0	*	0.0052	*	*
<b>D19S433</b>				
5.2	*	*	*	*
6.0	*	*	*	*
6.2	*	*	*	*
7.0	*	*	*	*
8.0	*	*	*	*
9.0	*	*	*	0.0021
9.2	*	*	*	*
10.0	0.0102	*	0.0014	0.0021
10.2	*	*	*	*
11.0	0.0629	*	0.0055	0.0148
11.2	*	*	*	*
12.0	0.1228	0.0361	0.0706	0.0657
12.1	*	*	*	*
12.2	0.0365	0.0258	0.0014	0.0127
13.0	0.2456	0.2835	0.2548	0.2225
13.1	*	*	*	*
13.2	0.0526	0.0206	0.0069	0.0445
14.0	0.2105	0.2990	0.3615	0.3538
14.2	0.0746	0.1031	0.0235	0.0381
14.3	*	*	*	*
15.0	0.0804	0.0619	0.1565	0.1356
15.2	0.0614	0.1237	0.0360	0.0551
16.0	0.0044	0.0103	0.0568	0.0254



**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
16.2	0.0263	0.0309	0.0152	0.0275
17.0	*	*	0.0069	*
17.2	0.0088	0.0052	0.0014	*
18.0	*	*	*	*
18.2	0.0029	*	0.0014	*
19.0	*	*	*	*
19.2	*	*	*	*
<b>D1S1656</b>				
8.0	*	*	*	*
9.0	*	*	*	*
10.0	0.0146	*	0.0028	0.0064
11.0	0.0453	0.0309	0.0776	0.0275
12.0	0.0643	0.0464	0.1163	0.0890
13.0	0.1009	0.1340	0.0665	0.1144
13.3	*	*	*	*
14.0	0.2573	0.0619	0.0789	0.1165
14.2	*	*	*	*
14.3	0.0073	*	0.0028	0.0042
15.0	0.1579	0.2784	0.1496	0.1377
15.3	0.0292	*	0.0582	0.0508
16.0	0.1096	0.2010	0.1357	0.1758
16.1	*	*	*	*
16.3	0.1023	0.0155	0.0609	0.0508
17.0	0.0278	0.0722	0.0471	0.0424
17.1	*	*	*	*
17.3	0.0497	0.0876	0.1330	0.1483
18.0	0.0029	0.0155	0.0055	0.0064

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
18.3	0.0234	0.0515	0.0499	0.0254
19.0	*	*	*	*
19.3	0.0073	0.0052	0.0152	0.0042
20.0	*	*	*	*
20.3	*	*	*	*
21.0	*	*	*	*
<b>D21S11</b>				
23.2	*	*	*	*
24.0	*	*	*	*
24.2	*	*	*	0.0021
24.3	*	*	*	*
25.0	*	*	*	*
25.2	*	*	0.0014	*
25.3	*	*	*	*
26.0	0.0015	*	*	*
26.2	*	*	*	0.0021
27.0	0.0746	*	0.0222	0.0275
27.1	*	*	*	*
27.2	*	*	*	*
27.3	*	*	*	*
28.0	0.2456	0.0567	0.1593	0.0996
28.1	*	*	*	*
28.2	*	0.0052	*	*
28.3	*	*	*	*
29.0	0.2047	0.2010	0.2022	0.2076
29.1	*	*	*	*
29.2	*	*	0.0028	0.0021

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
29.3	0.0015	*	*	*
30.0	0.1696	0.3299	0.2825	0.2733
30.1	*	*	*	*
30.2	0.0175	0.0052	0.0291	0.0233
30.3	*	0.0103	*	*
31.0	0.0789	0.1237	0.0720	0.0763
31.1	*	*	*	*
31.2	0.0512	0.0361	0.0983	0.0996
31.3	*	*	*	*
32.0	0.0088	0.0567	0.0055	0.0169
32.1	*	*	*	*
32.2	0.0614	0.1134	0.0900	0.1271
32.2	*	*	*	*
33.0	0.0044	0.0155	0.0014	0.0042
33.1	0.0029	*	*	0.0021
33.2	0.0351	0.0464	0.0263	0.0339
33.3	*	*	*	*
34.0	0.0058	*	*	0.0021
34.1	*	*	*	*
34.2	*	*	0.0042	*
35.0	0.0219	*	0.0014	*
35.1	*	*	*	*
35.2	*	*	*	*
36.0	0.0088	*	0.0014	*
36.1	*	*	*	*
36.2	*	*	*	*
37.0	0.0029	*	*	*

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
37.2	*	*	*	*
38.0	0.0015	*	*	*
38.2	*	*	*	*
39.0	0.0015	*	*	*
<b>D22S1045</b>				
7.0	*	*	*	*
8.0	0.0073	*	*	*
9.0	*	*	*	*
10.0	0.0409	*	*	0.0148
11.0	0.1447	0.2010	0.1399	0.0636
12.0	0.0541	0.0052	0.0125	0.0127
13.0	0.0029	*	0.0069	0.0085
14.0	0.0775	0.0103	0.0568	0.0275
15.0	0.2515	0.3093	0.3213	0.4258
16.0	0.1915	0.2268	0.3823	0.3496
17.0	0.2091	0.2216	0.0748	0.0911
18.0	0.0146	0.0206	0.0055	0.0064
19.0	0.0058	0.0052	*	*
<b>D2S1338</b>				
10.0	*	*	*	*
11.0	*	*	*	*
12.0	*	*	*	*
13.0	*	*	*	*
14.0	*	*	*	*
15.0	0.0015	*	0.0014	*
16.0	0.0556	0.0206	0.0374	0.0297
17.0	0.1009	0.0567	0.1856	0.1695

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
18.0	0.0424	0.1340	0.0734	0.0805
18.3	*	*	*	*
19.0	0.1389	0.1804	0.1205	0.1928
19.3	*	*	*	*
20.0	0.1038	0.1598	0.1565	0.1271
21.0	0.1360	0.0155	0.0374	0.0318
22.0	0.1374	0.0515	0.0346	0.0572
23.0	0.1038	0.1649	0.1053	0.1398
23.2	*	*	*	*
23.3	*	*	*	*
24.0	0.0833	0.1289	0.1150	0.0763
24.2	*	*	*	*
25.0	0.0775	0.0464	0.1025	0.0784
26.0	0.0146	0.0361	0.0305	0.0169
27.0	0.0044	0.0052	*	*
28.0	*	*	*	*
29.0	*	*	*	*
<b>D2S441</b>				
8.0	*	0.0052	*	*
8.1	*	*	*	*
9.0	0.0029	*	0.0014	*
9.1	*	0.0052	0.0014	*
10.0	0.0848	0.2680	0.2105	0.3369
10.1	*	*	*	*
11.0	0.3626	0.3505	0.3435	0.2987
11.1	*	*	*	*
11.3	0.0439	0.0309	0.0609	0.0445

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
12.0	0.1652	0.2165	0.0471	0.0360
12.2	*	*	*	*
12.3	0.0058	0.0052	0.0042	0.0021
13.0	0.0439	0.0258	0.0291	0.0233
13.3	0.0029	*	*	*
14.0	0.2675	0.0825	0.2410	0.2055
14.1	*	*	*	*
14.3	0.0015	*	*	*
15.0	0.0190	0.0103	0.0596	0.0487
16.0	*	*	0.0014	0.0021
17.0	*	*	*	0.0021
<b>D3S1358</b>				
8.0	*	*	*	*
9.0	*	*	*	*
10.0	*	*	*	*
11.0	*	*	0.0014	*
12.0	0.0044	*	*	*
13.0	0.0029	*	0.0014	0.0064
13.2	*	*	*	*
14.0	0.0906	0.0258	0.1066	0.0784
15.0	0.3085	0.3660	0.2729	0.3220
15.2	0.0015	*	*	*
16.0	0.3187	0.3299	0.2382	0.2797
16.2	*	*	*	*
17.0	0.2120	0.2010	0.2105	0.1843
17.1	*	*	*	*
17.2	*	*	*	*

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
18.0	0.0570	0.0670	0.1510	0.1229
18.2	*	*	*	*
19.0	0.0044	0.0103	0.0166	0.0042
20.0	*	*	0.0014	0.0021
20.1	*	*	*	*
21.0	*	*	*	*
<b>D5S818</b>				
6.0	*	*	*	*
7.0	0.0015	0.0155	0.0028	0.0339
8.0	0.0468	0.0052	0.0055	0.0085
9.0	0.0322	0.0979	0.0416	0.0530
10.0	0.0731	0.2268	0.0554	0.0572
10.1	*	*	*	*
11.0	0.2339	0.2680	0.3560	0.3898
11.1	*	*	*	*
11.3	*	*	*	*
12.0	0.3699	0.2062	0.3878	0.3390
12.1	*	*	*	*
12.3	*	*	*	*
13.0	0.2237	0.1649	0.1427	0.1081
14.0	0.0161	0.0155	0.0069	0.0085
14.3	*	*	*	*
15.0	0.0029	*	0.0014	0.0021
16.0	*	*	*	*
17.0	*	*	*	*
18.0	*	*	*	*
19.0	*	*	*	*

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
<b>D6S1043</b>				
7.0	*	*	*	*
8.0	*	*	0.0014	*
9.0	0.0015	*	*	*
10.0	0.0058	0.0309	0.0166	0.0042
11.0	0.1535	0.1495	0.2964	0.1780
12.0	0.2237	0.1237	0.2368	0.2055
12.3	*	0.0052	*	*
13.0	0.0965	0.1237	0.0859	0.1017
14.0	0.0585	0.1546	0.0554	0.1356
15.0	0.0541	0.0361	0.0125	0.0297
16.0	0.0161	0.0052	0.0042	0.0021
17.0	0.0570	0.0722	0.0609	0.0487
17.2	*	*	*	*
17.3	*	*	*	*
18.0	0.1067	0.1598	0.0886	0.1081
18.1	*	*	*	0.0021
18.3	0.0015	*	*	*
19.0	0.1126	0.0928	0.0983	0.0763
19.2	*	*	*	*
19.3	0.0015	*	*	0.0021
20.0	0.0731	0.0412	0.0319	0.0318
20.3	*	*	*	0.0127
21.0	0.0175	0.0052	0.0097	0.0064
21.3	0.0015	*	*	0.0403
22.0	*	*	0.0014	*
22.3	*	*	*	0.0127



**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
23.0	0.0102	*	*	*
23.3	*	*	*	0.0021
24.0	0.0058	*	*	*
24.3	*	*	*	*
25.0	0.0015	*	*	*
26.0	0.0015	*	*	*
<b>D7S820</b>				
5.0	*	*	*	*
5.2	*	*	*	*
6.0	0.0015	*	*	*
6.3	*	*	*	*
7.0	0.0117	0.0052	0.0277	0.0106
7.1	*	*	*	*
7.3	*	*	*	*
8.0	0.2281	0.1289	0.1440	0.1208
8.1	*	*	0.0014	*
8.2	*	*	*	*
8.3	*	*	*	*
9.0	0.1155	0.0464	0.1676	0.0911
9.1	*	*	*	*
9.2	*	*	*	*
9.3	*	*	*	*
10.0	0.3363	0.2577	0.2562	0.3072
10.1	*	*	*	*
10.3	*	*	*	0.0021
11.0	0.2032	0.3608	0.2050	0.2775
11.1	*	*	*	*

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
11.3	*	*	*	*
12.0	0.0877	0.1753	0.1593	0.1547
12.1	*	*	*	*
12.3	*	*	*	*
13.0	0.0146	0.0258	0.0346	0.0360
13.1	*	*	*	*
14.0	0.0015	*	0.0042	*
14.1	*	*	*	*
15.0	*	*	*	*
16.0	*	*	*	*
<b>D8S1179</b>				
4.0	*	*	*	*
5.0	*	*	*	*
6.0	*	*	*	*
7.0	*	*	*	*
8.0	0.0073	*	0.0139	0.0148
9.0	0.0044	*	0.0055	0.0064
10.0	0.0307	0.1237	0.1025	0.0932
10.2	*	*	*	*
11.0	0.0526	0.1186	0.0762	0.0530
12.0	0.1301	0.1186	0.1676	0.1292
12.3	*	*	*	*
13.0	0.2193	0.2010	0.3296	0.2733
13.3	*	*	*	*
14.0	0.2939	0.2010	0.1662	0.2627
15.0	0.1901	0.1289	0.1039	0.1292
15.3	*	*	*	*

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
16.0	0.0643	0.0928	0.0332	0.0318
17.0	0.0044	0.0103	0.0014	0.0042
18.0	0.0029	0.0052	*	0.0021
19.0	*	*	*	*
20.0	*	*	*	*
<b>FGA</b>				
12.3	*	*	*	*
13.0	*	*	*	*
14.0	*	*	*	*
15.0	*	*	*	*
15.2	*	*	*	*
16.0	*	*	*	*
16.1	*	*	*	*
16.2	0.0015	*	*	*
17.0	*	0.0103	*	0.0021
17.2	0.0015	*	*	*
18.0	0.0015	0.0258	0.0249	0.0127
18.2	0.0175	*	*	*
19.0	0.0512	0.0567	0.0499	0.0805
19.2	0.0029	*	*	*
19.3	*	*	*	*
20.0	0.0541	0.0876	0.1233	0.0847
20.1	*	*	*	*
20.2	*	*	*	*
20.3	*	*	*	*
21.0	0.1228	0.1031	0.1787	0.1525
21.2	*	*	0.0055	*

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
21.3	*	*	*	*
22.0	0.1988	0.2423	0.2050	0.1653
22.1	*	*	*	*
22.2	0.0044	*	0.0125	0.0042
22.3	0.0015	*	*	*
23.0	0.1696	0.2062	0.1524	0.1208
23.1	*	*	*	*
23.2	0.0015	*	0.0028	0.0042
23.3	*	*	*	*
24.0	0.1330	0.1495	0.1343	0.1419
24.1	*	*	*	*
24.2	*	0.0052	0.0014	*
24.3	*	*	*	*
25.0	0.1184	0.0722	0.0789	0.1186
25.1	*	*	*	*
25.2	0.0015	*	*	*
25.3	*	*	*	*
26.0	0.0702	0.0309	0.0263	0.0614
26.1	*	*	*	*
26.2	*	*	*	*
27.0	0.0234	0.0052	0.0042	0.0445
27.2	*	*	*	*
28.0	0.0146	*	*	0.0021
28.2	*	*	*	*
29.0	0.0058	*	*	0.0021
29.1	*	*	*	*
29.2	*	*	*	*

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
30.0	0.0015	*	*	0.0021
30.2	0.0015	*	*	*
31.0	*	*	*	*
31.2	0.0015	*	*	*
32	*	*	*	*
32.2	*	*	*	*
33.2	*	*	*	*
34.2	*	*	*	*
41.2	*	*	*	*
42.2	*	*	*	*
43.2	*	0.0052	*	*
44.2	*	*	*	*
45.2	*	*	*	*
46.2	*	*	*	*
47.2	*	*	*	*
48.2	*	*	*	*
49.2	*	*	*	*
50.2	*	*	*	*
51.2	*	*	*	*
<b>Penta D</b>				
2.2	0.1140	*	0.0042	0.0169
3.2	0.0088	*	*	0.0021
5.0	0.0439	*	*	0.0064
6.0	0.0102	0.0103	0.0042	0.0021
7.0	0.0439	*	0.0042	0.0021
7.4	*	*	*	*
8.0	0.1082	0.0052	0.0208	0.0191

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
9.0	0.1681	0.3144	0.2216	0.2415
9.2	*	*	*	*
9.4	*	*	*	*
10.0	0.0994	0.1856	0.1150	0.1568
11.0	0.1798	0.1804	0.1260	0.1568
11.2	*	*	*	*
12.0	0.1082	0.1804	0.2327	0.1631
12.2	*	*	*	*
13.0	0.0833	0.1031	0.1967	0.1441
13.4	0.0015	*	*	*
14.0	0.0249	0.0103	0.0609	0.0720
14.1	*	*	*	*
15.0	0.0044	0.0103	0.0097	0.0106
16.0	*	*	0.0028	0.0042
17.0	0.0015	*	0.0014	0.0021
18.0	*	*	*	*
<b>Penta E</b>				
4.0	*	*	*	*
5.0	0.0950	0.0722	0.0762	0.0360
6.0	0.0015	*	*	*
7.0	0.1038	*	0.1690	0.1186
8.0	0.1667	0.0052	0.0139	0.0254
9.0	0.0512	0.0258	0.0125	0.0169
10.0	0.0468	0.0619	0.0859	0.0847
11.0	0.0643	0.1598	0.0873	0.0742
11.4	*	*	*	*
12.0	0.1287	0.0928	0.1994	0.1737

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
13.0	0.1038	0.0515	0.0859	0.0932
14.0	0.0687	0.0515	0.0623	0.0720
14.2	*	*	*	*
15.0	0.0556	0.1134	0.0429	0.0911
15.2	*	*	*	*
15.4	*	*	0.0014	0.0021
16.0	0.0409	0.0567	0.0512	0.0614
16.2	*	*	*	*
16.4	*	*	*	*
17.0	0.0439	0.0825	0.0485	0.0551
17.4	*	*	*	*
18.0	0.0161	0.0773	0.0332	0.0339
18.4	*	*	*	*
19.0	0.0073	0.0412	0.0152	0.0212
19.4	*	0.0103	*	*
20.0	0.0044	0.0206	0.0097	0.0212
21.0	*	0.0206	0.0028	0.0064
22.0	*	0.0309	0.0014	0.0021
23.0	*	0.0206	*	0.0064
24.0	*	*	0.0014	*
25.0	0.0015	0.0052	*	0.0042
26.0	*	*	*	*
27.0	*	*	*	*
<b>TH01</b>				
3.0	*	*	*	*
4.0	*	*	*	*
5.0	0.0044	*	0.0014	*

**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
5.3	*	*	*	*
6.0	0.1316	0.1701	0.2355	0.2394
6.3	*	*	*	*
7.0	0.4079	0.2680	0.1939	0.2966
7.3	*	*	*	*
8.0	0.1959	0.0722	0.0956	0.0911
8.3	*	*	*	*
9.0	0.1594	0.4433	0.1191	0.1462
9.3	0.0965	0.0412	0.3449	0.2182
10.0	0.0044	0.0052	0.0083	0.0085
10.3	*	*	*	*
11.0	*	*	0.0014	*
12.0	*	*	*	*
13.0	*	*	*	*
13.3	*	*	*	*
<b>TPOX</b>				
4.0	*	*	*	*
5.0	*	*	0.0014	*
6.0	0.0892	*	0.0014	0.0085
7.0	0.0175	*	*	0.0064
7.1	*	*	*	*
7.3	*	*	*	*
8.0	0.3670	0.5515	0.5249	0.4852
9.0	0.1959	0.0773	0.1274	0.0932
10.0	0.0863	0.0258	0.0499	0.0487
10.1	*	*	*	*
10.3	*	*	*	*



**Table 6 Autosomal allele frequencies by population group for VeriFiler Express kit Kit STR loci.**  
(\*=Alleles not detected) (continued)

Allele	African American (n = 342)	Asian (n = 97)	U.S. Caucasian (n = 361)	U.S. Hispanic (n = 236)
11.0	0.2164	0.2990	0.2521	0.2542
12.0	0.0263	0.0464	0.0416	0.1038
13.0	0.0015	*	0.0014	*
14.0	*	*	*	*
15.0	*	*	*	*
16.0	*	*	*	*
<b>vWA</b>				
10.0	*	*	*	*
11.0	0.0029	*	*	0.0021
12.0	0.0015	*	0.0014	*
13.0	0.0088	*	0.0014	*
14.0	0.0804	0.1959	0.0928	0.0805
15.0	0.1915	0.0206	0.1053	0.1441
15.2	*	*	*	*
16.0	0.2500	0.1392	0.2008	0.2839
17.0	0.2354	0.3144	0.2839	0.2458
18.0	0.1491	0.2062	0.2022	0.1801
18.2	*	*	*	*
19.0	0.0629	0.1082	0.1039	0.0508
20.0	0.0161	0.0155	0.0069	0.0106
21.0	0.0015	*	0.0014	0.0021
22.0	*	*	*	*
23.0	*	*	*	*
24.0	*	*	*	*
25.0	*	*	*	*

Table 7 Probability of identity (PI) values for the VeriFiler™ Express kit STR loci

Locus	African American (n = 342)	Asian (n = 97)	Caucasian (n = 361)	Hispanic (n = 236)
CSF1PO	0.0828	0.0984	0.1291	0.1271
D10S1248	0.0666	0.0863	0.0979	0.1010
D12S391	0.0346	0.0444	0.0219	0.0268
D13S317	0.1339	0.0686	0.0767	0.0495
D16S539	0.0706	0.0873	0.0948	0.0742
D18S51	0.0268	0.0456	0.0284	0.0266
D19S433	0.0358	0.0676	0.0844	0.0661
D1S1656	0.0310	0.0424	0.0188	0.0242
D21S11	0.0395	0.0566	0.0497	0.0448
D22S1045	0.0538	0.0967	0.1221	0.1533
D2S1338	0.0212	0.0307	0.0257	0.0285
D2S441	0.0937	0.1027	0.0872	0.1035
D3S1358	0.1067	0.1350	0.0768	0.0942
D5S818	0.1012	0.0721	0.1437	0.1271
D6S1043	0.0266	0.0266	0.0517	0.0285
D7S820	0.0863	0.0992	0.0597	0.0808
D8S1179	0.0650	0.0405	0.0607	0.0611
FGA	0.0289	0.0403	0.0356	0.0261
Penta D	0.0257	0.0745	0.0542	0.0460
Penta E	0.0169	0.0124	0.0211	0.0149
TH01	0.1017	0.1405	0.0919	0.0853
TPOX	0.0892	0.2233	0.1783	0.1476
vWA	0.0615	0.0759	0.0639	0.0710
Combined	$2.546 \times 10^{-30}$	$4.693 \times 10^{-28}$	$1.092 \times 10^{-28}$	$2.947 \times 10^{-29}$

## Probability of paternity exclusion observation

The following table shows the Probability of paternity exclusion (PE) values of the VeriFiler™ Express 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 VeriFiler™ Express kit STR loci (Chakraborty, Stivers, and Zhong, 1996).

**Table 8** Probability of paternity exclusion values for the VeriFiler™ Express kit STR loci

Locus	African American (n = 342)	Asian (n = 97)	Caucasian (n = 361)	Hispanic (n = 236)
CSF1PO	0.5724	0.5351	0.4708	0.4756
D10S1248	0.6138	0.5619	0.5346	0.5286
D12S391	0.7214	0.6849	0.7794	0.7559
D13S317	0.4687	0.6068	0.5877	0.6657
D16S539	0.6018	0.5580	0.5436	0.5918
D18S51	0.7555	0.6807	0.7481	0.7563
D19S433	0.7167	0.6135	0.5698	0.6172
D1S1656	0.7356	0.6916	0.7957	0.7679
D21S11	0.7028	0.6440	0.6661	0.6829
D22S1045	0.6524	0.5350	0.4888	0.4388
D2S1338	0.7832	0.7382	0.7606	0.7474
D2S441	0.5471	0.5259	0.5616	0.5250
D3S1358	0.5158	0.4613	0.5842	0.5427
D5S818	0.5293	0.5973	0.4503	0.4825
D6S1043	0.7559	0.7562	0.6602	0.7475
D7S820	0.5615	0.5323	0.6330	0.5761
D8S1179	0.6186	0.6976	0.6302	0.6303
FGA	0.7457	0.6994	0.7173	0.7585
Penta D	0.7607	0.5908	0.6509	0.6782
Penta E	0.8069	0.8347	0.7830	0.8184
TH01	0.5259	0.4535	0.5476	0.5624
TPOX	0.5557	0.3428	0.3988	0.4452

vWA	0.6283	0.5872	0.6206	0.6009
Combined	0.99999999984527	0.999993438259661	0.999989850701339	0.999985788729133

## Performance of direct amplification using a 10- $\mu$ L PCR reaction volume

The VeriFiler™ Express PCR Amplification Kit is optimized for a 25- $\mu$ L PCR reaction 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 with certain sample types; however, we performed limited studies to demonstrate that the VeriFiler™ Express kit can be used for direct amplification of reference samples with a reduced reaction volume of 10  $\mu$ L. Laboratories should perform their own validation for use of the VeriFiler™ Express kit with a decreased reaction volume. A complete developmental validation at a reduced reaction volume of 10  $\mu$ L has not been conducted.

The performance evaluation of the VeriFiler™ Express kit, using a reduced reaction volume in direct amplification, was completed with the following samples:

- Blood stain cards collected on filter paper, 1.2-mm punch size
- Blood samples collected on Whatman FTA™ cards, 1.2-mm punch size
- Buccal samples collected on Whatman FTA™ cards, 1.2-mm punch size
- Buccal samples collected on COPAN 4N6FLOQSwabs™

Except where noted, the protocols described in this user guide for the developmentally validated 25- $\mu$ L PCR reaction volumes were followed for sample preparation, PCR, capillary electrophoresis, and analysis. Table 9 lists the reagent volumes used for each substrate tested for the 10- $\mu$ L PCR reaction volume. Because of the reduced reaction volume, optimization of the appropriate PCR cycle may require a lower cycle number to produce on-scale data than that used for a 25- $\mu$ L PCR reaction volume. See Table 9 for the cycle numbers used as part of this performance check.

**Table 9 PCR preparation—reagent components and conditions for a 10- $\mu$ L PCR reaction volume**

Sample and substrate type	Reagent volume per reaction					Cycle numbers
	Master mix	Primer mix	Low-TE buffer	Prep-n-Go™ Buffer	Total volume	
Blood on filter paper	4 $\mu$ L	4 $\mu$ L	0 $\mu$ L	2 $\mu$ L	10 $\mu$ L	24
Blood on FTA™ card	4 $\mu$ L	4 $\mu$ L	2 $\mu$ L	0 $\mu$ L	10 $\mu$ L	24
Buccal on FTA™ card	4 $\mu$ L	4 $\mu$ L	2 $\mu$ L	0 $\mu$ L	10 $\mu$ L	27
Buccal lysate (from swabs)	4 $\mu$ L	4 $\mu$ L	0 $\mu$ L	2 $\mu$ L	10 $\mu$ L	24

The success rate for the samples and substrates tested are shown in Table 10:

- The full profile success rate was defined as the percent of profiles in which all peaks were above a 175-RFU peak amplitude threshold (PAT).
- Off-scale peaks produced no artifacts that interfered with profile interpretation or artificial suppression of signal.
- Pull-up artifacts were within 0.5 bp of the parent allele.
- Peak morphology was intact with no split peaks.
- Stutter peaks were not over the 20% global filter used for analysis.

**Table 10 Success rate results for a 10- $\mu$ L PCR reaction volume, using a 20% global filter and a PAT of 175 RFU**

Sample and substrate type	Number of amplifications <sup>[1]</sup>	Partial profile	No profile	Off-scale alleles	Success rate (175 RFU)
Blood on filter paper	200	0	0	9	100%
Blood on FTA™ card	42	0	0	7	100%
Buccal on FTA™ card	70	2	2	2	94%
Buccal lysate (from swabs)	46	0	0	1	100%

<sup>[1]</sup> The number of amplifications refers to the number of unique donors tested for the particular sample type.

Our studies demonstrate that the VeriFiler™ Express kit can be used to generate full profiles from blood and buccal reference samples prepared using a reduced PCR reaction volume of 10  $\mu$ L.

There are multiple sample and substrate combinations that can be encountered that require different cycle numbers to produce optimal data within the dynamic range of the capillary electrophoresis instrument. In addition, sample collection techniques and collection efficiency impact the amount of sample on the collection device and subsequent downstream profile success. Therefore, we recommend that laboratories intending to implement a reduced reaction volume for a direct amplification workflow using the VeriFiler™ Express kit determine the optimum cycle number based on internal validation studies with their specific sample and substrate combinations.



# 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 $\mu$ L of Prep-n-Go™ Buffer.	
Positive signal from DNA Control 007 but partial or no signal from DNA test samples	Proper low-TE buffer not used for treated paper substrates.	Prepare low-TE buffer. See “Prepare low-TE buffer” on page 20.	
	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.
Insufficient lysis of the swab head occurred.	For buccal samples collected with the Bode Buccal DNA Collector™ device, punch from near the tip of the collector.		
	Ensure the swab heads are incubated for 20 minutes in 400 $\mu$ L of Prep-n-Go™ Buffer.		
More than two alleles present at a locus	Proper low-TE buffer not used for treated paper substrates.	Prepare low-TE buffer.	
	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) occurred.	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.	Incubate the amplification products at 60°C for an additional 10 minutes.		
	Ensure the cycle number is optimized. Use fewer PCR cycles or interpret the off-scale data according to your laboratory procedure.		

**Appendix A Troubleshooting**Performance of direct amplification using a 10- $\mu$ L PCR reaction volume

Observation	Possible cause	Recommended action
More than two alleles present at a locus <i>(continued)</i>	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.
	Expected amplification pattern observed in the TAZ™ dye channel.	Peak heights are typically elevated at D22S1045 for buccal samples. Follow laboratory guidelines for evaluation of the generated results.
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 $\mu$ L 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: 25 $\mu$ L
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 $\mu$ L 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 <i>GeneMapper™ ID-X Software v1.4 New Features and Installation Procedures User Bulletin</i> (Pub. No. 4477684), “Known issues: 3730 DNA Analyzer sizing failures”.



Observation	Possible cause	Recommended action
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 <b>Light</b> setting for Smoothing.	Use the <b>None</b> setting for Smoothing after performing appropriate validation studies.  For more information, see the <i>GeneMapper™ ID-X Software v1.4 New Features and Installation Procedures User Bulletin</i> (Pub. No. 4477684), “Known issues: 3730 DNA Analyzer sizing failures”.



# Required materials not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.fisherscientific.com) or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

## STR kit

Item	Source
VeriFiler™ Express PCR Amplification Kit, 200-reaction kit	A32014
GeneScan™ 600 LIZ™ Size Standard v2.0, 2 × 200 µL <b>IMPORTANT!</b> 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
<b>Collection system: NUCLEIC-CARD™ system or Whatman FTA™</b>	
NUCLEIC-CARD™ Sample Collection Device	A32607
NUCLEIC-CARD™ matrix, 1 spot	4474001
NUCLEIC-CARD™ COLOR matrix, 1 spot	4473974
Whatman™ FTA™ Classic Cards	MLS
Whatman™ EasiCollect™ system	MLS
<b>Sample preparation:</b>	
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

(continued)

Item	Source
<b>Punch tool:</b>	
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
<b>Collection system: Bode or paper</b>	
Buccal DNA Collector™ Device	Contact Bode Cellmark Forensics
903 paper	MLS
<b>Punch tool:</b>	
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
<b>Collection system: 4N6FLOQSwabs™ reference collection devices</b>	
4N6FLOQSwabs™ Lollipop Swab in peelpouch	4473997
4N6FLOQSwabs™, Regular size tip in peelpouch	4473979
4N6FLOQSwabs™, Regular size tip in plain tube	4479433 <sup>[1]</sup>
4N6FLOQSwabs™, Regular size tip in plastic tube with Active Drying System	4479439 <sup>[1]</sup>
4N6FLOQSwabs™, Regular size tip plus 2-mL Eppendorf™ cuvette in peelpouch	4479431 <sup>[1]</sup>
4N6FLOQSwabs™, Regular size tip plus 2-mL Eppendorf™ cuvette with evaporation duct in peelpouch	4479440 <sup>[1]</sup>
4N6FLOQSwabs™ Dual Swab Regular size tip in plain tube	4479432 <sup>[1]</sup>



(continued)

Item	Source
4N6FLOQSwabs™, Buccal shape tip in plastic tube with Active Drying System	4479436 <sup>[1]</sup>
Cotton swabs	MLS
<b>Sample preparation</b>	
Prep-n-Go™ Buffer (for use with buccal swab substrates)	4471406
<b>Heated lysis protocol only: 1.5 mL tube format or 96-well deep-well plate format</b>	
<b>1.5 mL tube format</b>	
1.5 mL tubes	MLS
Oven	VWR™ Scientific dry heat block or equivalent
<b>96-well deep-well plate format</b>	
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	Agilent Technologies 410094
<b>IMPORTANT!</b> Do not use a plastic plate adaptor.	

<sup>[1]</sup> To order, contact your local Thermo Fisher Scientific sales representative.

## Thermal cycler required materials

### ProFlex™ PCR System

Item	Source
ProFlex™ 96-well PCR System	<a href="#">4484075</a>
ProFlex™ 2 × 96-well PCR System	<a href="#">4484076</a>
ProFlex™ 3 × 32-Well PCR System	<a href="#">4484073</a>

## 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 v1.2.1	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	<a href="#">4316813</a>
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	<a href="#">4326659</a>
MicroAmp™ Optical 8-Tube Strip, 0.2 mL	<a href="#">4316567</a>
Septa for SeqStudio™ Genetic Analyzer, 96 well	A36541
Septa for SeqStudio™ Genetic Analyzer, 8 strip	A36543
DS-36 Matrix Standard Kit (Dye Set J6)	<a href="#">4425042</a>

### 3500 Series Genetic Analyzer

Item	Source
3500 Series HID Data Collection Software v4.0.1	A46085
3500 Series Data Collection Software 3.1	4405187 <sup>[1]</sup> , A26287
3500 Series Data Collection Software 3	4405186 <sup>[1]</sup>
3500 Series Data Collection Software 2	4475183 <sup>[1]</sup>
HID Updater 3500 Data Collection Software v2	4480670



(continued)

Item	Source
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)	<a href="#">4425042</a>
Conditioning reagent	4393718
8-Capillary array, 36 cm for 3500 Genetic Analyzers	4404683
24-Capillary array, 36 cm for 3500xL Genetic Analyzers	4404687
96-well retainer & base set (Standard) 3500/3500xL Genetic Analyzers	4410228
8-Tube retainer & base set (Standard) for 3500/3500xL Genetic Analyzers	4410231
8-Strip Septa for 3500/3500xL Genetic Analyzers	4410701
96-Well Septa for 3500/3500xL Genetic Analyzers	4412614
Septa Cathode Buffer Container, 3500 series	4410715

<sup>[1]</sup> Contact your Thermo Fisher Scientific HID representative.

## 3130 Series Genetic Analyzer

Item	Source
3130 Data Collection Software v4	4475105
3130x/ 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 x/ Genetic Analyzer Capillary Array, 36-cm	4315931
POP-4™ Polymer for 3130/3130 x/ Genetic Analyzers	4352755
Running Buffer, 10X	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
POP-7™ Polymer for 3730/3730xl DNA Analyzers	4335611
Running Buffer, 10X	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	<a href="#">N8010541</a>
MicroAmp™ Reaction Tube with Cap, 0.2 mL	<a href="#">N8010540</a>
MicroAmp™ 8-Tube Strip, 0.2 mL	<a href="#">N8010580</a>
MicroAmp™ Optical 8-Cap Strips	<a href="#">4323032</a>
MicroAmp™ 96-Well Tray/Retainer Set	<a href="#">403081</a>
MicroAmp™ 96-Well Base	<a href="#">N8010531</a>
MicroAmp™ Clear Adhesive Film	<a href="#">4306311</a>
MicroAmp™ Optical Adhesive Film	<a href="#">4311971</a>
MicroAmp™ Optical 96-Well Reaction Plate	<a href="#">N8010560</a>

### Laboratory supplies

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

<sup>[1]</sup> Major laboratory supplier





# Plate layouts

- Example PCR plate layout ..... 129
- Example electrophoresis plate layout ..... 130

## 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 4 different cycle numbers.

	1	2	3	4	5	6	7	8	9	10	11	12
	Sample 1	Sample 8	Sample 15									
A	Sample 2	Sample 9	Sample 16									
B	Sample 3	Sample 10	Sample 17									
C	Sample 4	Sample 11	Sample 18									
D	Sample 5	Sample 12	Sample 19									
E	Sample 6	Sample 13	Neg Control 007									
F	Sample 7	Sample 14										
G												
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	Sample 1	Sample 8	Sample 15	Sample 1	Sample 8	Sample 15	Sample 1	Sample 8	Sample 15	Sample 1	Sample 8	Sample 15
B	Sample 2	Sample 9	Sample 16	Sample 2	Sample 9	Sample 16	Sample 2	Sample 9	Sample 16	Sample 2	Sample 9	Sample 16
C	Sample 3	Sample 10	Sample 17	Sample 3	Sample 10	Sample 17	Sample 3	Sample 10	Sample 17	Sample 3	Sample 10	Sample 17
D	Sample 4	Sample 11	Sample 18	Sample 4	Sample 11	Sample 18	Sample 4	Sample 11	Sample 18	Sample 4	Sample 11	Sample 18
E	Sample 5	Sample 12	Sample 19	Sample 5	Sample 12	Sample 19	Sample 5	Sample 12	Sample 19	Sample 5	Sample 12	Sample 19
F	Sample 6	Sample 13	Neg Control	Sample 6	Sample 13	Neg Control	Sample 6	Sample 13	Neg Control	Sample 6	Sample 13	Neg Control
G	Sample 7	Sample 14	007	Sample 7	Sample 14	007	Sample 7	Sample 14	007	Sample 7	Sample 14	007
H	Allelic Ladder	CE Blank	CE Blank	Allelic Ladder	CE Blank	CE Blank	Allelic Ladder	CE Blank	CE Blank	Allelic Ladder	CE Blank	CE Blank
	Cycle 1			Cycle 2			Cycle 3			Cycle 4		



# PCR work areas

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■ PCR setup work area materials .....	131
■ Amplified DNA work area .....	132

## 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.

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## PCR setup work area materials

---

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

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- 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

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

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

## Chemical safety



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

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



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



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

## Biological hazard safety



**WARNING! Potential Biohazard.** Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



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

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

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## Related documentation

Document title	Pub. No.
<b>STR kits</b>	
<i>VeriFiler™ Express Kit – PCR and CE Quick Reference</i>	100043592
<i>VeriFiler™ Express Kit – PCR Setup – Swab Substrate Quick Reference</i>	100043591
<i>VeriFiler™ Express Kit – PCR Setup – Treated Paper Substrate Quick Reference</i>	100043589
<i>VeriFiler™ Express Kit – PCR Setup – Untreated Paper Substrate Quick Reference</i>	100043590
<b>Thermal cyclers</b>	
<i>ProFlex™ PCR System User Guide</i>	MAN0007697
<i>GeneAmp™ PCR System 9700 Base Module User Manual</i>	4303481
<b>SeqStudio™ Genetic Analyzer</b>	
<i>SeqStudio™ Genetic Analyzer Instrument and Software User Guide</i>	MAN0018646
<i>SeqStudio™ Genetic Analyzer for HID Instrument and Software v1.2.1 User Bulletin—New Features and Developmental Validation</i>	100086084
<b>3500 Series Genetic Analyzer</b>	
<i>3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v1 User Guide</i>	4401661
<i>3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v2 User Guide</i>	4476988
<i>HID Updater 3500 Data Collection Software v2.0 User Bulletin</i>	NA
<i>3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 3 User Guide</i>	100025036
<i>3500 Series Data Collection Software v3 User Bulletin: New Features and HID Validation Summary</i>	MAN0010812
<i>3500 Series Data Collection Software v3.1 User Bulletin: New Features and HID Validation Summary</i>	MAN0014110



(continued)

Document title	Pub. No.
<i>3500 Series Data Collection Software 4 User Bulletin: New Features and Developmental Validation</i>	100075298
<b>3130 Series Genetic Analyzer</b>	
<i>3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide</i>	4352716
<i>3130/3130xl Genetic Analyzers Using Data Collection Software v3.0 User Bulletin</i>	4363787
<i>3130/3130xl Genetic Analyzers Getting Started Guide</i>	4352715
<i>3130/3130xl Genetic Analyzers Quick Reference Card</i>	4362825
<i>3130/3130xl Genetic Analyzers AB Navigator Software Administrator Guide</i>	4359472
<b>3730 Series Genetic Analyzer</b>	
<i>3730/3730xl Genetic Analyzer Getting Started Guide</i>	4359476
<i>3730xl Data Collection Software 5 for HID User Bulletin: New Features and Developmental Validation</i>	MAN0019461
<i>3730xl DNA Analyzer with 3730xl Data Collection Software 5 User Guide</i>	100077621
<b>GeneMapper™ ID-X Software v1.3</b>	
<i>GeneMapper™ ID-X Software v1.3 Verification Experiments and Installation Procedures User Bulletin</i>	4470483
<b>GeneMapper™ ID-X Software v1.4</b>	
<i>GeneMapper™ ID-X Software v1.4 New Features and Installation Procedures User Bulletin</i>	4477684
<b>GeneMapper™ ID-X Software v1.5</b>	
<i>GeneMapper™ ID-X Software v1.5 New Features and Verification User Bulletin</i>	100031708
<i>GeneMapper™ ID-X Software v1.5 Getting Started Guide— Basic Features</i>	100031701
<i>GeneMapper™ ID-X Software v1.5 Quick Reference— Basic Features</i>	100031702
<i>GeneMapper™ ID-X Software v1.5 Getting Started Guide— Mixture Analysis Tool</i>	100031704
<i>GeneMapper™ ID-X Software v1.5 Quick Reference— Mixture Analysis Tool</i>	100031705
<i>GeneMapper™ ID-X Software v1.5 Installation Guide</i>	100031706
<i>GeneMapper™ ID-X Software v1.5 Administrator Guide</i>	100031703
<i>GeneMapper™ ID-X Software v1.5 Reference Guide</i>	100031707
<b>GeneMapper™ ID-X Software v1.6</b>	
<i>GeneMapper™ ID-X Software v1.6 New Features and Software Verification User Bulletin</i>	100073905

## Customer and technical support

For support:

- **In North America**—Send an email to [HIDTechSupport@thermofisher.com](mailto: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](https://www.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](https://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](https://www.thermofisher.com/support).

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