applied biosystems

# AmpFℓSTR™ Identifiler™ PCR Amplification Kit

## USER GUIDE

for use with:
200 reaction kit

**Catalog Number** 4322288
**Publication Number** 4323291
**Revision** K

**For Forensic or Paternity Use Only.**

ThermoFisher SCIENTIFIC

**Manufacturer:** Thermo Fisher Scientific | 7 Kingsland Grange | Warrington, Cheshire WA1 4SR | United Kingdom

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**Revision history:** Pub. No. 4323291

| Revision | Date | Description |
|---|---|---|
| K | 24 August 2018 | Updated branding and trademarks, no technical changes. |
| J | August 2012 | Add validation experiments and results for primer manufacturing process improvements and buffer and enzyme kit component changes. |
| H | May 2012 | • Remove Mac OS™ procedures.<br>• Add 3100, 3100-Avant, 3130, 3130xl, 3500, 3500xL Genetic Analyzer information.<br>• Add GeneMapper™ ID Software and GeneMapper™ *ID-X* Software information. |
| G | March 2012 | Change to limited licensing information. |
| F | April 2011 | Change to limited licensing information. |
| E | September 2010 | Change to limited licensing information. |
| D | August 2006 | Change to limited licensing information. |
| C | May 2005 | Change to limited licensing information. |
| B | May 2001 | Update GeneAmp™ PCR System 9700 part numbers. |
| A | May 2001 | New document. |

# Contents

# About This Guide

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

## Purpose

The Applied Biosystems *AmpFℓSTR™ Identifiler™ PCR Amplification Kit User Guide* provides information about the Applied Biosystems instruments, chemistries, and software associated with the AmpFℓSTR™ Identifiler™ PCR Amplification Kit.

# 1 Overview



## Product overview

### Purpose

The AmpFℓSTR™ Identifiler™ PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that amplifies 15 tetranucleotide repeat loci and the Amelogenin gender-determining marker in a single PCR amplification:

- All thirteen of the required loci for the Combined DNA Index System (CODIS) (Budowle *et al.*, 1998).
- Two additional loci, D2S1338 and D19S433.

### Product description

The Identifiler™ Kit contains all the necessary reagents for the amplification of human genomic DNA.

The reagents are designed for use with the following Applied Biosystems instruments:

- 3100/3100-*Avant* Genetic Analyzer
- Applied Biosystems 3130/3130*xl* Genetic Analyzer
- Applied Biosystems 3500/3500xL Genetic Analyzer
- 310 Genetic Analyzer
- GeneAmp™ PCR System 9700 with the Silver 96-Well Block
- GeneAmp™ PCR System 9700 with the Gold-plated Silver 96-Well Block
- Veriti™ 96-Well Thermal Cycler

### About the primers

The Identifiler™ Kit employs the same primer sequences for all loci common to other AmpFℓSTR™ kits (except the MiniFiler™ kit). A degenerate unlabeled primer for the D8S1179 locus was added to the AmpFℓSTR™ Identifiler™ Primer Set in order to address a mutation observed in a population of Chamorros and Filipinos from Guam (Budowle *et al.*,1998b and Budowle *et al.*, 2000). The addition of the degenerate primer allows for the amplification of those alleles in samples containing this mutation without altering the overall performance of the Identifiler™ Kit. The original validation data in this guide (Section 5.1 on page 66) were generated prior to the addition of the degenerate primer. Data showing equivalence with the degenerate primer has been published.

Non-nucleotide linkers are used in primer synthesis for the following loci: CSF1PO, D13S317, D16S539, D2S1338, and TPOX. For these primers, non-nucleotide linkers are placed between the primers and the fluorescent dye during oligonucleotide synthesis (Butler, 2005, Grossman *et al.*, 1994, and Baron *et al.*, 1996). Non-nucleotide linkers enable reproducible positioning of the alleles to facilitate interlocus spacing. The combination of a five-dye fluorescent system and the inclusion of non-nucleotide linkers allows for simultaneous amplification and efficient separation of the 15 STR loci and Amelogenin during automated DNA fragment analysis.

## Loci amplified by the kit

The following table shows the loci amplified, their chromosomal locations, and the corresponding fluorescent marker dyes. The AmpFℓSTR™ Identifiler™ Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the allelic ladder and the genotype of the AmpFℓSTR™ Control DNA 9947A are also listed in the table.

**Table 1** Identifiler™ Kit loci and alleles

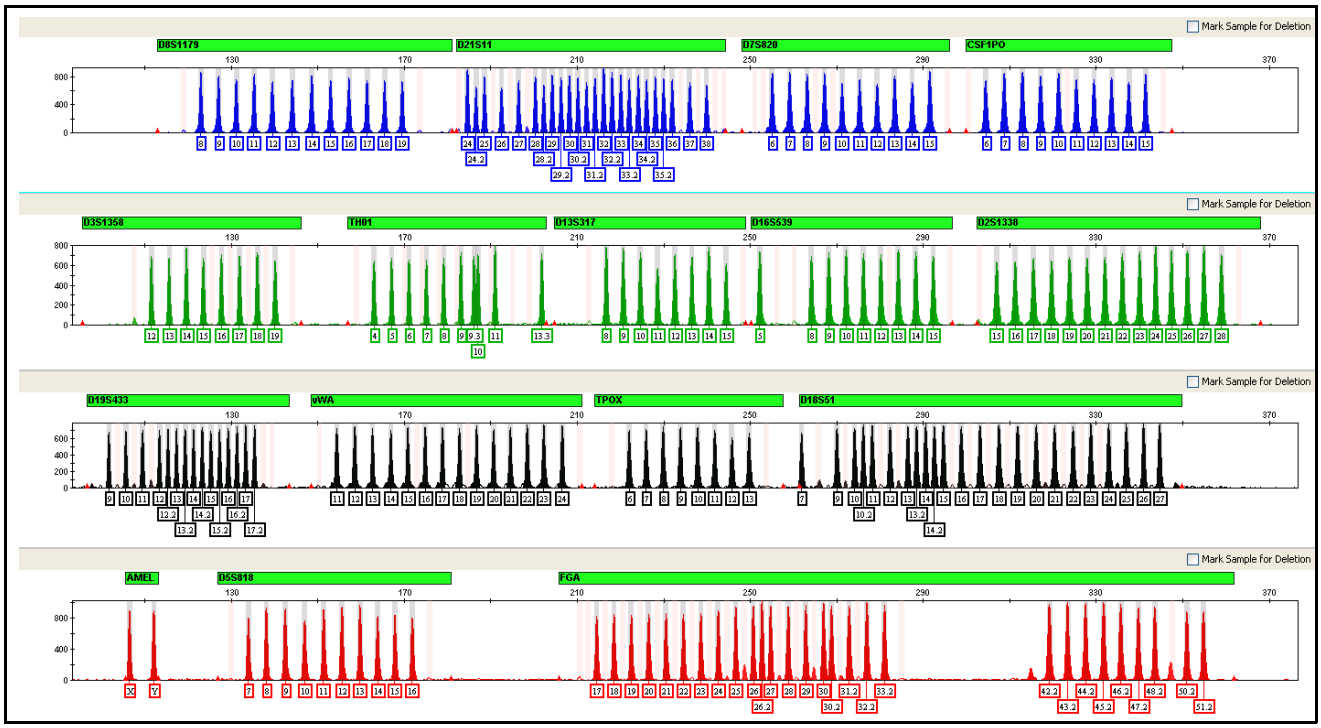
| Locus designation | Chromosome location | Alleles included in AmpFℓSTR™ Identifiler™ Allelic Ladder | Dye label | Control DNA 9947A |
|---|---|---|---|---|
| D8S1179 | 8 | 8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 | 6-FAM™ | 13† |
| D21S11 | 21q11.2-q21 | 24, 24.2, 25, 26, 27, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 37, 38 | | 30‡ |
| D7S820 | 7q11.21-22 | 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 | | 10, 11 |
| CSF1PO | 5q33.3-34 | 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 | | 10, 12 |
| D3S1358 | 3p | 12, 13, 14, 15, 16, 17, 18, 19 | VIC™ | 14, 15 |
| TH01 | 11p15.5 | 4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3 | | 8, 9.3 |
| D13S317 | 13q22-31 | 8, 9, 10, 11, 12, 13, 14, 15 | | 11§ |
| D16S539 | 16q24-qter | 5, 8, 9, 10, 11, 12,13, 14, 15 | | 11, 12 |
| D2S1338 | 2q35-37.1 | 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 | | 19, 23 |
| D19S433 | 19q12-13.1 | 9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2 | NED™ | 14, 15 |
| vWA | 12p12-pter | 11,12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 | | 17, 18 |
| TPOX | 2p23-2per | 6, 7, 8, 9, 10, 11, 12, 13 | | 8†† |
| D18S51 | 18q21.3 | 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 | | 15, 19 |
| Amelogenin | X: p22.1-22.3<br>Y: p11.2 | X, Y | PET™ | X |
| D5S818 | 5q21-31 | 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 | | 11‡‡ |
| FGA | 4q28 | 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 | | 23, 24 |

† For CODIS purposes, profile reported as 13, 13.
‡ For CODIS purposes, profile reported as 30, 30.
§ For CODIS purposes, profile reported as 11, 11.
†† For CODIS purposes, profile reported as 8, 8.
‡‡ For CODIS purposes, profile reported as 11, 11.

## Allelic ladder profile

Figure 1 shows the allelic ladder for the Identifiler™ Kit. See "Allelic ladder requirements" on page 25 for information on ensuring accurate genotyping.

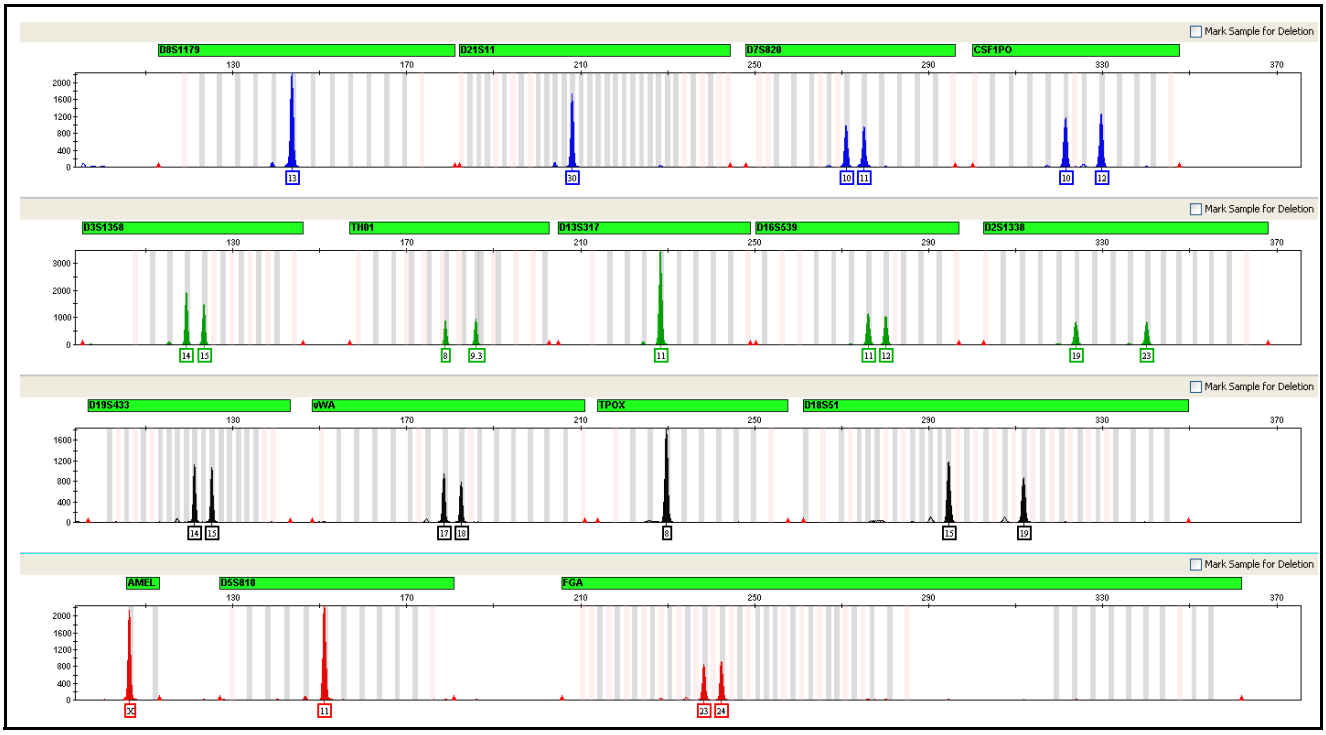
**Figure 1** GeneMapper™ *ID-X* Software plot of the AmpFℓSTR™ Identifiler™ Allelic Ladder



## Control DNA 9947A profile

Figure 2 shows amplification of Control DNA 9947A using the Identifiler™ Kit.

**Figure 2** 1 ng of Control DNA 9947A amplified with the Identifiler™ Kit and analyzed on the Applied Biosystems 3130*xl* Genetic Analyzer



# Workflow overview

**Perform PCR**

**Extract DNA**


AutoMate *Express*™ System + PrepFiler™ Express Kit

**Quantify DNA**

Quantifiler™ Duo DNA Quantification Kit

**Prepare reactions**

AmpFℓSTR™ Identifiler™ PCR Amplification Kit

**Perform PCR**

GeneAmp™ PCR System 9700 Cycler

Veriti™ 96-Well Thermal Cycler

**Perform electro-phoresis**

3100/3100-*Avant* Genetic Analyzer

3130/3130*xl* Genetic Analyzer

3500/3500xL Genetic Analyzer

310 Genetic Analyzer

**Analyze data**

GeneMapper™ *ID-X* or GeneMapper™ *ID* Software

# Instrument and software overview

This section provides information about the Data Collection Software versions required to run the AmpFℓSTR™ Identifiler™ PCR Amplification Kit on specific instruments.

## Data Collection and GeneMapper™ *ID* or *ID-X* Software

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

## Instrument and software compatibility

**Table 2** Software specific to each instrument

| Instrument | Operating system | Data Collection Software | Analysis software |
|---|---|---|---|
| 3500/3500xL | • Windows™ XP<br>• Windows Vista™ | 3500 Series Data Collection Software v1.0 | GeneMapper™ *ID-X* Software v1.2 or higher |
| 3130/3130*xl* | Windows™ XP | 3.0 | • GeneMapper™ *ID* Software v3.2.1 *and*<br>• GeneMapper™ *ID-X* Software v1.0.1 or higher |
| 3100/3100-*Avant* | Windows™ NT | 1.1 (3100)<br>1.0 (3100-*Avant*) | |
| | Windows 2000 | 2.0 | |
| 310 | Windows XP | 3.1 | |
| | • Windows™ NT<br>• Windows 2000 | 3.0 | |

**Note:** We conducted validation studies for the AmpFℓSTR™ Identifiler™ PCR Amplification Kit using the 310 Genetic Analyzer running Mac OS™. This configuration is now obsolete.

## About multicomponent analysis

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

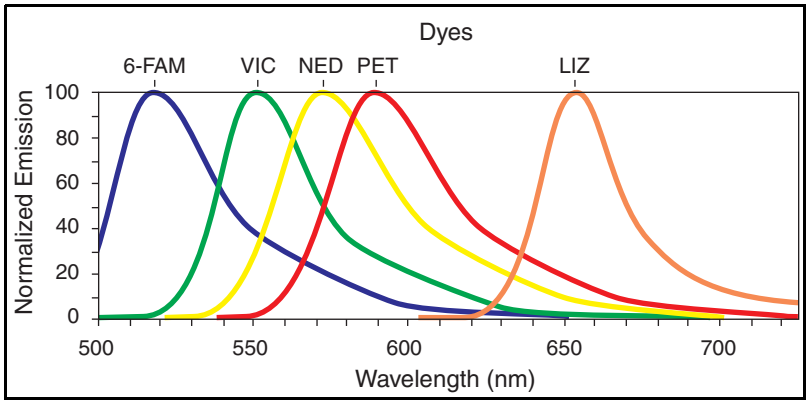
Multicomponent analysis is the process that separates the five different fluorescent dye colors into distinct spectral components. The four dyes used in the Identifiler™ Kit to label samples are 6-FAM™, VIC™, NED™, and PET™ dyes. The fifth dye, LIZ™, is used to label the GeneScan™ 500 LIZ™ Size Standard or the GeneScan™ 600 LIZ™ Size Standard v2.0.

## How multicomponent analysis works

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

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

**Figure 3** Emission spectra of the five dyes used in the AmpFℓSTR™ Identifiler™ Kit



# Materials and equipment

## Kit contents and storage

The AmpFℓSTR™ Identifiler™ PCR Amplification Kit (Part No. 4322288) contains materials sufficient to perform 200 amplifications at 25 µL/ amplification.

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

| Component | Description | 200× Volume | Storage |
|---|---|---|---|
| AmpFℓSTR™ PCR Reaction Mix | Contains $MgCl_2$, deoxynucleotide triphosphates, and bovine serum albumin in buffer with 0.05% sodium azide. | 2 tubes, 1.1 mL each | –15 to –25°C on receipt, 2 to 8°C after initial use |
| AmpFℓSTR™ Identifiler™ Primer Set | Contains fluorescently labeled primers and non-labeled primers. | 1 tube, 1.1 mL | |
| AmpFℓSTR™ Identifiler™ Allelic Ladder | Contains amplified alleles.<br>See Table 1 on page 12 for a list of alleles included in the allelic ladder. | 1 tube, 0.05 mL | |
| AmpliTaq Gold™ DNA Polymerase | Contains enzyme, with an activity of 5 U/µL. | 2 tubes, 0.05 mL/tube | –15 to –25°C |
| AmpFℓSTR™ Control DNA 9947A | Contains 0.10 ng/µL human female 9947A DNA in 0.05% sodium azide and buffer†.<br>See Table 1 on page 12 for profile. | 1 tube, 0.3 mL | 2 to 8 °C |

† The AmpFℓSTR™ Control DNA 9947A is included at a concentration appropriate to its intended use as an amplification control (i.e., to provide confirmation of the capability of the kit reagents to generate a profile of expected genotype). The AmpFℓSTR™ Control DNA 9947A is not designed to be used as a DNA quantitation control, and you may see variation from the labelled concentration when quantitating aliquots of the AmpFℓSTR™ Control DNA 9947A.

## Standards for samples

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

- **AmpFℓSTR™ Control DNA 9947A** – A positive control for evaluating the efficiency of the amplification step and STR genotyping using the AmpFℓSTR™ Identifiler™ Allelic Ladder.
- **GeneScan™ 500 LIZ™ Size Standard or GeneScan™ 600 LIZ™ Size Standard v2.0** – Used for obtaining sizing results. These standards, which have been evaluated as internal size standards, yield precise sizing results for Identifiler™ Kit PCR products. Order the GeneScan™ 500 LIZ™ Size Standard (Part No. 4322682) or the GeneScan™ 600 LIZ™ Size Standard v2.0 (Part No. 4408399) separately.
- **AmpFℓSTR™ Identifiler™ Allelic Ladder** – Allelic ladder developed by Life Technologies for accurate characterization of the alleles amplified by the Identifiler™ Kit. The AmpFℓSTR™ Identifiler™ Allelic Ladder contains most of the alleles reported for the 15 autosomal loci. Refer to Table 1 on page 12 for a list of the alleles included in the AmpFℓSTR™ Identifiler™ Allelic Ladder.

# 2 Perform PCR



## Required user-supplied reagents

In addition to the Identifiler™ Kit reagents, the use of low-TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) is recommended. You can prepare the buffer as described in the procedure below or order it from Teknova (Cat No. T0223).

To prepare low-TE buffer:

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

   **Note:** Adjust the volumes accordingly for specific needs.
2. Aliquot and autoclave the solutions.
3. Store at room temperature.

## DNA quantification

### Importance of quantification

Quantifying the amount of DNA in a sample before amplification allows you to determine whether or not sufficient DNA is present to permit amplification and to calculate the optimum amount of DNA to add to the reaction. The optimum amount of DNA for the Identifiler™ Kit is 1.0 ng in a maximum input volume of 10 µL for 28 PCR cycles.

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

- Fluorescence intensity that exceeds the linear dynamic range for detection by the instrument ("off-scale" data). Off-scale data are problematic because:
  - Quantitation (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause the corresponding stutter peak to appear higher in relative intensity, thus increasing the calculated percent stutter.
  - Multicomponent analysis of off-scale data is not accurate, and it results in poor spectral separation ("pull-up").
- Incomplete A-nucleotide addition.

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

## Methods of quantifying DNA

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

| Product | Description |
|---|---|
| Quantifiler™ Human DNA Quantification Kit (Part No. 4343895)<br>*and*<br>Quantifiler™ Y Human Male DNA Quantification Kit (Part No. 4343906)<br>For more information, see *Quantifiler™ Human DNA Quantification Kits User's Manual* (Pub. No. 4344790) | **Properties:**<br>The Quantifiler™ Human and Quantifiler™ Y Human Male Kits are highly specific for human DNA, and they individually detect total human or male DNA, respectively. The kits detect single-stranded and degraded DNA.<br>**How they work:**<br>The Quantifiler™ DNA Quantification Kits consist of target-specific and internal control 5' nuclease assays.<br>The Quantifiler™ Human and Quantifiler™ Y Human Male Kits contain different target-specific assays (human DNA or human male DNA, respectively) that each consist of two locus-specific PCR primers and one TaqMan™ MGB probe labeled with FAM™ dye for detecting the amplified sequence. The kits each contain a separate internal PCR control (IPC) assay, which consists of an IPC template DNA (a synthetic sequence not found in nature), two primers for amplifying the IPC template, and one TaqMan™ MGB probe labeled with VIC™ dye for detecting the amplified IPC. |
| Quantifiler™ Duo DNA Quantification Kit (Part No. 4387746)<br>For more information, see *Quantifiler™ Duo DNA Quantification Kit User's Manual* (Pub. No. 4391294) | **Properties:**<br>The Quantifiler™ Duo Kit is highly specific for human DNA. This kit combines the detection of both total human and male DNA in one PCR reaction.The kit detects single-stranded and degraded DNA.<br>**How it works:**<br>The Quantifiler™ Duo DNA Quantification Kit consists of target-specific and internal control 5' nuclease assays.<br>The Quantifiler™ Duo kit combines two human-specific assays in one PCR reaction (for total human DNA and human male DNA). The two human DNA specific assays each consist of two PCR primers and a TaqMan™ probe. The TaqMan™ probes for the human DNA and human male DNA assays are labeled with VIC™ and FAM™ dyes, respectively. In addition, the kit contains an internal PCR control (IPC) assay similar in principle to that used in the other Quantifiler kits, but labeled with NED™ dye. |

# Prepare the amplification kit reactions

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

| DNA sample | Volume per reaction |
|---|---|
| AmpFℓSTR™ PCR Reaction Mix | 10.5 µL |
| AmpliTaq Gold™ DNA Polymerase | 0.5 µL |
| AmpFℓSTR™ Identifiler™ Primer Set | 5.5 µL |

**Note:** The volumes indicated above include a slight overfill to account for the loss that occurs during reagent transfers.

2. Prepare reagents. Thaw the PCR Reaction Mix and the Identifiler™ Primer Set, then vortex all reagent tubes, including the enzyme, for 3 seconds and centrifuge briefly before opening the tubes.

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

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

| DNA sample | To prepare... |
|---|---|
| Negative control | Add 10 µL of low-TE buffer (10mM Tris, 0.1mM EDTA, pH 8.0). |
| Test sample | Dilute a portion of the test DNA sample with low-TE buffer so that 1.0 ng of total DNA is in a final volume of 10 µL. Add 10 µL of the diluted sample to the reaction mix. |
| Positive control | Add 10 µL of 9947A control DNA (0.1 ng/µL). |

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

7. Seal the plate with MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical Adhesive Film, or cap the tubes.
8. Centrifuge the tubes at 3000 rpm for about 20 seconds in a tabletop centrifuge (with plate holders if using 96-well plates).
9. Amplify the samples in a GeneAmp™ PCR System 9700 with the silver or gold-plated silver 96-well blocks or a Veriti™ 96-Well Thermal Cycler.
**Note:** The Identifiler™ Kit is not validated for use with the GeneAmp PCR System 9700 with the aluminium 96-well block. Use of this thermal cycling platform may adversely affect performance of the Identifiler™ Kit.

# Perform PCR

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

| Initial incubation step | Denature | Anneal | Extend | Final extension | Final hold |
|---|---|---|---|---|---|
| HOLD | CYCLE (28) | | | HOLD | HOLD |
| 95°C<br>11 min | 94°C<br>1 min | 59°C<br>1min | 72°C<br>1min | 60°C<br>60 min | 4–25°C<br>∞ |

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

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

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

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

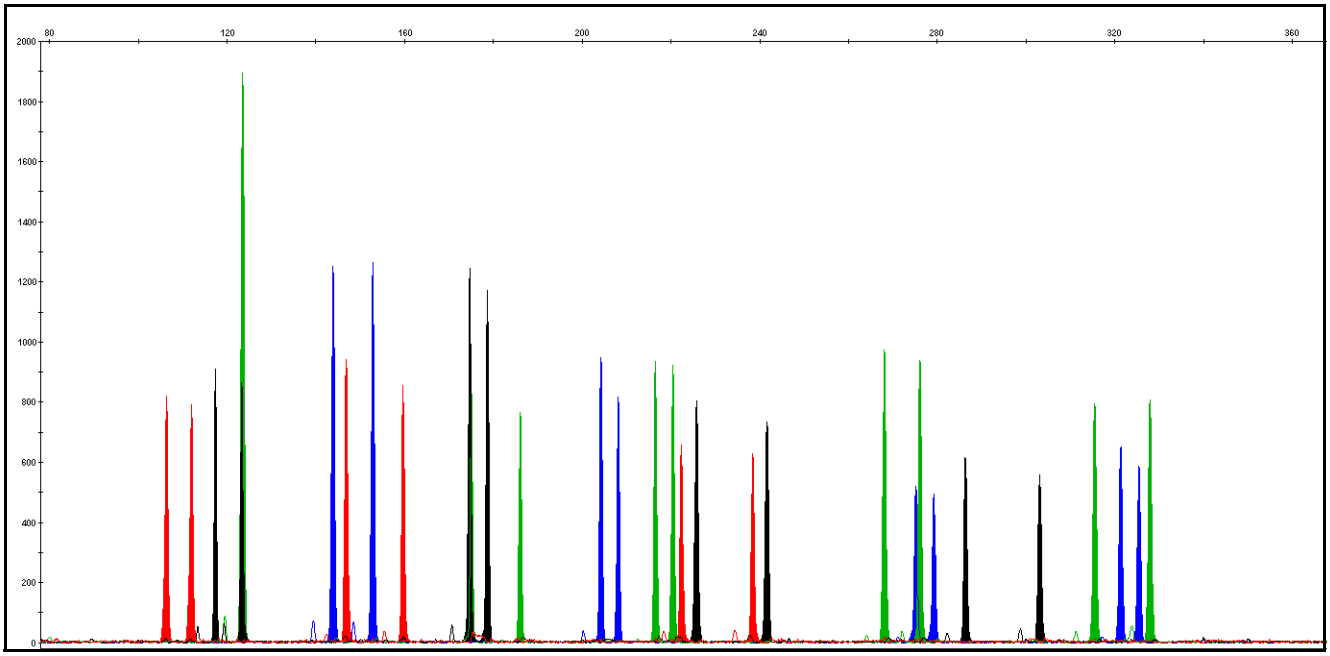
**IMPORTANT!** Store the amplified products so that they are protected from light.

# Amplification using bloodstained FTA™ cards

FTA™ cards can be useful for collecting, storing, and processing biological samples. A small punch disc of the card containing the sample can be placed directly into an amplification tube, purified, and amplified, without transferring the disc. Our studies indicate that a 1.2-mm bloodstained disc contains approximately 5–20 ng DNA. An appropriate cycle number for this high quantity of DNA is 25 cycles as determined by our validation studies. However, it is recommended that each laboratory determine the optimum cycle number based on internal validation studies.

In the example shown in Figure 4, a 1.2-mm disc of a bloodstained FTA card was purified using three washes with FTA Purification Reagent and two washes with 1X low-TE buffer. The purified punch disc was then amplified in the MicroAmp™ tube for 25 cycles.

**Figure 4** AmpFℓSTR™ Identifiler™ Kit PCR Amplification Kit results from a 1.2-mm FTA bloodstain disc (25-cycle amplification), analyzed on the Applied Biosystems 3130*xl* Genetic Analyzer



# 3 Perform Electrophoresis



## Allelic ladder requirements

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

| Instrument | Number of allelic ladders to run | One injection equals | Number of samples per allelic ladder(s) |
|---|---|---|---|
| 3100-*Avant* or 3130 | 1 per 4 injections | 4 samples | 15 samples + 1 allelic ladder |
| 3100 or 3130*xl* | 1 per injection | 16 samples | 15 samples + 1 allelic ladder |
| 3500 | 1 per 3 injections | 8 samples | 23 samples + 1 allelic ladder |
| 3500xL | 1 per injection | 24 samples | 23 samples + 1 allelic ladder |
| 310 | 1 per 10 injections | 1 sample | 9 samples + 1 allelic ladder |

**IMPORTANT!** Variation in laboratory temperature can cause changes in fragment migration speed and sizing variation between both single- and multiple-capillary runs (with larger size variations seen between samples injected in multiple-capillary runs). We recommend the above frequency of allelic ladder injections, which should account for normal variation in run speed. However, during internal validation studies, verify the required allelic ladder injection frequency to ensure accurate genotyping of all samples in your laboratory environment.

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

# Section 3.1 3100/3100-*Avant* and 3130/3130*xl* instruments

## Set up the 3100/3100-*Avant* or 3130/3130*xl* instrument for electrophoresis

### Reagents and parts

"Ordering Information" on page 121 lists the required materials not supplied with the AmpFℓSTR™ Identifiler™ PCR Amplification 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. Keep freeze-thaw cycles to a minimum.

### Electrophoresis software setup and reference documents

The following table lists Data Collection Software and the run modules that can be used to analyze Identifiler™ Kit PCR products. For details on the procedures, refer to the documents listed in the table.

| Genetic Analyzer | Data Collection Software | Operating System | Run modules and conditions | References |
|---|---|---|---|---|
| Applied Biosystems 3130/3130*xl* | 3.0 | Windows™ XP | • HIDFragmentAnalysis36_POP4_1<br>Injection conditions:<br>– 3130 = 3 kV/5 sec<br>– 3130*xl* = 3 kV/10 sec<br>• Dye Set G5 | *Applied Biosystems 3130/3130xl Genetic Analyzers Using Data Collection Software v3.0, Protocols for Processing AmpFℓSTR™ PCR Amplification Kit PCR Products User Bulletin* (Pub. No. 4363787) |
| 3100 | 2.0 | Windows™ 2000 | • HIDFragmentAnalysis36_POP4_1<br>Injection condition: 3kV/10 sec<br>• Dye Set G5 | *3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0, Protocols for Processing AmpFℓSTR™ PCR Amplification Kit PCR Products User Bulletin* (Pub. No. 4350218) |
| | 1.1 | Windows™ NT | • GeneScan36vb_DyeSetG5Module<br>Injection condition: 3kV/10 sec<br>• GS600v2.0Analysis.gsp | *3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR™ PCR Amplification Kit PCR Products User Bulletin* (Pub. No. 4332345) |
| 3100-*Avant* | 1.0 | Windows™ NT | • GeneScan36Avb_DyeSetG5Module<br>Injection condition: 3 kV/5sec<br>• GS600v2.0Analysis.gsp | *3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR™ PCR Amplification Kit PCR Products User Bulletin* (Pub. No. 4332345) |

# Prepare samples for electrophoresis on the 3100/3100-*Avant* or 3130/3130*xl* instrument

Prepare the samples for electrophoresis immediately before loading.

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

| Reagent | Volume per reaction | | Reagent | Volume per reaction |
|---|---|---|---|---|
| GeneScan™ 500 LIZ™ Size Standard | 0.3 µL | *OR* | GeneScan™ 600 LIZ™ Size Standard v2.0 | 0.5 µL |
| Hi-Di™ Formamide | 8.7 µL | | Hi-Di™ Formamide | 8.5 µL |

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

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

2. Pipette the required volumes of components into an appropriately sized polypropylene tube.
3. Vortex the tube, then centrifuge briefly.
4. Into each well of a MicroAmp™ Optical 96-Well Reaction Plate, add:
   - 9 µL of the formamide:size standard mixture
   - 1 µL of PCR product or allelic ladder

   **Note:** For blank wells, add 10 µL of Hi-Di™ Formamide.
5. Seal the reaction plate with appropriate septa, then centrifuge the plate to ensure that the contents of each well are collected at the bottom.
6. Heat the reaction plate in a thermal cycler for 3 minutes at 95°C.
7. Immediately place the plate on ice for 3 minutes.
8. Prepare the plate assembly, then place on the autosampler.
9. Ensure that a plate record is completed and link the plate record to the plate.
10. Start the electrophoresis run.

# Section 3.2 3500/3500xL Series instruments

## Set up the 3500/3500xL instrument for electrophoresis

### Reagents and parts

"Ordering Information" on page 121 lists the required materials not supplied with the AmpFℓSTR™ Identifiler™ PCR Amplification 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. Keep freeze-thaw cycles to a minimum.

### Electrophoresis software setup and reference documents

The following table lists Data Collection Software and the run modules that can be used to analyze Identifiler™ Kit PCR products. For details on the procedures, refer to the documents listed in the table.

| Genetic Analyzer | Data Collection Software | Operating System | Run modules and conditions | References |
|---|---|---|---|---|
| Applied Biosystems 3500 | 3500 Data Collection Software v1.0 | Windows™ XP *or* Windows Vista ™ | • HID36_POP4<br>Injection conditions: 1.2kV/15 sec<br>• Dye Set G5 | *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Pub. No. 4401661)<br>*Applied Biosystems 3500 and 3500xL Genetic Analyzers Quick Reference Card* (Pub. No. 4401662) |
| Applied Biosystems 3500xL | | | • HID36_POP4<br>Injection conditions: 1.2kV/24 sec<br>• Dye Set G5 | |

## Prepare samples for electrophoresis on the 3500/3500xL instrument

Prepare the samples for electrophoresis immediately before loading.

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

| Reagent | Volume per reaction |
|---|---|
| GeneScan™ 600 LIZ™ Size Standard v2.0 | 0.5 µL |
| Hi-Di™ Formamide | 8.5 µL |

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

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

2. Pipette the required volumes of components into an appropriately sized polypropylene tube.
3. Vortex the tube, then centrifuge briefly.
4. Into each well of a MicroAmp™ Optical 96-Well Reaction Plate, or each MicroAmp™ optical strip tube, add:
   - 9 µL of the formamide:size standard mixture
   - 1 µL of PCR product or allelic ladder

   **Note:** For blank wells, add 10 µL of Hi-Di™ Formamide.
5. Seal the reaction plate or strip tubes with the appropriate septa, then centrifuge to ensure that the contents of each well are collected at the bottom.
6. Heat the reaction plate or strip tubes in a thermal cycler for 3 minutes at 95°C.
7. Immediately put the plate or strip tubes on ice for 3 minutes.
8. Prepare the plate assembly, then place on the autosampler.
9. Ensure that a plate record is completed and link the plate record to the plate.
10. Start the electrophoresis run.

# Section 3.3 310 Instrument

## Set up the 310 instrument for electrophoresis

### Reagents and parts

"Ordering Information" on page 121 lists the required materials not supplied with the AmpFℓSTR™ Identifiler™ PCR Amplification 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. Keep freeze-thaw cycles to a minimum.

### Electrophoresis software setup and reference documents

The following table lists Data Collection Software and the run modules that can be used to analyze Identifiler™ Kit PCR products. For details on the procedures, refer to the documents listed in the table.

| Data Collection Software | Operating System | Run modules and conditions | References |
|---|---|---|---|
| 3.1† *or* 3.0† | Windows XP *or* Windows™ NT and Windows 2000 | • GS STR POP4 (1mL) G5 v2.md5<br>Injection condition:<br>15 kV/5 sec | *310 Genetic Analyzer User's Manual (Windows)* (Pub. No. 4317588)<br>*310 Protocols for Processing AmpFℓSTR™ PCR Amplification Kit Products with Microsoft Windows NT Operating System: User Bulletin* (Pub. No. 4341742) |

† We conducted concordance studies for the Identifiler™ Kit using this configuration.

## Prepare samples for electrophoresis on the 310 instrument

Prepare the samples for electrophoresis immediately before loading.

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

| Reagent | Volume per reaction |
|---|---|
| GeneScan™ 500 LIZ™ Size Standard or GeneScan™ 600 LIZ™ Size Standard | 0.75 µL |
| Hi-Di™ Formamide | 24.5 µL |

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

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

2. Pipette the required volumes of components into an appropriately sized polypropylene tube.
3. Vortex the tube, then centrifuge briefly.
4. Into each 0.2 mL sample tube, add:
   - 25 µL of the formamide:size standard mixture
   - 1.5 µL of PCR product or allelic ladder

   **Note:** For blank wells, add 25 µL of Hi-Di™ Formamide.
5. Seal the tubes with the appropriate septa, then briefly centrifuge to ensure that the contents of each tube are mixed and collected at the bottom.
6. Heat the tubes in a thermal cycler for 3 minutes at 95°C.
7. Immediately place the tubes on ice for 3 minutes.
8. Place the sample tray on the autosampler.
9. Ensure that an injection list is prepared.
10. Start the electrophoresis run.

# 4 Analyze Data



# Section 4.1 GeneMapper™ *ID* Software

## Overview of GeneMapper™ *ID* Software

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

After electrophoresis, the data collection software stores information for each sample in a .fsa file. Using GeneMapper™ *ID* Software v3.2.1 software, you can then analyze and interpret the data from the .fsa files.

### Instruments

Refer to "Instrument and software overview" on page 16 for a list of compatible instruments.

### Before you start

When using GeneMapper™ *ID* Software v3.2.1 to perform human identification (HID) analysis with AmpFℓSTR™ kits, be aware that:

- HID analysis requires at least one allelic ladder sample per run folder. Your laboratory can use multiple ladder samples in an analysis, provided individual laboratories conduct the appropriate validation studies.
  For multiple ladder samples, the GeneMapper™ *ID* Software calculates allelic bin offsets by using an average of all ladders that use the same panel within a run folder.
- Allelic ladder samples in an individual run folder are considered to be from a single run.
  When the software imports multiple run folders into a project, only the ladder(s) within their respective run folders are used for calculating allelic bin offsets and subsequent genotyping.
- Allelic ladder samples must be labeled as "Allelic Ladder" in the Sample Type column in a project. Failure to apply this setting for ladder samples results in failed analysis.
- Injections containing the allelic ladder must be analyzed with the same analysis method and parameter values that are used for samples to ensure proper allele calling.
- Alleles that are not in the AmpFℓSTR™ Allelic Ladders do exist. Off-ladder (OL) alleles may contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the ±0.5-nt bin window of any known allelic ladder allele or virtual bin.
  **Note:** If a sample allele peak is called as an off-ladder allele, the sample result needs to be verified according to the laboratory's protocol.

## Set up GeneMapper™ *ID* Software for data analysis

### File names

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

### Before using the software for the first time

Before you can analyze sample (.fsa) files using GeneMapper™ *ID* Software v3.2.1 for the first time, you need to:

- Import panels and bins into the Panel Manager, as explained in "Import panels and bins" on page 35.
- Create an analysis method, as explained in , "Create an analysis method" on page 39.
- Create a size standard, as explained in "Create size standard" on page 44.
- Define custom views of analysis tables.
  Refer to Chapter 1 of the *GeneMapper™ ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (Pub. No. 4335523) for more information.
- Define custom views of plots.
  Refer to Chapter 1 of the *GeneMapper™ ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (Pub. No. 4335523) for more information.

## Import panels and bins

To import the Identifiler™ panel and bin set into the GeneMapper™ *ID* Software v3.2.1 database:

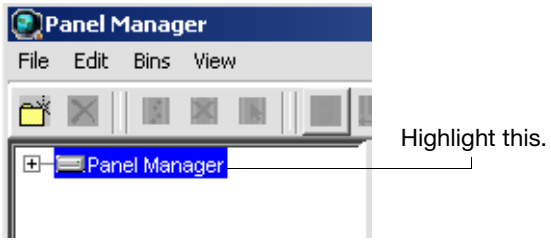
1. Start the GeneMapper™ *ID* Software, then log in with the appropriate user name and password.

   **IMPORTANT!** If you need logon instructions, refer to page 2-7 of the *GeneMapper™ ID Software Version 3.1 Human Identification Analysis User Guide* (Pub. No. 4338775).

2. Select **Tools** ▸ **Panel Manager**.

3. Find, then open the folder containing the panels and bins:

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

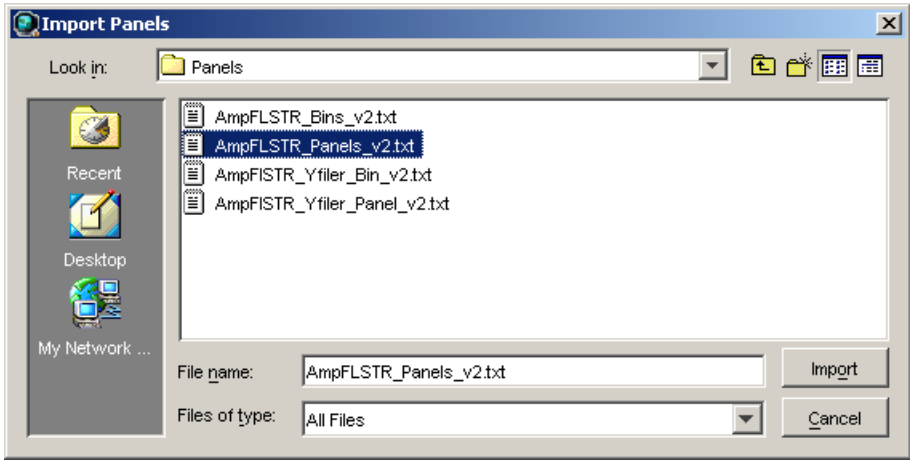



   b. Select **File** ▸ **Import Panels** to open the Import Panels dialog box.

   c. Navigate to, then open the ***x***:\Applied Biosystems\GeneMapper\Panels folder, where *x* is the drive on which the GeneMapper™ *ID* Software is installed.

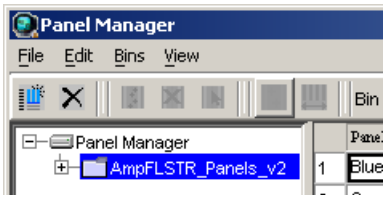
4. Select **AmpFLSTR_Panels_v2.txt**, then click **Import**.

   **Note:** Importing this file creates a new folder in the navigation pane of the Panel Manager, AmpFLSTR_Panels_v2. This folder contains the panels and associated markers.




5. Import AmpFLSTR_Bins_v2.txt:

   a. Select the **AmpFLSTR_Panels_v2** folder in the navigation pane.

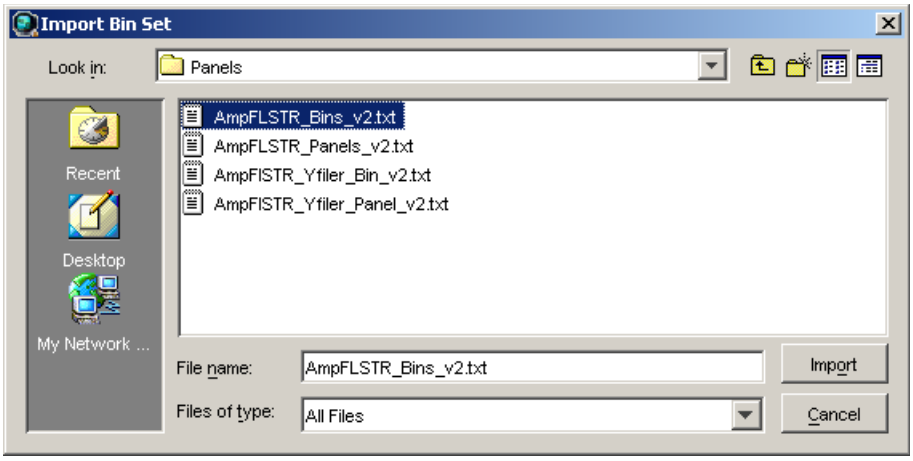



b. Select **File ▸ Import Bin Set** to open the Import Bin Set dialog box.

c. Navigate to, then open the ***x***:\Applied Biosystems\GeneMapper\Panels folder.

d. Select **AmpFLSTR_Bins_v2.txt**, then click **Import**.

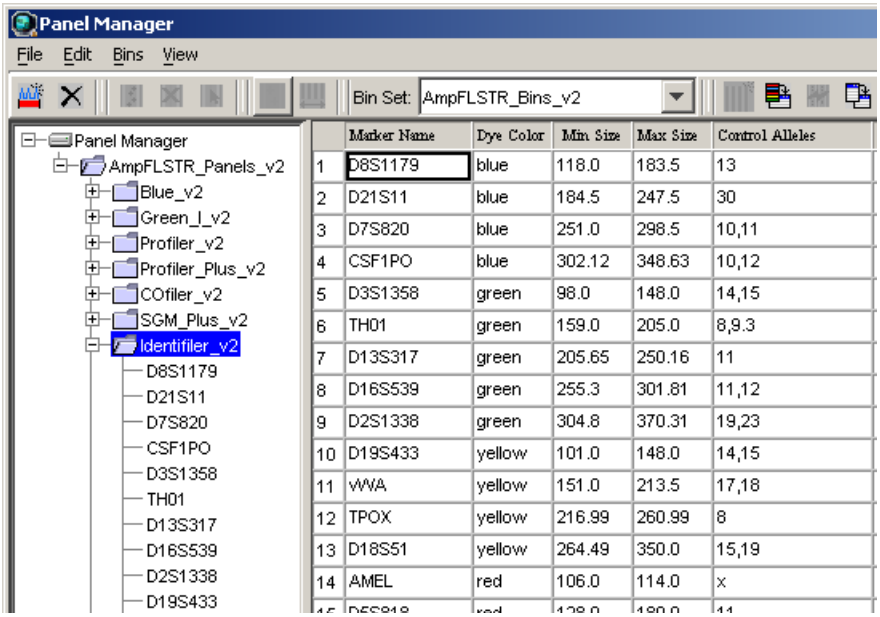
**Note:** Importing this file associates the bin set with the panels in the AmpFLSTR_Panels_v2 folder.




6. View the imported panels in the navigation pane:

   a. Double-click the **AmpFLSTR_Panels_v2** folder.

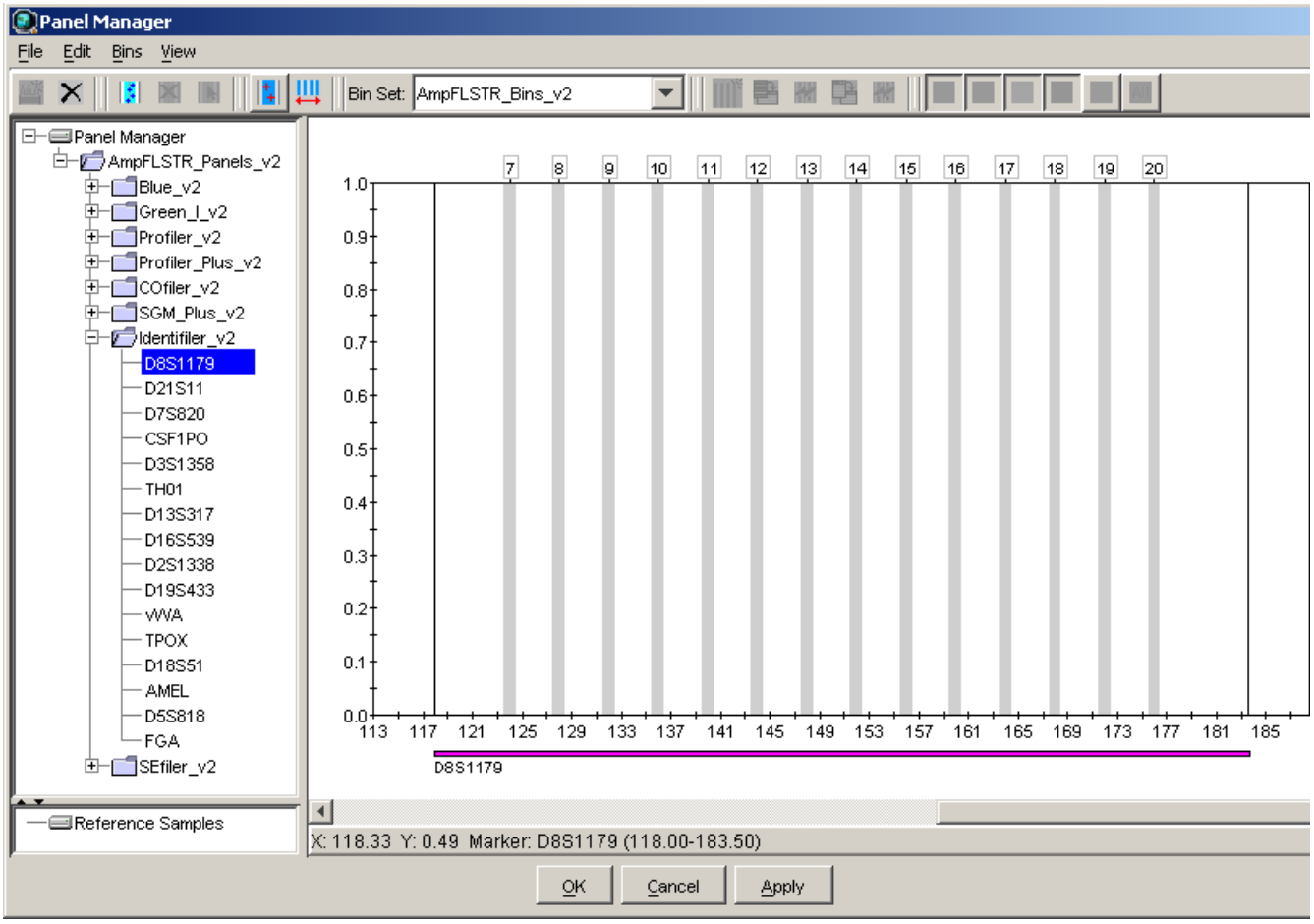
b. Double-click the **Identifiler_v2** folder to display the panel information in the right pane and the markers below it.




| | Marker Name | Dye Color | Min Size | Max Size | Control Alleles |
|---|---|---|---|---|---|
| 1 | D8S1179 | blue | 118.0 | 183.5 | 13 |
| 2 | D21S11 | blue | 184.5 | 247.5 | 30 |
| 3 | D7S820 | blue | 251.0 | 298.5 | 10,11 |
| 4 | CSF1PO | blue | 302.12 | 348.63 | 10,12 |
| 5 | D3S1358 | green | 98.0 | 148.0 | 14,15 |
| 6 | TH01 | green | 159.0 | 205.0 | 8,9.3 |
| 7 | D13S317 | green | 205.65 | 250.16 | 11 |
| 8 | D16S539 | green | 255.3 | 301.81 | 11,12 |
| 9 | D2S1338 | green | 304.8 | 370.31 | 19,23 |
| 10 | D19S433 | yellow | 101.0 | 148.0 | 14,15 |
| 11 | vWA | yellow | 151.0 | 213.5 | 17,18 |
| 12 | TPOX | yellow | 216.99 | 260.99 | 8 |
| 13 | D18S51 | yellow | 264.49 | 350.0 | 15,19 |
| 14 | AMEL | red | 106.0 | 114.0 | X |

7. View the markers and display the Bin view in the navigation pane:
   a. Select the **AmpFLSTR_Panels_v2** folder to display its list of kits in the right pane.
   b. Double-click the **Identifiler_v2** folder to display its list of markers below it.

c. Select **D8S1179** to display the Bin view for the marker in the right pane.




8. Click **Apply**, then **OK** to add the AmpF*l*STR™ panel and bin set to the GeneMapper™ *ID* Software database.

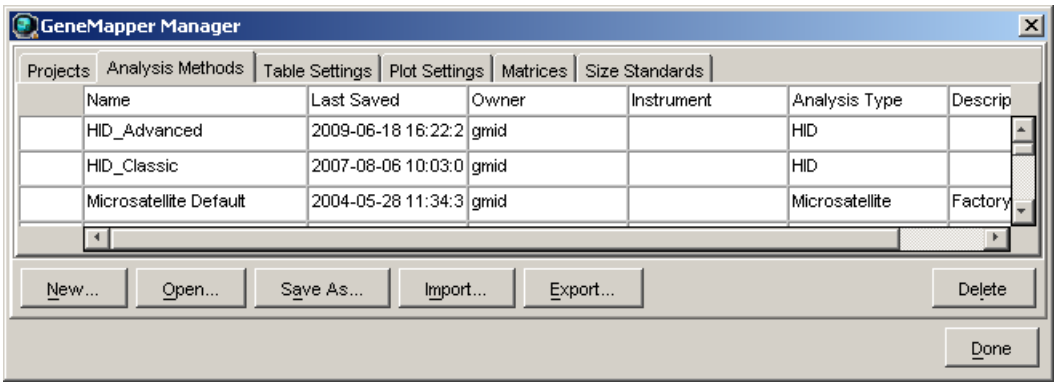
**IMPORTANT!** If you close the Panel Manager without clicking OK, the panels and bins are not imported into the GeneMapper™ *ID* Software database.

## Create an analysis method

The HID Advanced analysis method for the Identifiler™ Kit uses the AmpFLSTR_Bins_v2 file described in step 5 on page 35.

Use the following procedure to create a HID analysis method for the Identifiler™ Kit.

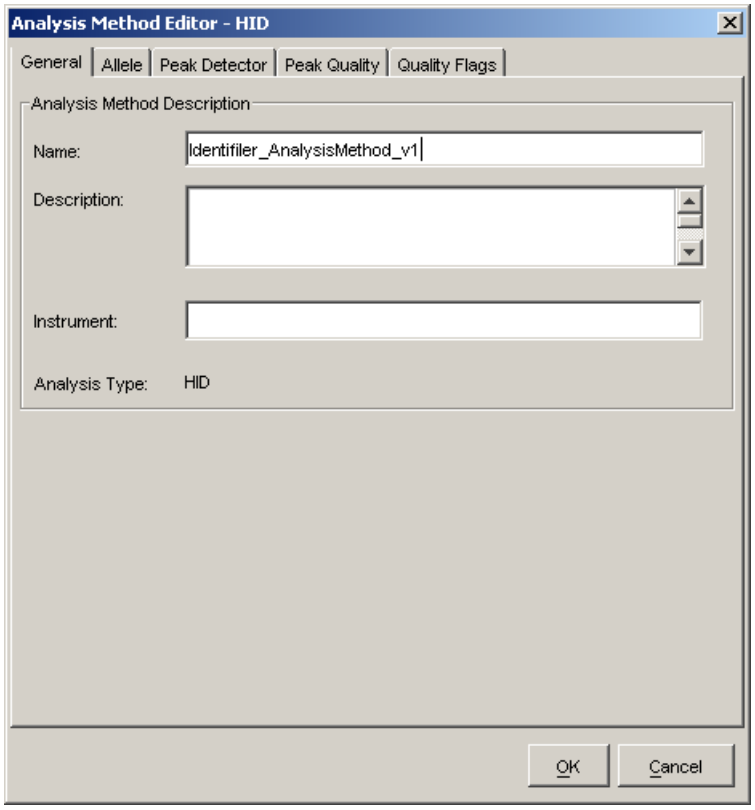
1. Select **Tools** ▸ **GeneMapper Manager** to open the GeneMapper Manager.




2. Select the **Analysis Methods** tab, then click **New** to open the New Analysis Method dialog box.
3. Select **HID** and click **OK** to open the Analysis Method Editor with the General Tab selected.
4. Enter the settings in each tab of the Analysis Method Editor as shown in the figures below unless the instructions state otherwise.

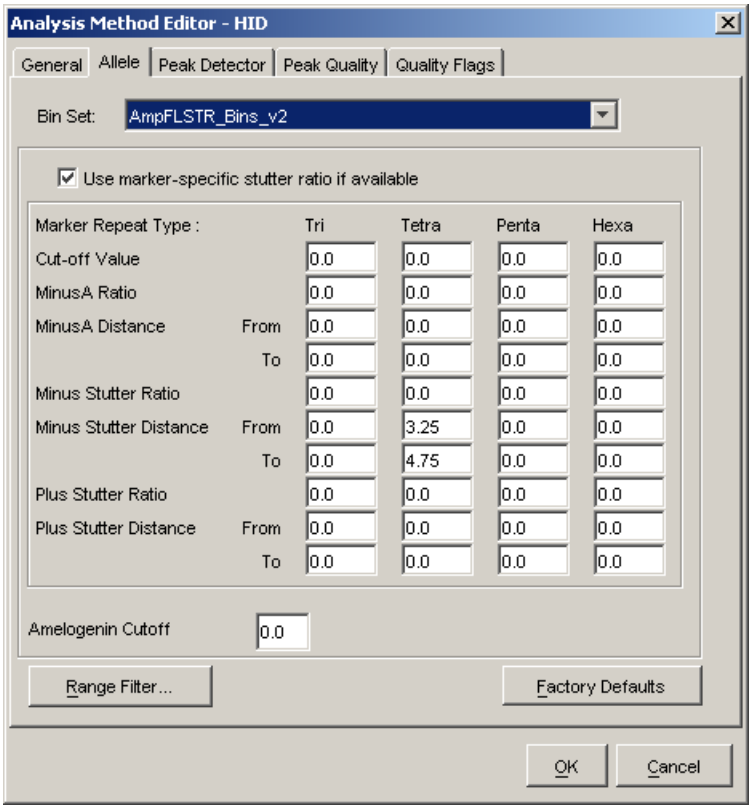
   **Note:** The Analysis Method Editor closes when you save your settings. To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.
5. After you enter settings in all tabs, click **Save**.

## General tab settings



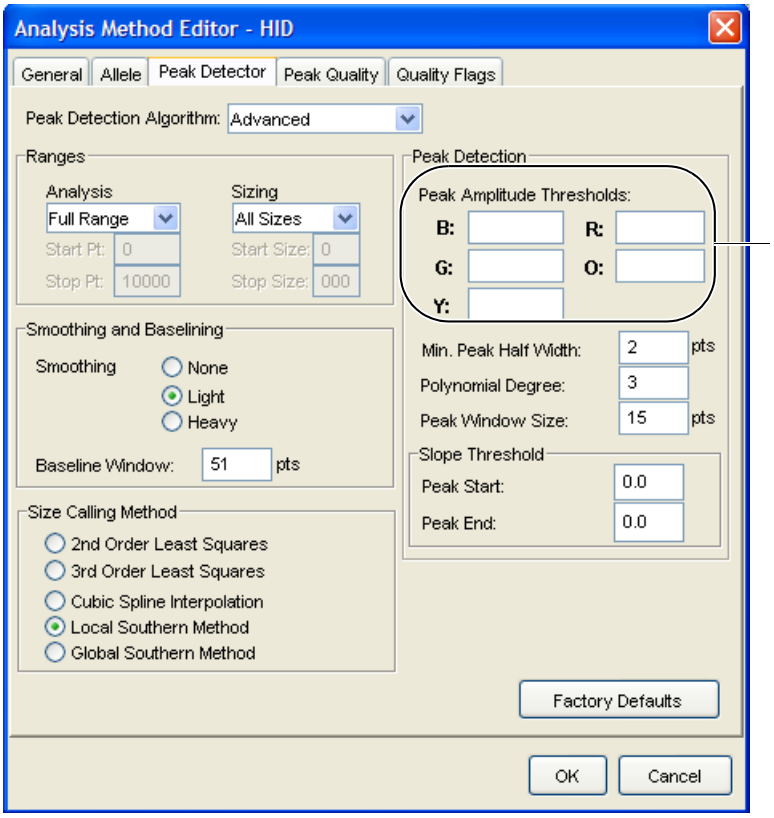

In the Name field, either type the name as shown, or enter a name of your choosing. The Description and Instrument fields are optional.

## Allele tab settings




- In the Bin Set field, select the **AmpFLSTR_Bins_v2** bin set imported previously and configure the stutter distance parameters as shown.
- GeneMapper™ *ID* Software v3.2.1 allows you to specify four types of marker repeat motifs: tri, tetra, penta, and hexa. You can enter parameter values for each type of repeat in the appropriate column.
- Specify the stutter ratio:
  - To apply the stutter ratios listed in the Allele tab for single-source data, deselect the "Use marker-specific stutter ratio if available" check box (selected by default). Perform appropriate internal validation studies to determine the appropriate filter setting to use.
    **Note:** Applying global stutter ratios may reduce the editing required for single-source sample data.
  - To apply the stutter ratios contained in the AmpFLSTR_Panels_v2.txt file, select the "Use marker-specific stutter ratio if available" check box (selected by default). Perform appropriate internal validation studies to determine the appropriate filter setting to use.

## Peak Detector tab settings

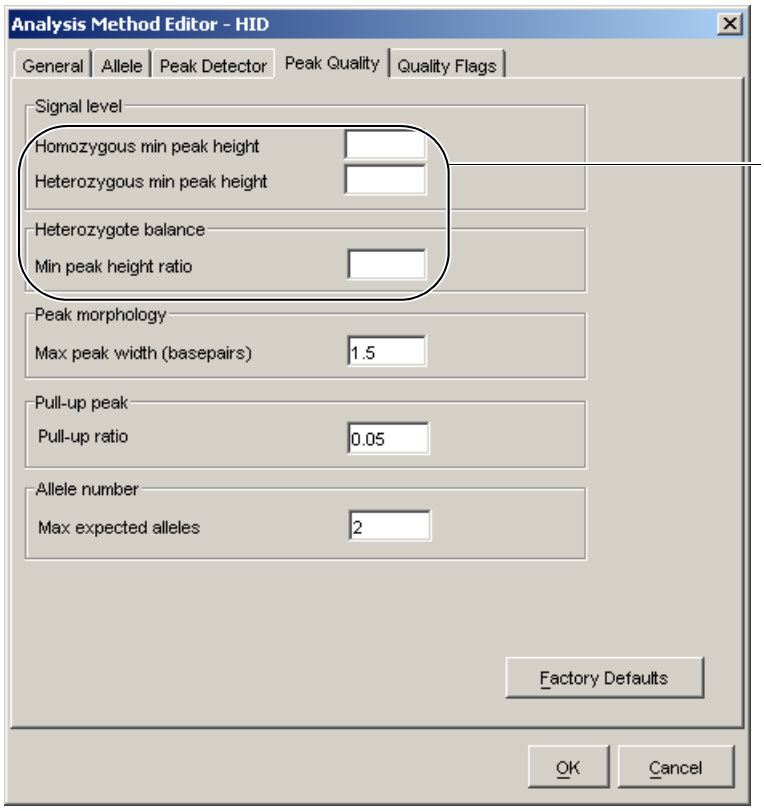



**IMPORTANT!** Perform the appropriate internal validation studies to determine the peak amplitude thresholds for interpretation of Identifiler™ Kit data.

Fields include:

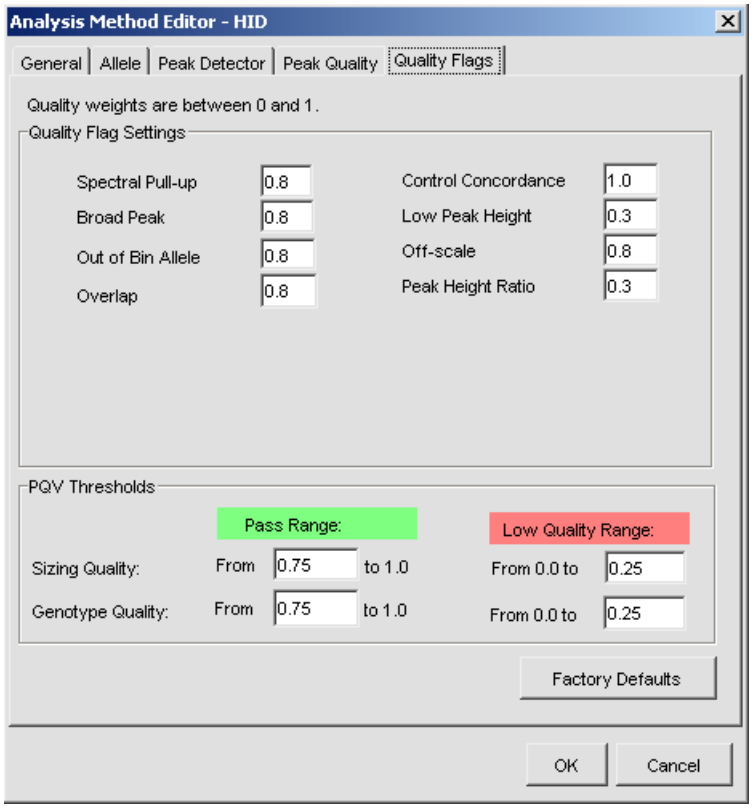
- **Peak amplitude thresholds** – The software uses these parameters to specify the minimum peak height, in order to limit the number of detected peaks. Although GeneMapper™ *ID* Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the genotype of these peaks.
- **Size calling method** – The Identifiler™ Kit has been validated using the Local Southern sizing method. Before using other sizing methods, perform internal validation studies.

## Peak Quality tab settings




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

## Quality Flags tab settings




**IMPORTANT!** The values shown are the software defaults and are the values we used during developmental validation. Perform the appropriate internal validation studies to determine the appropriate values for interpretation of Identifiler™ Kit data.

## Create size standard

The size standards for the Identifiler™ Kit use the following size standard peaks in their definitions:

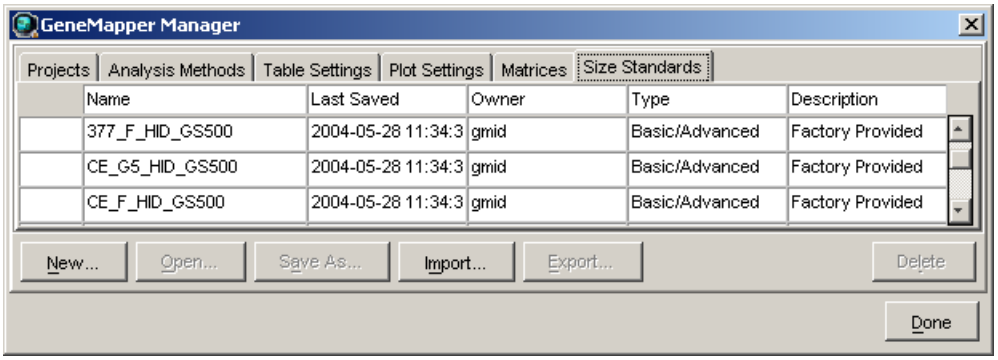
| GeneScan™ 500 LIZ™ Size Standard | GeneScan™ 600 LIZ™ Size Standard v2.0 |
|---|---|
| 75, 100, 139, 150, 160, 200, 300, 340, 350, 400, and 450 | 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440 and 460 |

**Note:** The 250-nt peak in the GeneScan™ 500 LIZ™ Size Standard is not included in the size standard definition. This peak can be used as an indicator of precision within a run.

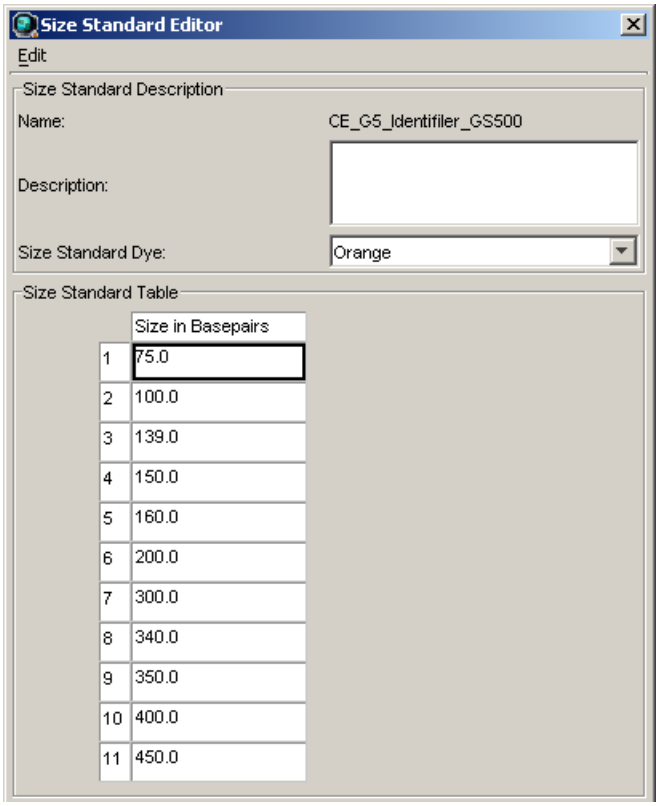
Use the following procedure to create the appropriate size standard:

1. Select **Tools** ▸ **GeneMapper Manager** to open the GeneMapper Manager.

2. Select the **Size Standards** tab, click **New**, select the **Basic or Advanced** radio button, then click **OK**.




3. Enter a name (for example, CE_G5_Identifiler_GS500 as shown below). In the Size Standard Dye field, select **Orange**. In the Size Standard Table, enter the sizes specified in on page 44. The example below is for the GeneScan™ 500 LIZ™ Size Standard.



# Analyze and edit sample files with GeneMapper™ *ID* Software

1. In the Project window, select **File ▸ Add Samples** to Project, then navigate to the disk or directory containing the sample files.
2. Apply analysis settings to the samples in the project.

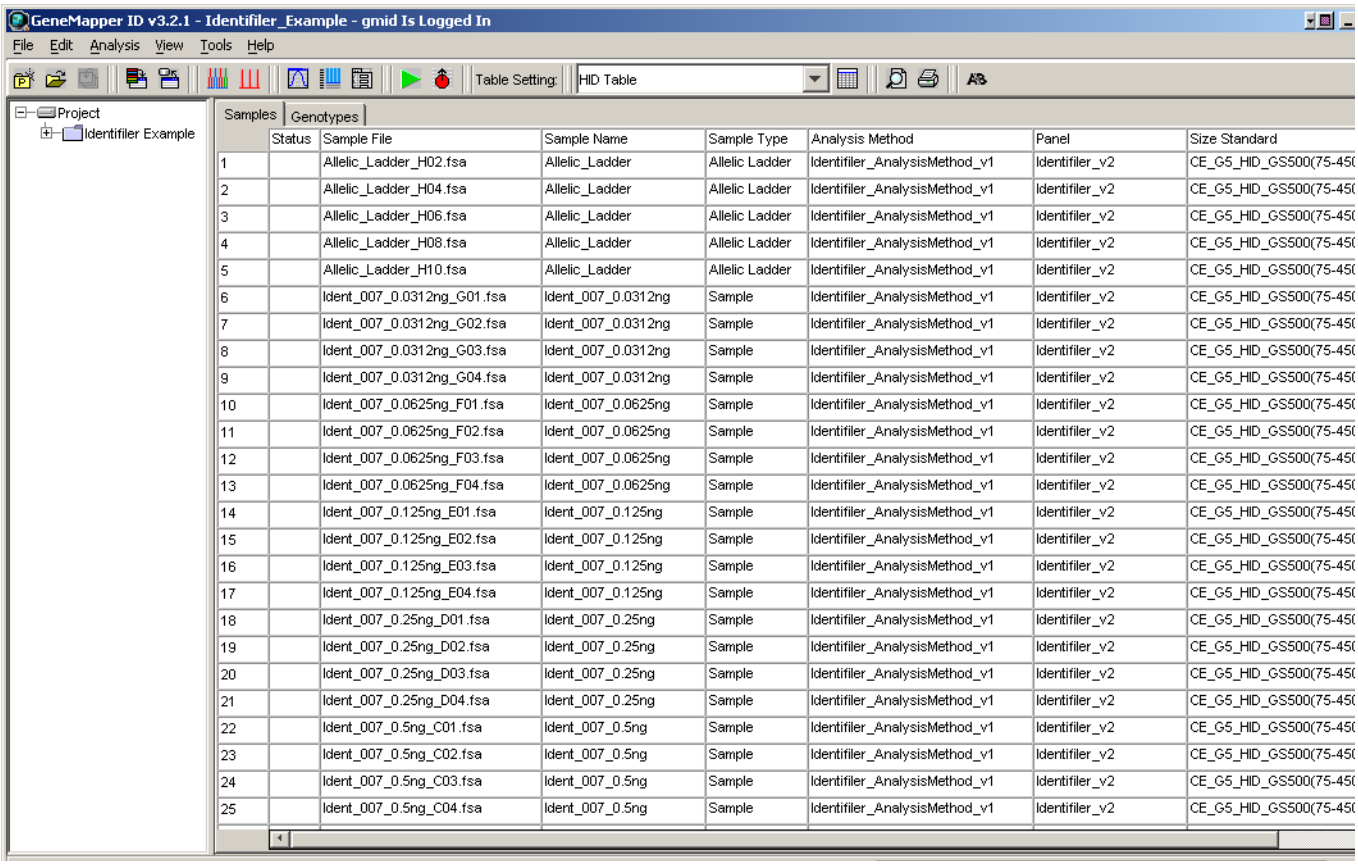
| Parameter | Settings |
|---|---|
| Sample Type | Select the sample type. |
| Analysis Method | Identifiler_AnalysisMethod_v1 (or the name of the analysis method you created) |
| Panel | Identifiler_v2 |
| Size Standard | CE_G5_Identifiler_GS500† (or the name of the size standard you created) |

† The Identifiler™ Kit was originally validated using the GeneScan™ 500 LIZ™ Size Standard. If you use the GeneScan™ 600 LIZ™ Size Standard v2.0 as an alternative, perform the appropriate internal validation studies to support the use of this size standard with the Identifiler™ Kit.

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

3. Click (Analyze), enter a name for the project (in the Save Project dialog box), then click **OK** to start analysis.
   - The status bar displays the progress of analysis:
     - As a completion bar extending to the right with the percentage indicated
     - With text messages on the left
   - The table displays the row of the sample currently being analyzed in green (or red if analysis failed for the sample).
   - The Genotypes tab becomes available after analysis (see the figure on the next page).

**Project window after analysis**




| | Status | Sample File | Sample Name | Sample Type | Analysis Method | Panel | Size Standard |
|---|---|---|---|---|---|---|---|
| 1 | | Allelic_Ladder_H02.fsa | Allelic_Ladder | Allelic Ladder | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 2 | | Allelic_Ladder_H04.fsa | Allelic_Ladder | Allelic Ladder | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 3 | | Allelic_Ladder_H06.fsa | Allelic_Ladder | Allelic Ladder | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 4 | | Allelic_Ladder_H08.fsa | Allelic_Ladder | Allelic Ladder | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 5 | | Allelic_Ladder_H10.fsa | Allelic_Ladder | Allelic Ladder | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 6 | | Ident_007_0.0312ng_G01.fsa | Ident_007_0.0312ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 7 | | Ident_007_0.0312ng_G02.fsa | Ident_007_0.0312ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 8 | | Ident_007_0.0312ng_G03.fsa | Ident_007_0.0312ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 9 | | Ident_007_0.0312ng_G04.fsa | Ident_007_0.0312ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 10 | | Ident_007_0.0625ng_F01.fsa | Ident_007_0.0625ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 11 | | Ident_007_0.0625ng_F02.fsa | Ident_007_0.0625ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 12 | | Ident_007_0.0625ng_F03.fsa | Ident_007_0.0625ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 13 | | Ident_007_0.0625ng_F04.fsa | Ident_007_0.0625ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 14 | | Ident_007_0.125ng_E01.fsa | Ident_007_0.125ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 15 | | Ident_007_0.125ng_E02.fsa | Ident_007_0.125ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 16 | | Ident_007_0.125ng_E03.fsa | Ident_007_0.125ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 17 | | Ident_007_0.125ng_E04.fsa | Ident_007_0.125ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 18 | | Ident_007_0.25ng_D01.fsa | Ident_007_0.25ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 19 | | Ident_007_0.25ng_D02.fsa | Ident_007_0.25ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 20 | | Ident_007_0.25ng_D03.fsa | Ident_007_0.25ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 21 | | Ident_007_0.25ng_D04.fsa | Ident_007_0.25ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 22 | | Ident_007_0.5ng_C01.fsa | Ident_007_0.5ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 23 | | Ident_007_0.5ng_C02.fsa | Ident_007_0.5ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 24 | | Ident_007_0.5ng_C03.fsa | Ident_007_0.5ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |
| 25 | | Ident_007_0.5ng_C04.fsa | Ident_007_0.5ng | Sample | Identifiler_AnalysisMethod_v1 | Identifiler_v2 | CE_G5_HID_GS500(75-45( |

For more information about any of these tasks, refer to the GeneMapper™ *ID* Software *Version 3.1 Human Identification Analysis User Guide* (Pub. No. 4338775).

# Examine and edit a project

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

# For more information

For details about GeneMapper™ *ID* Software features, allele filters, peak detection algorithms, and project editing, refer to:

- *GeneMapper™ ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial (Pub. No. 4335523)*
- *GeneMapper™ ID Software Version 3.1 Human Identification Analysis User Guide (Pub. No. 4338775)*
- *Installation Procedures and New Features for GeneMapper™ ID Software Software Version v3.2 User Bulletin* (Pub. No. 4352543)

# Section 4.2 GeneMapper™ *ID-X* Software

## Overview of GeneMapper™ *ID-X* Software

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

After electrophoresis, the data collection software stores information for each sample in a .fsa file or a .hid file. Using GeneMapper™ *ID-X* Software, you can then analyze and interpret the data from .fsa files (GeneMapper™ *ID-X* Software v1.0.1 or higher) or .hid files (GeneMapper™ *ID-X* Software v1.2 or higher).

### Instruments

Refer to "Instrument and software overview" on page 16 for a list of compatible instruments.

### Before you start

When using GeneMapper™ *ID-X* Software v1.0.1 or higher to perform human identification (HID) analysis with AmpFℓSTR™ kits, be aware that:

- HID analysis requires at least one allelic ladder sample per run folder. Your laboratory can use multiple ladder samples in an analysis, provided individual laboratories conduct the appropriate validation studies.
  For multiple ladder samples, the GeneMapper™ *ID-X* Software calculates allelic bin offsets by using an average of all ladders that use the same panel within a run folder.
- Allelic ladder samples in an individual run folder are considered to be from a single run.
  When the software imports multiple run folders into a project, only the ladder(s) within their respective run folders are used for calculating allelic bin offsets and subsequent genotyping.
- Allelic ladder samples must be labeled as "Allelic Ladder" in the Sample Type column in a project. Failure to apply this setting for ladder samples results in failed analysis.
- Injections containing the allelic ladder must be analyzed with the same analysis method and parameter values that are used for samples to ensure proper allele calling.
- Alleles that are not in the AmpFℓSTR™ Allelic Ladders do exist. Off-ladder (OL) alleles may contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the ±0.5-nt bin window of any known allelic ladder allele or virtual bin.
  **Note:** If a sample allele peak is called as an off-ladder allele, the sample result needs to be verified according to the laboratory's protocol.

# Set up GeneMapper™ *ID-X* Software for data analysis

## Panel, bin, and stutter file version

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

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

## Before using the software for the first time

Before you use GeneMapper™ *ID-X* Software (v1.0.1 or higher for .fsa files, v1.2 or higher for .hid files) to analyze data for the first time, you must do the following:

1. Check the version of panel, bin, and stutter files installed with the GeneMapper™ *ID-X* Software as explained in "Check panel, bin, and stutter file version" below.
2. Check **www.lifetechnologies.com/support** ▸ **Software, Patches & Updates** ▸ **GeneMapper™ *ID-X* Software** to determine if newer files are available.
3. If updated files are available, download and import the files into the GeneMapper™ *ID-X* Software, as explained in "Import panels, bins, and marker stutter" on page 50.
   **Note:** When downloading new versions of analysis files, refer to the associated Read Me file for details of changes between software file versions. If you have validated previous file versions for data analysis, conduct the appropriate internal verification studies before using new file versions for operational analysis.
4. Create an analysis method, as explained in "Create an analysis method" on page 55.
5. Define custom views of analysis tables.
   Refer to Chapter 1 of the *GeneMapper™ ID-X Software Version 1.0 Getting Started Guide* (Pub. No. 4375574) for more information.
6. Define custom views of plots.
   Refer to Chapter 1 of the *GeneMapper™ ID-X Software Version 1.0 Getting Started Guide* (Pub. No. 4375574) for more information.

## For more information

For quick set up instructions, refer to the *GeneMapper™ ID-X Software Version 1.0 Getting Started Guide* (Pub. No. 4375574).

For details about GeneMapper™ *ID-X* Software features, refer to:

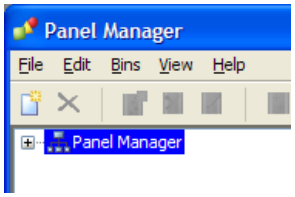
- *GeneMapper™ ID-X Software Version 1.0 Getting Started Guide* (Pub. No. 4375574)
- *GeneMapper™ ID-X Software Version 1.0 Quick Reference Guide* (Pub. No. 4375670)
- *GeneMapper™ ID-X Software Version 1.0 Reference Guide* (Pub. No. 4375671)

## Check panel, bin, and stutter file version

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

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

2. Select **Tools** ▸ **Panel Manager**.
3. Check the version of files imported into the Panel Manager:
   a. Select **Panel Manager** in the navigation pane.
   b. Expand the Panel Manager folder and any sub-folders to identify the analysis file version already installed for your kit choice.




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

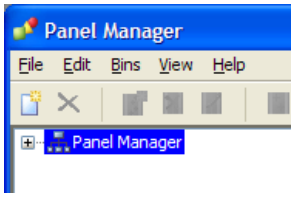
## Import panels, bins, and marker stutter

To import the Identifiler™ Kit panel, bin set, and marker stutter from our web site into the GeneMapper™ *ID-X* Software database:

1. Download and open the file containing panels, bins, and marker stutter:
   a. Go to **www.lifetechnologies.com/support** ▸ **Software, Patches & Updates** ▸ **GeneMapper™ *ID-X* Software**. Download the file AmpFLSTR Analysis Files GMIDX.
   b. Unzip the file.
2. Start the GeneMapper™ *ID-X* Software, then log in with the appropriate user name and password.

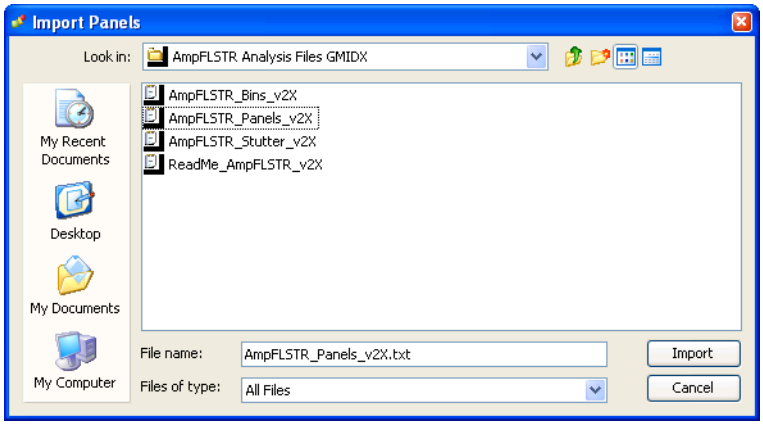
   **IMPORTANT!** For logon instructions, refer to the *GeneMapper™ ID-X Software Version 1.0 Getting Started Guide* (Pub. No. 4375574).

3. Select **Tools** ▸ **Panel Manager**.
4. Find, then open the folder containing the panels, bins, and marker stutter:
   a. Select **Panel Manager** in the navigation pane.
   b. Select **File** ▸ **Import Panels** to open the Import Panels dialog box.
   c. Navigate to, then open the **AmpFLSTR Analysis Files GMIDX** folder that you unzipped in step 1 on page 50.



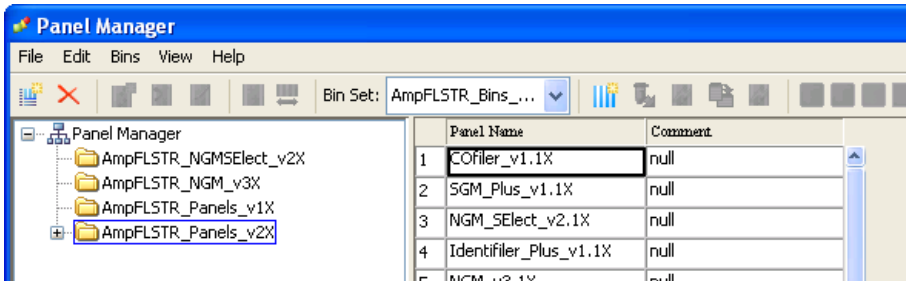
5. Select **AmpFLSTR_Panels_v2X** (or the version you installed), then click **Import**.

   **Note:** Importing this file creates a new folder in the navigation pane of the Panel Manager "Identifiler_v1.1X". This folder contains the panel and associated markers.




6. Import AmpFLSTR_Bins_V2X.txt:

   a. Select the **AmpFLSTR_Panels_v2X** folder in the navigation pane.

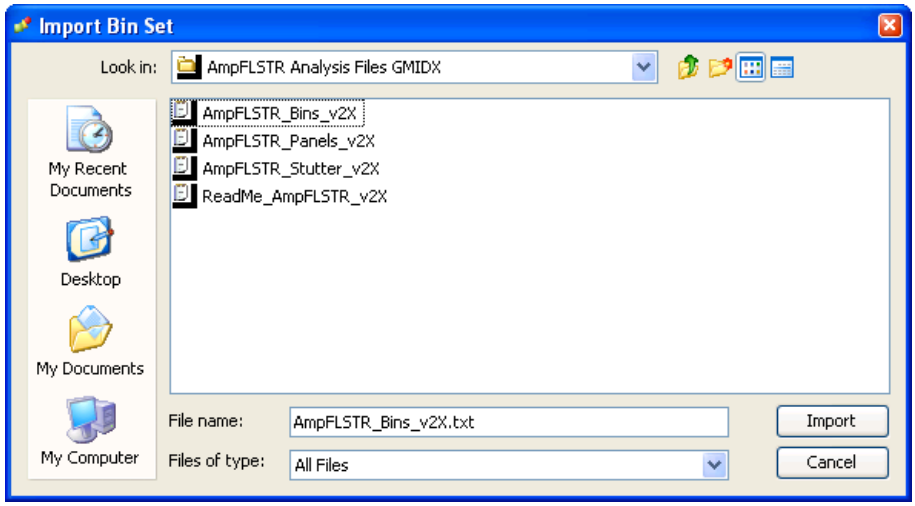



   b. Select **File** ▸ **Import Bin Set** to open the Import Bin Set dialog box.

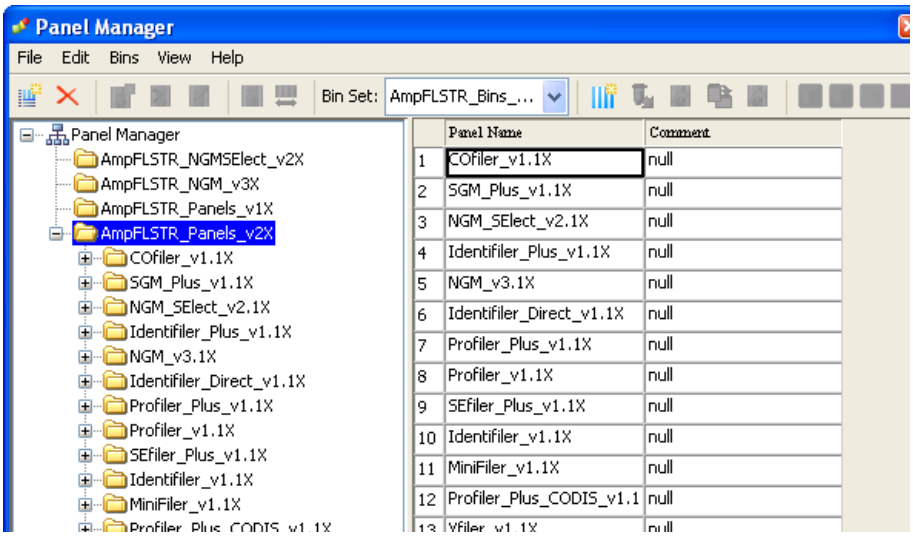
   c. Navigate to, then open the **AmpFLSTR Analysis Files GMIDX** folder.

d. Select **AmpFLSTR_Bins_V2X.txt**, then click **Import**.

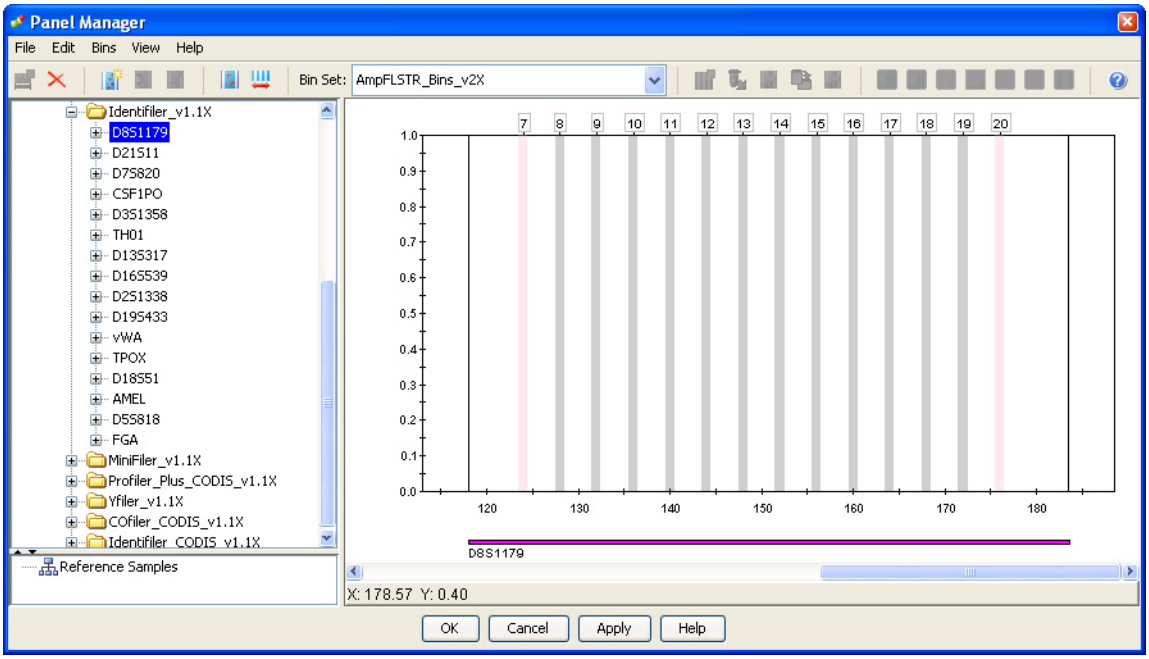
**Note:** Importing this file associates the bin set with the panels in the AmpFLSTR_Panels_v2X folder.




7. View the imported panels in the navigation pane:
   a. Double-click the **AmpFLSTR_Panels_v2X** folder.
   b. Double-click the **Identifiler_v1.1X** folder to display the panel information in the right pane.

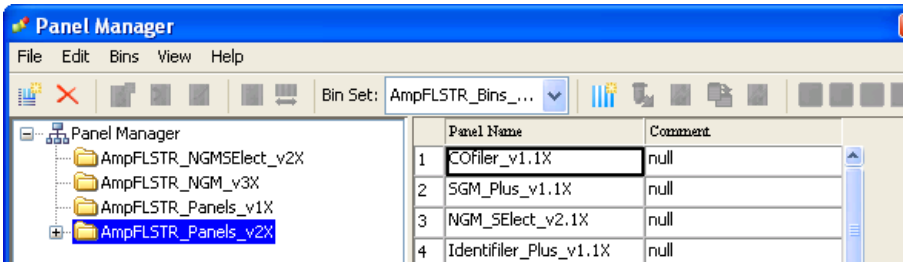


8. Select and expand **Identifiler_v1.1X** in the navigation pane, then select **D8S1179** to display the Bin view for the marker in the right pane.




9. Import AmpFLSTR_Stutter_v2X:

   a. Select the **AmpFLSTR_Panels_v2X** folder in the navigation panel.

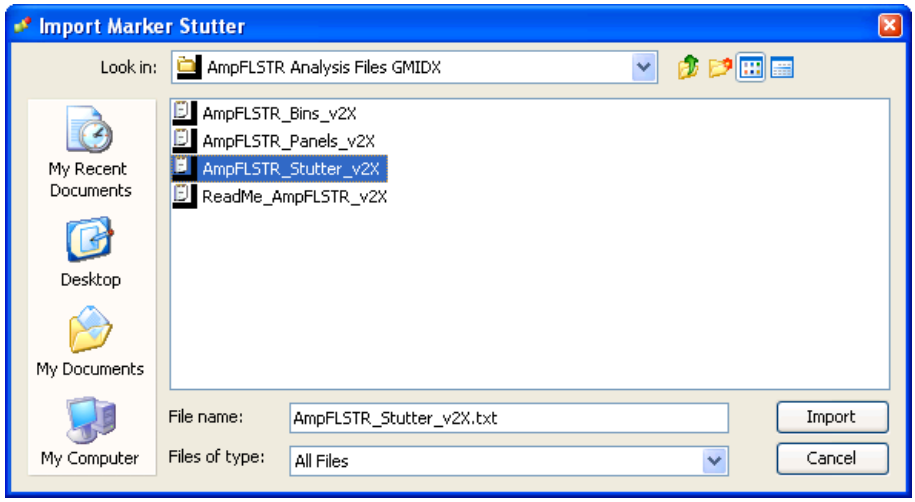



   b. Select File ▸ **Import Marker Stutter** to open the Import Marker Stutter dialog box.

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

d. Select **AmpFLSTR_Stutter_v2X**, then click **Import**.

**Note:** Importing this file associates the marker stutter ratio with the bin set in the AmpFLSTR_v2X folder.

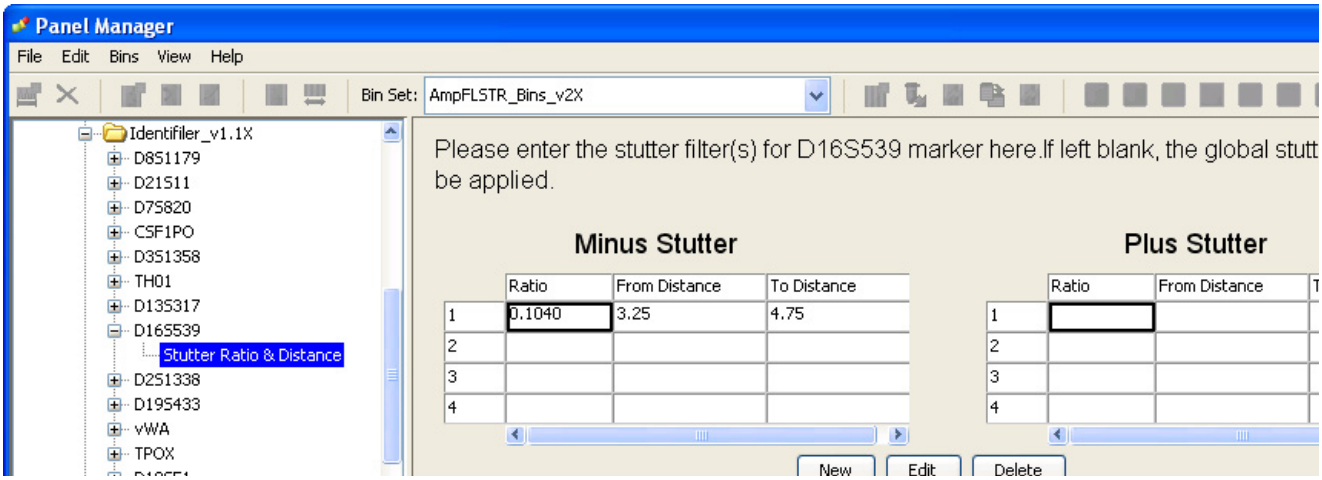



10. View the imported marker stutters in the navigation pane:

a. Double-click the **AmpFLSTR_Panels_v2X** folder to display its list of kits in the right pane.

b. Double-click the **Identifiler_v1.1X** folder to display its list of markers below it.

c. Double-click **D16S539** to display the Stutter Ratio & Distance view for the marker in the right pane.




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

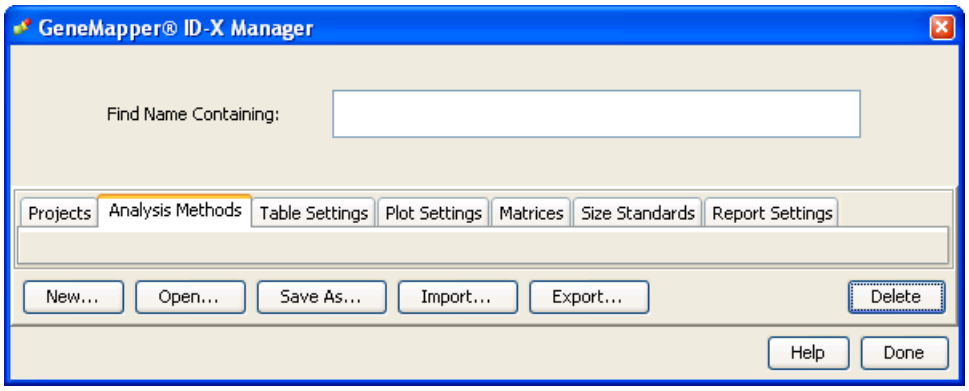
**IMPORTANT!** If you close the Panel Manager without clicking **Apply**, the panels, bin sets, and marker stutter will not be imported into the GeneMapper™ *ID-X* Software database.

## Create an analysis method

Use the following procedure to create an analysis method for the Identifiler™ Kit.

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

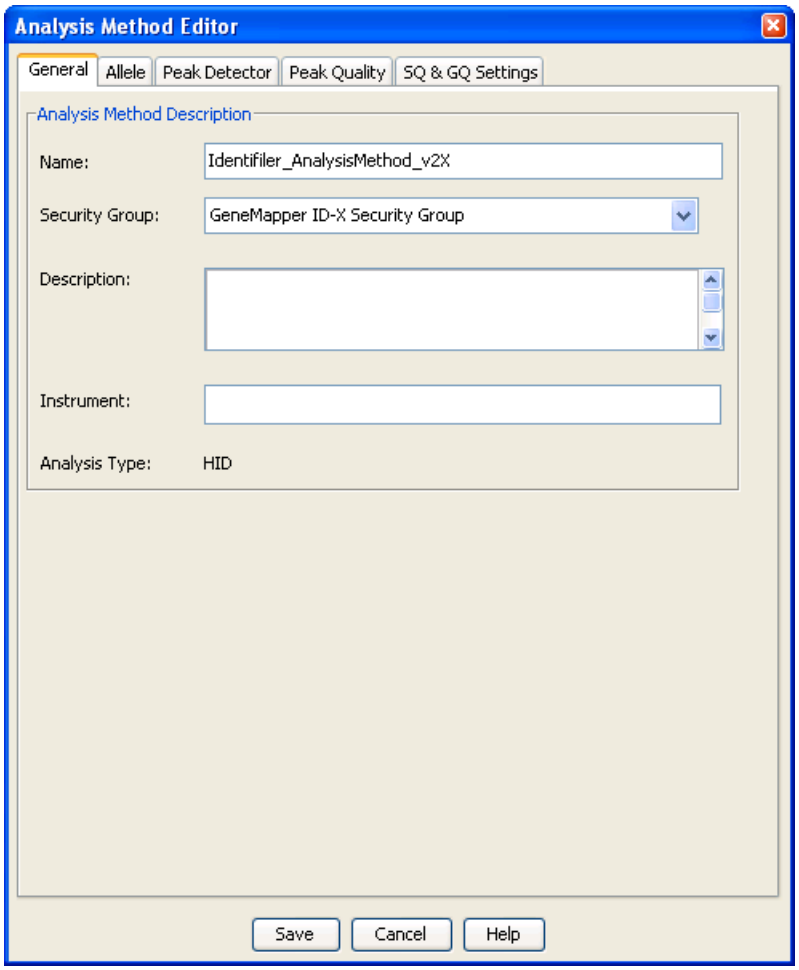
1. Select **Tools** ▸ **GeneMapper™ ID-X Manager** to open the GeneMapper™ *ID-X* Manager.




2. Select the **Analysis Methods** tab, then click **New** to open the Analysis Method Editor with the **General** tab selected.
3. The figures below show the settings for each tab of the Analysis Method Editor. Configure the Analysis Method Editor tab settings as shown in the figures below, unless the instructions state otherwise.

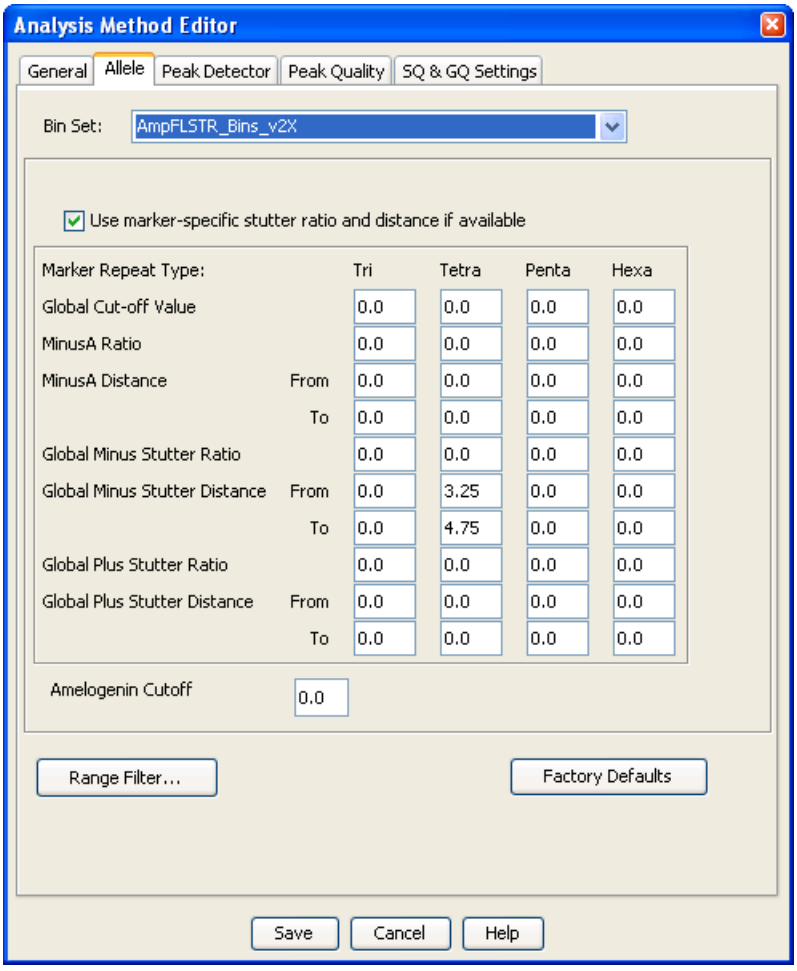
   **Note:** The Analysis Method Editor closes when you save your settings. To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.
4. After you enter settings in all tabs, click **Save**.

## General tab settings



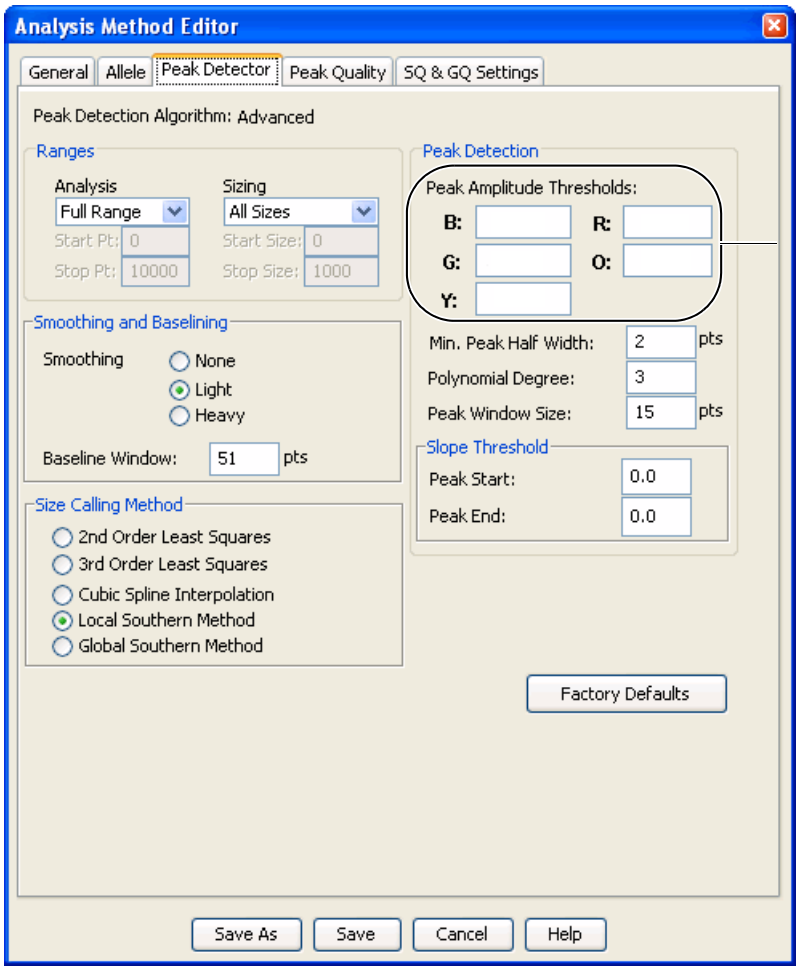

In the Name field, either type the name as shown or enter a name of your choosing. In the Security Group field, select the Security Group appropriate to your software configuration from the dropdown list. The Description and Instrument fields are optional.

## Allele tab settings




- In the Bin Set field, select the **AmpFLSTR_Bins_v2X** bin set and configure the stutter distance parameters as shown.
- GeneMapper™ *ID-X* Software v1.0.1 or higher allows you to specify 4 types of marker repeat motifs: tri, tetra, penta and hexa. You can enter parameter values for each type of repeat in the appropriate column.
- Specify the stutter ratio:
  - To apply the stutter ratios listed in the Allele tab for single-source data, deselect the "Use marker-specific stutter ratio if available" check box (selected by default). Perform appropriate internal validation studies to determine the appropriate filter setting to use.
    **Note:** Applying global stutter ratios may reduce the editing required for single-source sample data.
  - To apply the stutter ratios contained in the AmpFLSTR_Panels_v2.txt file, select the "Use marker-specific stutter ratio if available" check box (selected by default). Perform appropriate internal validation studies to determine the appropriate filter setting to use.

## Peak Detector tab settings



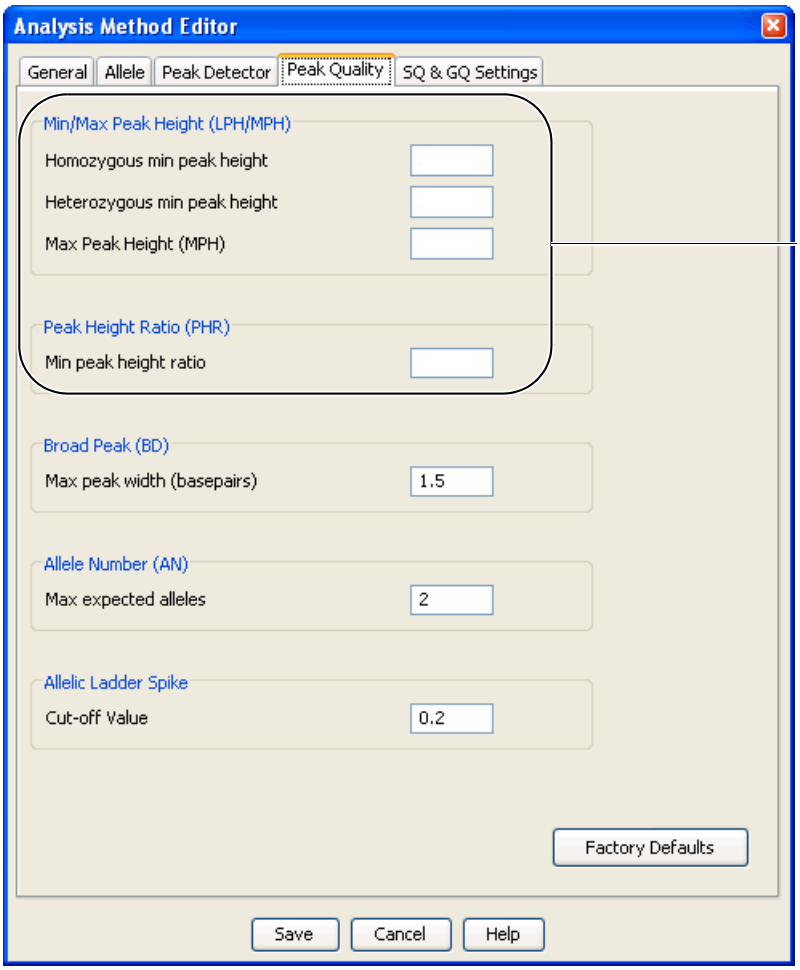

Perform internal validation studies to determine settings

**IMPORTANT!** Perform the appropriate internal validation studies to determine the appropriate peak amplitude thresholds for interpretation of Identifiler™ Kit data.

Fields include:

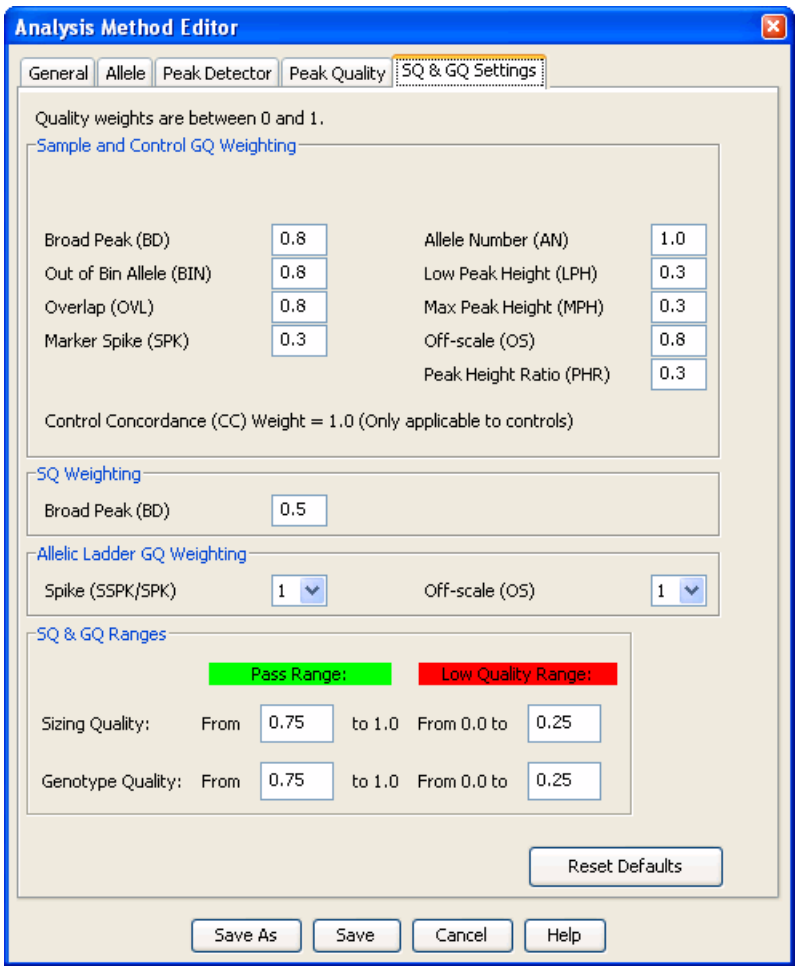
- **Peak amplitude thresholds** – The software uses these parameters to specify the minimum peak height, in order to limit the number of detected peaks. Although GeneMapper™ *ID-X* Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the genotype of these peaks.
- **Size calling method** – The Identifiler™ Kit has been validated using the Local Southern sizing method. Select alternative sizing methods only after you perform the appropriate internal validation studies.
- **Normalization** – A Normalization checkbox is available on this tab in GeneMapper™ *ID-X* Software v1.2 for use in conjunction with data run on the Applied Biosystems 3500 Series Genetic Analyzers. Users of this version of software should perform laboratory evaluations to determine whether to use the Normalization feature for analysis of Identifiler™ Kit data.

## Peak Quality tab settings




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

## SQ & GQ tab settings




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

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

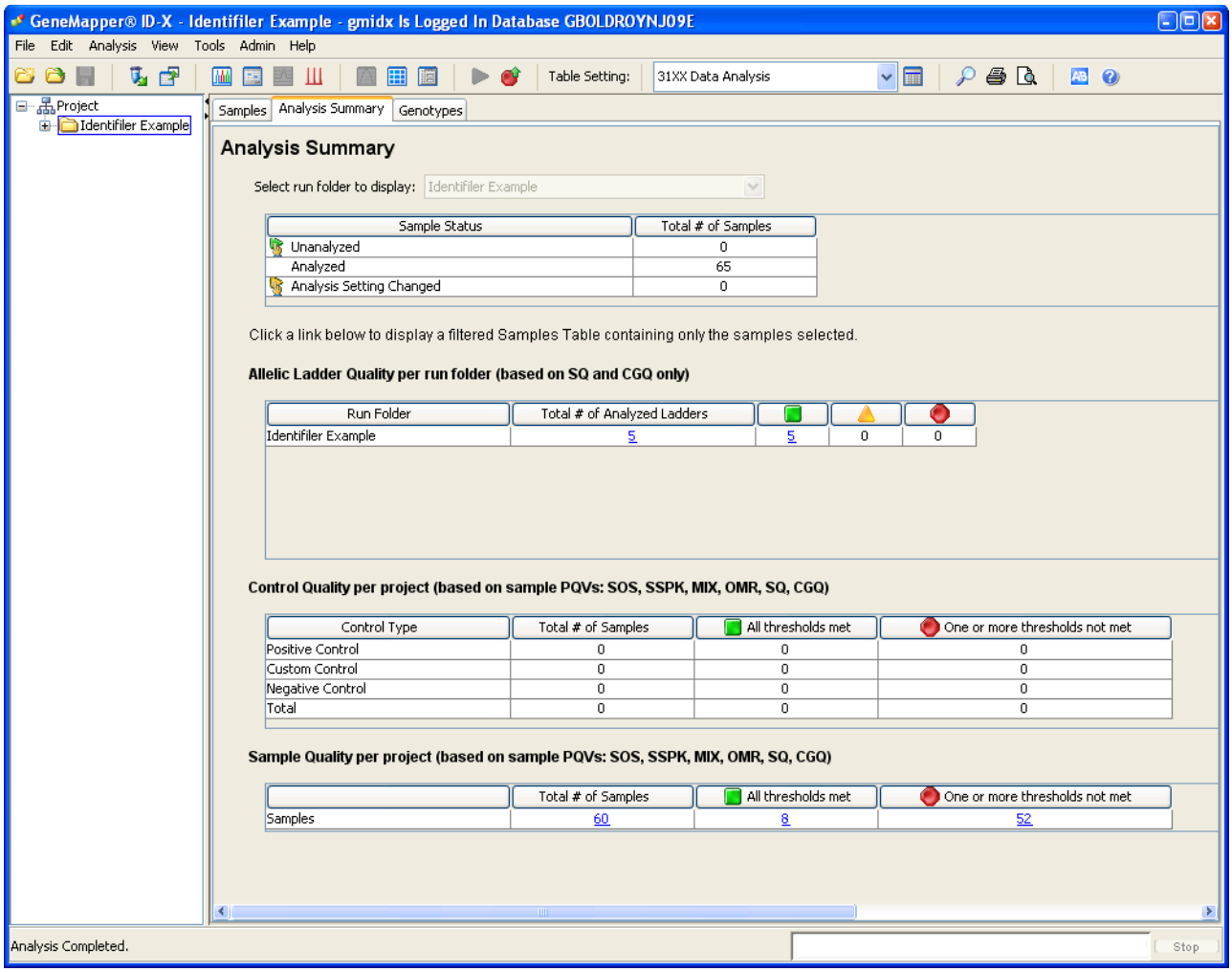
1. In the Project window, select **File ▸ Add Samples to Project**, then navigate to the disk or directory containing the sample files.
2. Apply analysis settings to the samples in the project.

| Parameter | Settings |
|---|---|
| Sample Type | Select the sample type. |
| Analysis Method | Identifiler_AnalysisMethod_v2X (or the name of the analysis method you created) |
| Panel | Identifiler_v1.1X |
| Size Standard | CE_G5_GS500(75-450) |

For more information about how the Size Caller works, or about size standards, refer to the *GeneMapper™ ID-X Software v1.2 Reference Guide* (Pub. No. 4426481A).

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

**Analysis summary window after analysis**




# Examine and edit a project

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

# For more information

For more information, refer to:

- *GeneMapper™ ID-X Software Version 1.0 Getting Started Guide* (Pub. No. 4375574)
- *GeneMapper™ ID-X Software Version 1.0 Quick Reference Guide* (Pub. No. 4375670)
- *GeneMapper™ ID-X Software Version 1.0 Reference Guide* (Pub. No. 4375671)
- *GeneMapper™ ID-X Software Version 1.1(Mixture Analysis) Getting Started Guide* (Pub. No. 4396773)

- *GeneMapper™ ID-X Software Version 1.2 Reference Guide* (Pub. No. 4426481)
- *GeneMapper™ ID-X Software Version 1.2 Quick Reference Guide* (Pub. No. 4426482)
- *GeneScan™ Analysis Software for the Windows NT™ Operating System Overview of the Analysis Parameters and Size Caller User Bulletin* (Pub. No. 4335617).

# 5 Experiments and Results

# Section 5.1 Developmental Validation

## Overview

This chapter provides results of the developmental validation experiments we performed using the AmpFℓSTR™ Identifiler™ PCR Amplification Kit.

The data contained in this section was generated during the original developmental validation of the Identifiler™ Kit before its release in 2001. Since that time, we have made a series of improvements to the Identifiler™ Kit. For information on changes to the Identifiler™ Kit since 2001 and associated data, see:

- "Performance Verification After Primer Manufacturing Process Improvements" on page 107
- "Performance Validation After Buffer and Enzyme Component Replacement" on page 108

### Importance of validation

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

### Experiment conditions

We performed experiments to evaluate the performance of the Identifiler™ Kit according to the DNA Advisory Board (DAB) Quality Assurance Standards, effective October 1, 1998 (DNA Advisory Board, 1998). The DAB standards describe the quality assurance requirements that a laboratory should follow to ensure the quality and integrity of the data and competency of the laboratory.

These DAB standards describe the quality assurance requirements that a laboratory should follow to ensure the quality and integrity of the data and competency of the laboratory. DAB defines a laboratory as a facility in which forensic DNA testing is performed.

Based on these standards, we conducted experiments which comply with Standards 8.1.1 and 8.1.2 and its associated subsections. Whereas this DNA methodology is not novel, Standard 8.1.2 and its related subsections have been addressed (Holt *et al.*, 2001 and Wallin *et al.*, 2001). This chapter will discuss many of the experiments we performed and examples of the results we obtained. We used conditions that produced maximum PCR product yield and a window in which reproducible performance characteristics were met. These experiments, while not exhaustive, are appropriate for a manufacturer, in our opinion. Each laboratory using the Identifiler™ Kit should perform appropriate validation studies.

# Developmental validation

## DAB 8.1.1 Developmental Validation

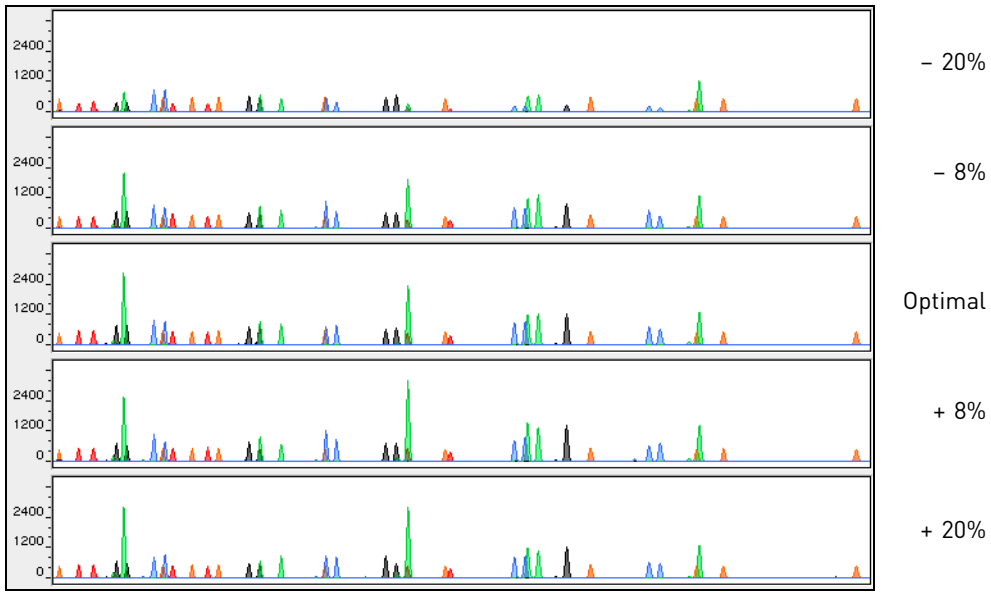
*"Developmental validation that is conducted shall be appropriately documented."* (DNA Advisory Board, 1998).

Critical reagent concentrations and reaction conditions (such as thermal cycling parameters, AmpliTaq Gold™ DNA polymerase activation, cycle number) to produce reliable, locus-specific amplification and appropriate sensitivity have been determined.

## PCR components

The concentration of each component of the Identifiler™ Kit was examined. The PCR components are Tris-HCl (pH 8.3), KCl, dNTPs, primers, AmpliTaq Gold™ DNA Polymerase, $MgCl_2$, bovine serum albumin, and sodium azide. The concentration for a particular component was established to be in the window that meets the reproducible performance characteristics of specificity and sensitivity (Figure 5).

**Figure 5** A 1 ng amplification of genomic DNA varying the $MgCl_2$ concentration, analyzed on the 310 Genetic Analyzer




## Thermal cycler parameters

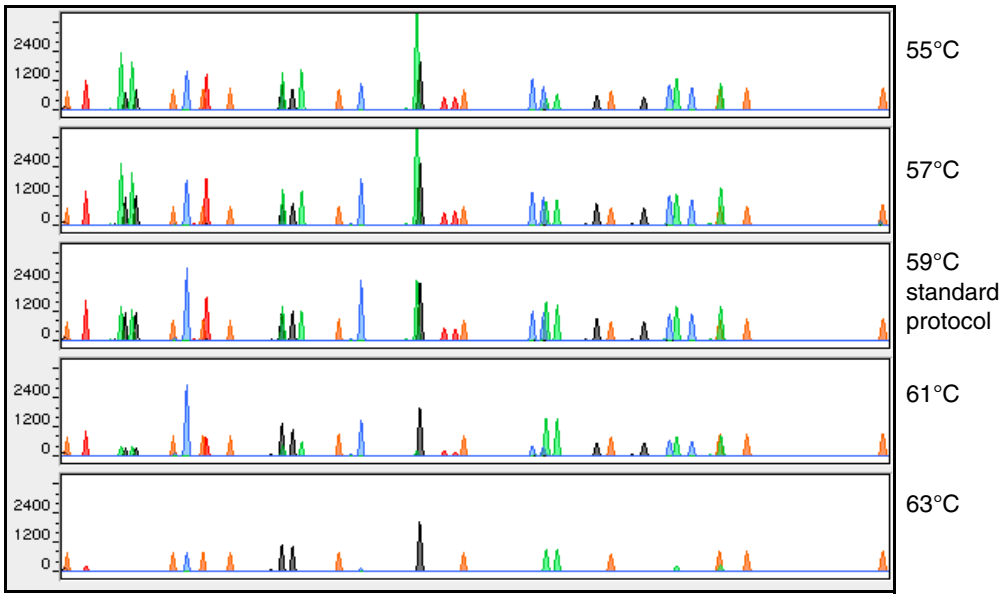
Thermal cycling parameters were established for amplification of the Identifiler™ Kit in the GeneAmp™ PCR Systems 9600 and 9700. Thermal cycling times and temperatures of GeneAmp PCR systems were verified. Annealing and denaturation temperature windows were tested around each stipend to verify that a ±1.5°C window produced a specific PCR product with the desired sensitivity of at least 1 ng of AmpFℓSTR™ Control DNA 9947A.

The effects of denaturation and annealing temperatures on the amplification of Identifiler™ Kit loci were examined using AmpFℓSTR™ Control DNA 9947A and two DNA samples.

The denaturation temperatures tested were 92.5, 94, and 95.5°C, all for 1-minute hold times on the GeneAmp PCR System 9700. The annealing temperatures tested were 55, 57, 59, 61, and 63°C (Figure 6), also for 1-minute hold times in the GeneAmp PCR System 9700. The PCR products were analyzed using the 310 Genetic Analyzer.

Neither preferential nor differential amplification was observed in the denaturation temperature experiments. Of the tested annealing temperatures, 55, 57, 59, and 61°C produced robust profiles. At 63°C, the yield of the majority of loci was significantly reduced. This should pose no problem with routine thermal cycler calibration and when following the recommended amplification protocol. Preferential amplification was not observed at any of the tested annealing temperatures.

**Figure 6** An amplification of 1 ng of genomic DNA, amplified while varying the annealing temperature, analyzed on the 310 Genetic Analyzer




## AmpliTaq Gold™ DNA Polymerase activation

Identifiler™ Kit reactions were amplified for 27, 28, 29, 30, and 31 cycles on the GeneAmp™ PCR System 9700 using 1.0 ng of three DNA samples. As expected, PCR product increased with the number of cycles. A full profile was generated at 27 cycles; off-scale data were collected for several allele peaks at 31 cycles.

While none of the cycle numbers tested produced nonspecific peaks, 28 cycles was found to give optimal sensitivity when the amplified products were examined on 310 Genetic Analyzers. Additionally, the cycle number was set to avoid detection of low quantities of DNA (20 pg or less). At 28 cycles, 1.0 ng of AmpFℓSTR™ Control DNA 9947A amplifies reliably and specifically following the conditions outlined in this guide.

# Accuracy, precision, and reproducibility

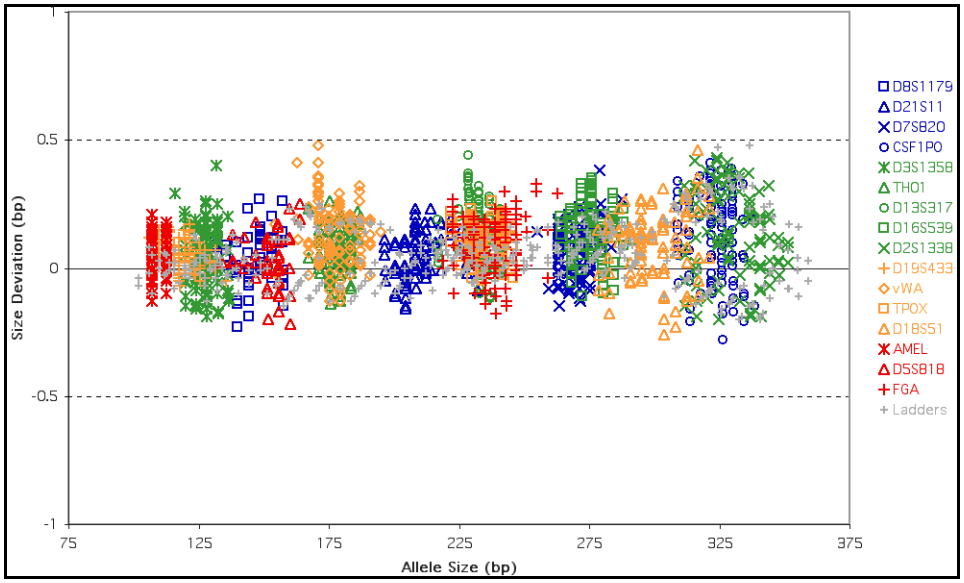
## DAB 8.1.2 Accuracy

*"Novel forensic DNA methodologies shall undergo developmental validation to ensure the accuracy, precision and reproducibility of the procedure."* (DAB, 1998).

Laser-induced fluorescence detection systems of length polymorphism at short tandem repeat loci is not a novel methodology (Holt *et al.*, 2001 and Wallin *et al.*, 2001). However, accuracy and reproducibility of Identifiler™ Kit profiles have been determined from various sample types.

Figure 7 illustrates the size differences that are typically observed between sample alleles and allelic ladder alleles on the 310 Genetic Analyzer with POP-4™ polymer. The x-axis in Figure 7 represents the nominal base pair sizes for the AmpFℓSTR™ Identifiler™ Allelic Ladder, and the dashed lines parallel to the x-axis represent the ±0.5-bp windows. The y-axis is the deviation of each sample allele size from the corresponding allelic ladder allele size. The data include a total of 2269 alleles from 70 population database samples. All sample alleles are within 0.5 bp of a corresponding allele in an allelic ladder.

**Figure 7** Size deviation of 70 samples and two allelic ladders from one injection of allelic ladder on a single 310 Genetic Analyzer run




## Precision and size windows

Sizing precision allows for determining accurate and reliable genotypes. Sizing precision was measured on the 310 Genetic Analyzer. The recommended method for genotyping is to use a ±0.5-bp "window" around the size obtained for each allele in the AmpFℓSTR™ Identifiler™ Allelic Ladder. A ±0.5-bp window allows for the detection and correct assignment of alleles. An allele that sizes only one base pair different from an allele in the allelic ladder will not be incorrectly typed and will be identified as off-ladder. Any sample allele that sizes outside a window could be either of the following:

- An "off-ladder" allele, for example, an allele of a size that is not represented in the AmpFℓSTR™ Identifiler™ Allelic Ladder
- An allele that does correspond to an allelic ladder allele, but whose size is just outside a window because of measurement error

The measurement error inherent in any sizing method can be defined by the degree of precision in sizing an allele multiple times. Precision is measured by calculating the standard deviation in the size values obtained for an allele that is run in several injections in one capillary run. Table 3 on page 70 indicates typical precision results obtained from the seven injections of the AmpFℓSTR™ Identifiler™ Allelic Ladder analyzed on the 310 Genetic Analyzer (47-cm capillary and POP-4™ polymer). The internal size standard used was GeneScan™ 500 LIZ™ Size Standard. These results were obtained within a set of injections on a single capillary.

As indicated above, sample alleles may occasionally size outside of the ±0.5-bp window for a respective allelic ladder allele because of measurement error. The frequency of such an occurrence is lowest in detection systems having the smallest standard deviations in sizing. Figure 7 on page 69 illustrates the tight clustering of allele sizes obtained on the 310 Genetic Analyzer, where the standard deviation in sizing is typically less than 0.15 bp. The instance of a sample allele sizing outside of the ±0.5-bp window because of measurement error is relatively rare when the standard deviation in sizing is approximately 0.15 bp or less (Smith, 1995).

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

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

1187 population database DNA samples have been typed using the Identifiler™ Kit (see"About the primers" on page 11). These samples have been previously genotyped with concordant results of the same loci, using other AmpFℓSTR™ kits.

**Table 3** Example of precision results of seven injections of the AmpFℓSTR™ Identifiler™ Allelic Ladder

| | 310 Genetic Analyzer | |
|---|---|---|
| **Allele** | **Mean** | **Standard Deviation** |
| **Amelogenin** | | |
| X | 107.02 | 0.04 |
| Y | 112.61 | 0.02 |
| **CSF1PO** | | |
| 6 | 304.69 | 0.08 |
| 7 | 309.01 | 0.10 |

| 310 Genetic Analyzer | | |
|---|---|---|
| **Allele** | **Mean** | **Standard Deviation** |
| 8 | 313.30 | 0.10 |
| 9 | 317.55 | 0.11 |
| 10 | 321.97 | 0.12 |
| 11 | 325.86 | 0.11 |
| 12 | 329.97 | 0.13 |
| 13 | 334.00 | 0.10 |
| 14 | 338.04 | 0.11 |
| 15 | 341.84 | 0.08 |
| **D2S1338** | | |
| 15 | 307.30 | 0.11 |
| 16 | 311.65 | 0.11 |
| 17 | 315.91 | 0.12 |
| 18 | 320.16 | 0.12 |
| 19 | 324.34 | 0.12 |
| 20 | 328.44 | 0.08 |
| 21 | 332.58 | 0.11 |
| 22 | 336.62 | 0.09 |
| 23 | 340.57 | 0.11 |
| 24 | 344.18 | 0.07 |
| 25 | 347.78 | 0.07 |
| 26 | 351.39 | 0.07 |
| 27 | 355.08 | 0.07 |
| 28 | 358.77 | 0.05 |
| **D3S1358** | | |
| 12 | 111.96 | 0.06 |
| 13 | 116.04 | 0.04 |
| 14 | 119.99 | 0.04 |
| 15 | 123.89 | 0.02 |
| 16 | 128.06 | 0.05 |
| 17 | 132.24 | 0.05 |
| 18 | 136.30 | 0.06 |
| 19 | 140.43 | 0.03 |
| **D5S818** | | |
| 7 | 134.14 | 0.05 |
| 8 | 138.21 | 0.04 |
| 9 | 142.56 | 0.04 |

| 310 Genetic Analyzer | | |
|---|---|---|
| **Allele** | **Mean** | **Standard Deviation** |
| 10 | 147.02 | 0.06 |
| 11 | 151.31 | 0.01 |
| 12 | 155.63 | 0.05 |
| 13 | 159.81 | 0.06 |
| 14 | 164.04 | 0.07 |
| 15 | 167.95 | 0.05 |
| 16 | 172.09 | 0.05 |
| **D7S820** | | |
| 6 | 255.15 | 0.08 |
| 7 | 259.21 | 0.07 |
| 8 | 263.24 | 0.07 |
| 9 | 267.26 | 0.09 |
| 10 | 271.32 | 0.08 |
| 11 | 275.35 | 0.06 |
| 12 | 279.42 | 0.07 |
| 13 | 283.42 | 0.06 |
| 14 | 287.48 | 0.10 |
| 15 | 291.58 | 0.06 |
| **D8S1179** | | |
| 8 | 123.29 | 0.07 |
| 9 | 127.32 | 0.05 |
| 10 | 131.41 | 0.05 |
| 11 | 135.49 | 0.04 |
| 12 | 139.73 | 0.04 |
| 13 | 144.25 | 0.03 |
| 14 | 148.71 | 0.06 |
| 15 | 153.16 | 0.07 |
| 16 | 157.51 | 0.07 |
| 17 | 161.72 | 0.05 |
| 18 | 165.84 | 0.07 |
| 19 | 169.92 | 0.05 |
| **D13S317** | | |
| 8 | 216.87 | 0.05 |
| 9 | 220.83 | 0.05 |
| 10 | 224.77 | 0.07 |
| 11 | 228.88 | 0.07 |

| 310 Genetic Analyzer | | |
|---|---|---|
| **Allele** | **Mean** | **Standard Deviation** |
| 12 | 232.81 | 0.05 |
| 13 | 236.68 | 0.07 |
| 14 | 240.69 | 0.06 |
| 15 | 244.68 | 0.09 |
| **D16S539** | | |
| 5 | 252.37 | 0.08 |
| 8 | 264.30 | 0.07 |
| 9 | 268.32 | 0.08 |
| 10 | 272.32 | 0.06 |
| 11 | 276.37 | 0.07 |
| 12 | 280.37 | 0.09 |
| 13 | 284.34 | 0.07 |
| 14 | 288.44 | 0.09 |
| 15 | 292.51 | 0.07 |
| **D18S51** | | |
| 7 | 262.07 | 0.08 |
| 9 | 270.22 | 0.06 |
| 10 | 274.34 | 0.09 |
| 10.2 | 276.36 | 0.06 |
| 11 | 278.41 | 0.08 |
| 12 | 282.49 | 0.05 |
| 13 | 286.57 | 0.06 |
| 13.2 | 288.63 | 0.05 |
| 14 | 290.77 | 0.04 |
| 14.2 | 292.78 | 0.05 |
| 15 | 294.91 | 0.07 |
| 16 | 299.07 | 0.06 |
| 17 | 303.50 | 0.07 |
| 18 | 307.94 | 0.09 |
| 19 | 312.40 | 0.11 |
| 20 | 316.71 | 0.09 |
| 21 | 320.99 | 0.14 |
| 22 | 325.24 | 0.11 |
| 23 | 329.40 | 0.11 |
| 24 | 333.54 | 0.15 |
| 25 | 337.67 | 0.11 |

| 310 Genetic Analyzer | | |
|---|---|---|
| **Allele** | **Mean** | **Standard Deviation** |
| 26 | 341.56 | 0.09 |
| 27 | 345.24 | 0.08 |
| **D19S433** | | |
| 9 | 101.99 | 0.05 |
| 10 | 105.88 | 0.05 |
| 11 | 109.78 | 0.04 |
| 12 | 113.64 | 0.02 |
| 12.2 | 115.61 | 0.02 |
| 13 | 117.56 | 0.03 |
| 13.2 | 119.55 | 0.02 |
| 14 | 121.46 | 0.03 |
| 14.2 | 123.47 | 0.02 |
| 15 | 125.45 | 0.05 |
| 15.2 | 127.43 | 0.05 |
| 16 | 129.44 | 0.05 |
| 16.2 | 131.46 | 0.05 |
| 17 | 133.42 | 0.03 |
| 17.2 | 135.44 | 0.06 |
| **D21S11** | | |
| 24 | 184.86 | 0.04 |
| 24.2 | 186.82 | 0.02 |
| 25 | 188.77 | 0.03 |
| 26 | 192.69 | 0.05 |
| 27 | 196.56 | 0.04 |
| 28 | 200.41 | 0.05 |
| 28.2 | 202.36 | 0.05 |
| 29 | 204.32 | 0.03 |
| 29.2 | 206.31 | 0.02 |
| 30 | 208.29 | 0.07 |
| 30.2 | 210.24 | 0.05 |
| 31 | 212.23 | 0.05 |
| 31.2 | 214.14 | 0.06 |
| 32 | 216.14 | 0.04 |
| 32.2 | 218.10 | 0.04 |
| 33 | 220.14 | 0.05 |
| 33.2 | 222.07 | 0.04 |

| 310 Genetic Analyzer | | |
|---|---|---|
| **Allele** | **Mean** | **Standard Deviation** |
| 34 | 224.10 | 0.07 |
| 34.2 | 226.02 | 0.06 |
| 35 | 228.07 | 0.06 |
| 35.2 | 230.01 | 0.07 |
| 36 | 232.04 | 0.07 |
| 37 | 236.00 | 0.03 |
| 38 | 239.94 | 0.08 |
| **FGA** | | |
| 17 | 214.81 | 0.07 |
| 18 | 218.80 | 0.06 |
| 19 | 222.79 | 0.07 |
| 20 | 226.81 | 0.06 |
| 21 | 230.76 | 0.08 |
| 22 | 234.78 | 0.07 |
| 23 | 238.81 | 0.05 |
| 24 | 242.83 | 0.07 |
| 25 | 246.88 | 0.06 |
| 26 | 250.96 | 0.06 |
| 26.2 | 253.00 | 0.09 |
| 27 | 254.97 | 0.08 |
| 28 | 259.02 | 0.10 |
| 29 | 263.12 | 0.08 |
| 30 | 267.26 | 0.09 |
| 30.2 | 269.07 | 0.10 |
| 31.2 | 273.17 | 0.09 |
| 32.2 | 277.24 | 0.08 |
| 33.2 | 281.33 | 0.09 |
| 42.2 | 319.83 | 0.14 |
| 43.2 | 324.04 | 0.14 |
| 44.2 | 328.26 | 0.13 |
| 45.2 | 332.42 | 0.16 |
| 46.2 | 336.43 | 0.14 |
| 47.2 | 340.42 | 0.14 |
| 48.2 | 344.15 | 0.10 |
| 50.2 | 351.45 | 0.05 |
| 51.2 | 355.13 | 0.05 |

| 310 Genetic Analyzer | | |
|---|---|---|
| **Allele** | **Mean** | **Standard Deviation** |
| **TH01** | | |
| 4 | 163.29 | 0.04 |
| 5 | 167.36 | 0.03 |
| 6 | 171.40 | 0.05 |
| 7 | 175.40 | 0.03 |
| 8 | 179.38 | 0.04 |
| 9 | 183.36 | 0.05 |
| 9.3 | 186.93 | 0.02 |
| 10 | 187.29 | 0.04 |
| 11 | 191.23 | 0.03 |
| 13.3 | 201.94 | 0.05 |
| **TPOX** | | |
| 6 | 222.07 | 0.04 |
| 7 | 226.02 | 0.06 |
| 8 | 229.91 | 0.03 |
| 9 | 233.86 | 0.06 |
| 10 | 237.88 | 0.07 |
| 11 | 241.83 | 0.06 |
| 12 | 245.77 | 0.07 |
| 13 | 249.78 | 0.08 |
| **vWA** | | |
| 11 | 154.59 | 0.08 |
| 12 | 158.87 | 0.07 |
| 13 | 163.00 | 0.05 |
| 14 | 167.27 | 0.05 |
| 15 | 171.15 | 0.05 |
| 16 | 175.15 | 0.04 |
| 17 | 179.15 | 0.04 |
| 18 | 183.08 | 0.04 |
| 19 | 187.00 | 0.04 |
| 20 | 190.93 | 0.05 |
| 21 | 194.80 | 0.05 |
| 22 | 198.62 | 0.06 |
| 23 | 202.44 | 0.05 |
| 24 | 206.69 | 0.08 |

# Extra Peaks in the electropherogram

## Causes of extra peaks

To further demonstrate reproducibility, 1187 population database DNA samples have been typed using the Identifiler™ Kit. These samples have been previously genotyped with concordant results of the same loci using other AmpFℓSTR™ kits.

Peaks other than the target alleles may be detected on the electropherogram displays. Several causes for the appearance of extra peaks, including the stutter product (at the n–4 position), incomplete 3′ A nucleotide addition (at the n–1 position), artifacts, and mixed DNA samples (see "DAB 8.1.2.2 Mixture Studies" on page 91).

## Stutter products

The PCR amplification of tetranucleotide STR loci typically produces a minor product peak four bases shorter (n–4) than the corresponding main allele peak. This is referred to as the stutter peak or product. Sequence analysis of stutter products at tetranucleotide STR loci has revealed that the stutter product is missing a single tetranucleotide core repeat unit relative to the main allele (Walsh *et al.*,1996).

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. Such measurements have been made for amplified samples at the loci used in the Identifiler™ Kit. All data were generated on the 310 Genetic Analyzer.

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

- For each Identifiler™ Kit locus, the percent stutter generally increases with allele length, as shown in Figure 8 through Figure 12 on the following pages. Smaller alleles display a lower level of stutter relative to the longer alleles within each locus. This is reflected in Figure 8 through Figure 11, where minimal data points are plotted for some smaller alleles, as stutter could not be detected for many of these samples.
- For the alleles within a particular locus, the percent stutter is generally greater for the longer allele in a heterozygous sample (this is related to the first point above).
- Each allele within a locus displays percent stutter that is reproducible.
- The highest percent stutter observed for each allele is as follows: CSF1PO, 9.2%; D2S1338, 11.1%; D3S1358, 10.7%; D5S818, 6.8%; D7S820, 8.2%; D8S1179, 8.2%; D13S317, 8.0%; D16S539, 10.4%; D18S51, 17.0%; D19S433, 13.3%; D21S11, 9.4%; FGA, 14.7%; TH01, 5.1%; TPOX, 4.8% and vWA, 12.6%.
- The highest observed percent stutter for each locus is included as the filter in the GeneMapper™ *ID* Software and the GeneMapper™ *ID-X* Software. Peaks in the stutter position that are above the highest observed percent stutter will not be filtered. Peaks in the stutter position that have not been filtered and remain labeled can be further evaluated. For evaluation of mixed samples, see "Mixture studies" on page 91.

- The percent stutter does not change significantly with the recommended quantity of input DNA, for on-scale data. The measurement of percent stutter may be unusually high for main peaks that are off-scale.
- The percent stutter for allele 15 in D3S1358 (Figure 9) is artificially increased due to a reproducible artifact (Figure 4-8) observed in the green dye lanes at this position. When analyzing samples which contain a D3S1358 allele 15, we recommend careful examination due to the contribution that this identified artifact may add to the observed peak height or area. The highest percent stutter for D3S1358 is not inconclusive of allele 15.

**Figure 8** Stutter percentages for the D8S1179, D21S11, D7S820, and CSF1PO loci

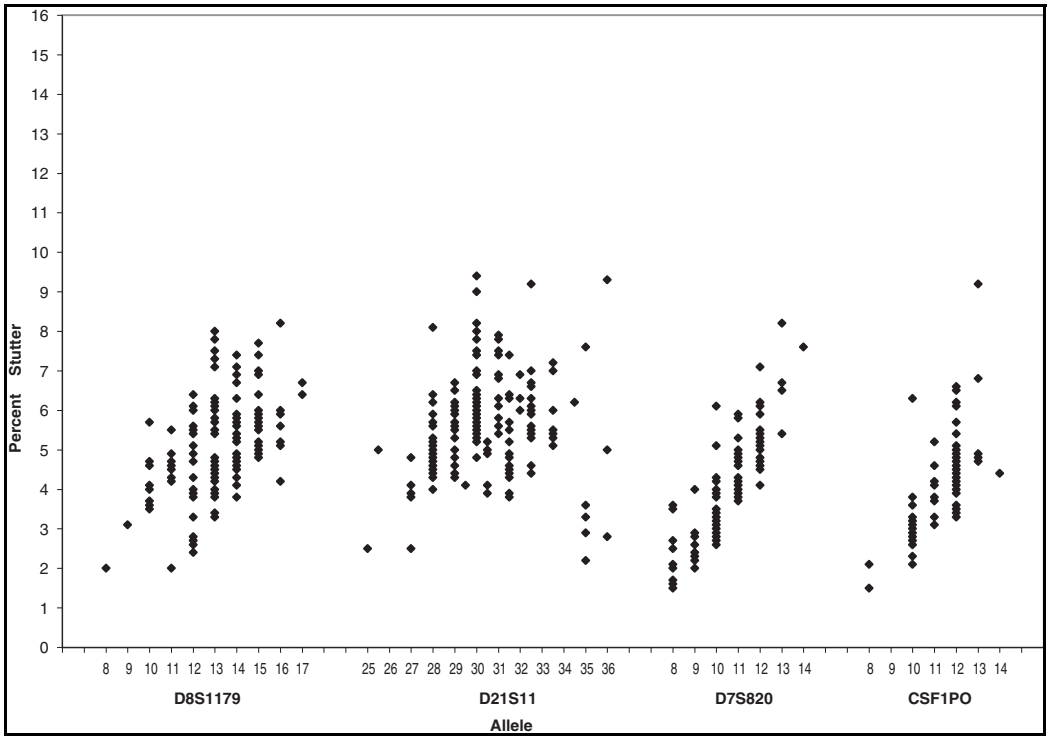


**Figure 9** Stutter percentages for the D3S1358, TH01, D13S317, D16S539, and D2S1338 loci. See the comment on page 78 regarding stutter at allele 15 of D3S1358

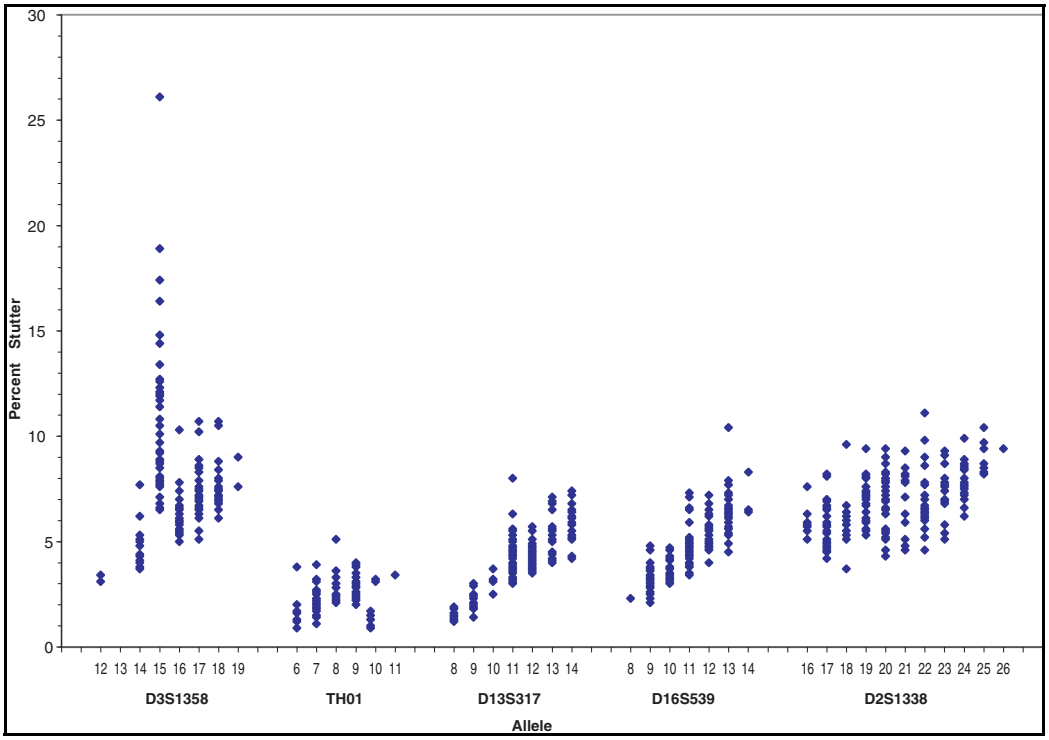



**Figure 10** Stutter percentages for the D19S433, vWA, TPOX, and D18S51 loci

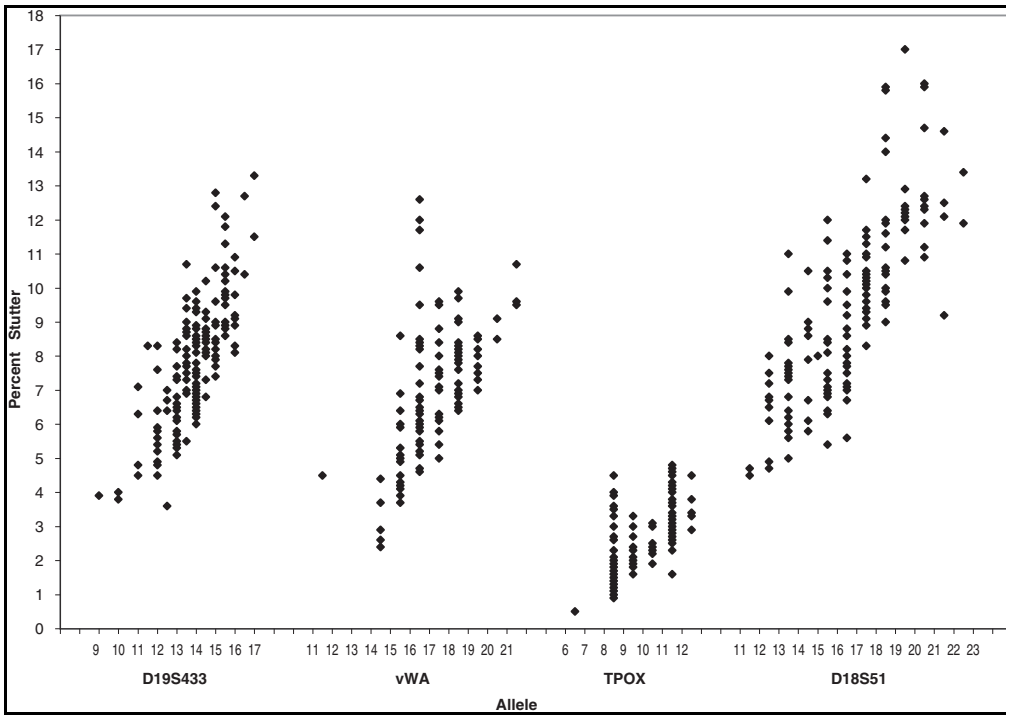


**Figure 11** Stutter percentages for the D5S818 and FGA loci

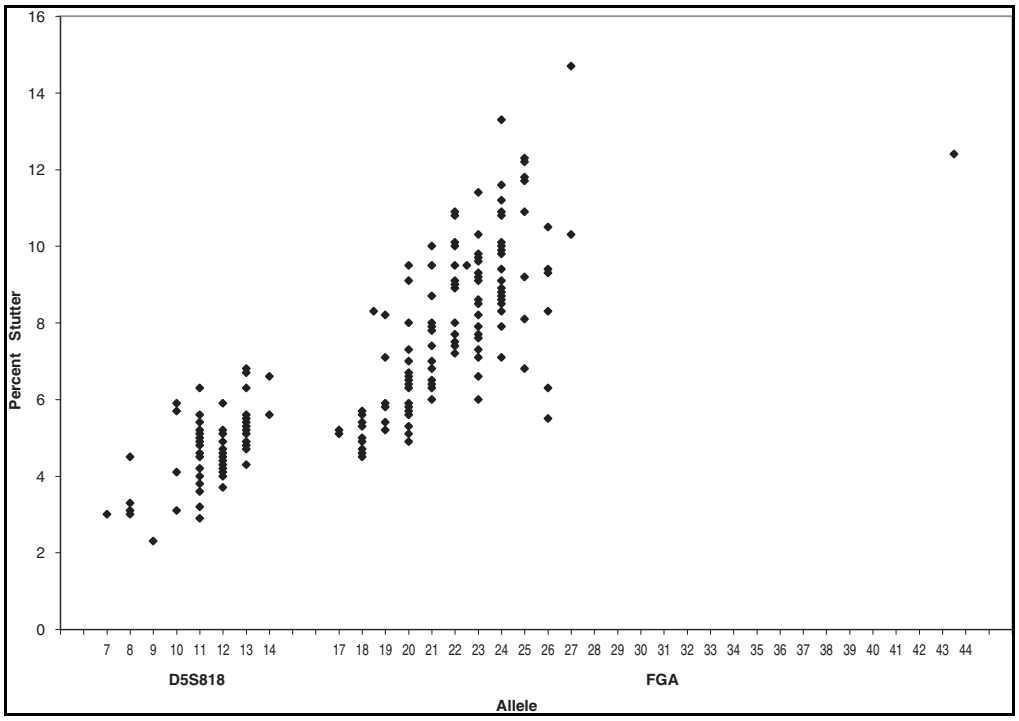
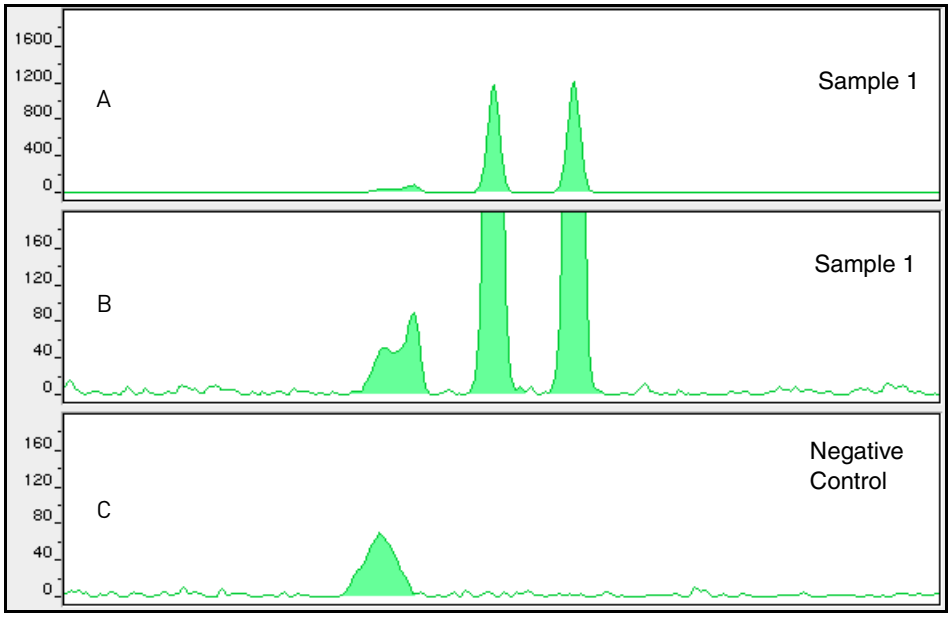



**Figure 12** Sample 1 in panel A and panel B has a profile of 15, 16 for D3S1358. The amount of stutter can not be accurately measured due to the VIC™ dye artifact. Note the degree of magnification (y-axis) used in panels B and C to illustrate the artifact. Data was produced on the 310 Genetic Analyzer.



## Addition of 3′ A nucleotide

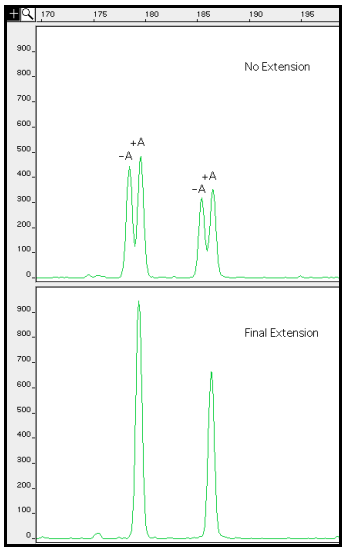
AmpliTaq Gold™ enzyme, like many other DNA polymerases, can catalyze the addition of a single nucleotide (predominately adenosine) to the 3′ ends of double-stranded PCR products (Clark, 1988; Magnuson *et al.*,1996). This non-template addition results in a PCR product that is one base pair longer than the actual target sequence, and the PCR product with the extra nucleotide is referred to as the "+A" form (Figure 13).

The efficiency of "A addition" is related to the particular sequence of the DNA at the 3′ end of the PCR product. The Identifiler™ Kit includes two main design features that promote maximum A addition:

- The primer sequences have been optimized to encourage A addition.
- The final extension step is 60°C for 60 minutes.

This final extension step gives the AmpliTaq Gold™ DNA Polymerase extra time to complete A addition to all double-stranded PCR product. STR systems that have not been optimized for maximum A addition may have "split peaks", where each allele is represented by two peaks one base pair apart.

**Figure 13** Split peaks resulting from incomplete A nucleotide addition due to omission of the 60-minute extension step




The AmpliTaq Gold™ DNA Polymerase generally requires extra time to complete the A nucleotide addition at the 3′ end of the PCR products.

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

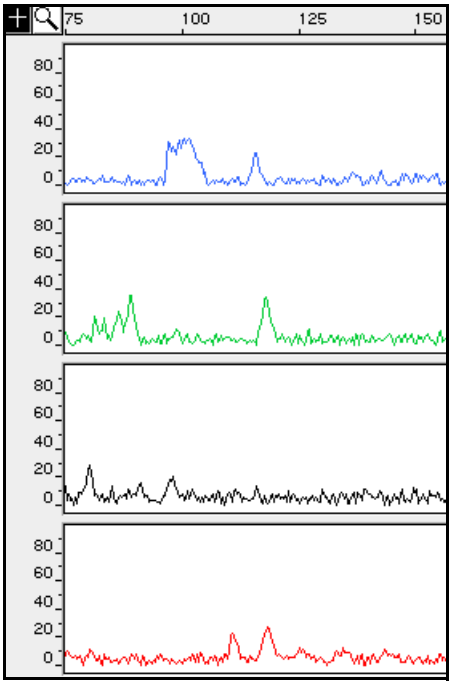
## Artifacts

Artifacts, or anomalies, have been seen in data produced on the 310 Genetic Analyzer when using the Identifiler™ Kit. The shape of these artifacts is not consistent with the shape of labeled DNA fragments as seen on the 310 Genetic Analyzer. Artifacts may or not be reproducible.

Artifacts can be intermittent and are not always reproducible. In our experience, non-reproducible artifacts can be correlated to sources other than the kit (that is, spikes). An intermittent artifact is not observed in the same position upon re-injection.

Figure 14 demonstrates reproducible artifacts while using the Identifiler™ Kit. Consider these artifacts when interpreting data.

**Figure 14** Reproducible anomalies in the blue, green, yellow, and red dye electropherograms when using the Identifiler™ Kit. Genotyping may result in the detection of these artifacts as off-ladder alleles, or "OL Alleles". Note the degree of magnification (y-axis) used in this figure to illustrate these artifacts. Data produced on the 310 Genetic Analyzer.




# Characterization of loci

## DAB 8.1.2.1 Documentation

*"Documentation exists and is available which defines and characterizes the locus."* (DAB, 1998).

## Overview

This section describes basic characteristics of the 16 loci that are amplified with the Identifiler™ Kit. These loci have been previously characterized.

## Nature of the polymorphisms

The primers for the Amelogenin locus flank a six-base pair deletion within intron 1 of the X homologue. Amplification results in 107-bp and 113-bp products from the X and Y chromosomes, respectively. (Sizes are the actual base pair size according to sequencing results, including 3' A nucleotide addition.) The remaining Identifiler™ Kit loci are all tetranucleotide short tandem repeat (STR) loci. The length differences among alleles of a particular locus result from differences in the number of 4–bp repeat units.

We have subjected to DNA sequencing some alleles in the AmpFℓSTR™ Identifiler™ Allelic Ladder containing partial repeat units in population database and nonhuman primate DNA samples (Lazaruk, *et al.*, 2001). In addition, other groups in the forensic 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 Identifiler™ Kit loci, there is consensus on the repeat patterns and structure of the STRs.

### Inheritance

The AmpFℓSTR™ loci have been validated by family studies to demonstrate their mode(s) of inheritance.

The Centre d'Etude du Polymorphisme Humain (CEPH) has collected DNA from 39 families of Utah Mormon, French Venezuelan, and Amish descent. These DNA sets have been extensively studied all over the world and are routinely used to characterize the mode of inheritance of various DNA loci. Each family set contains three generations, generally including four grandparents, two parents, and several offspring. Consequently, the CEPH family DNA sets are ideal for studying inheritance patterns (Begovich *et al.*,1992).

Four CEPH family DNA sets were examined. One and a half nanograms of DNA from each sample were amplified using the AmpFℓSTR™ SGM Plus™ kit, followed by analysis using an 377 DNA Sequencer. The families examined included #1331 (11 offspring), #13291 (9 offspring), #13292 (9 offspring), and #13294 (8 offspring), representing 37 meiotic divisions. The results confirmed that the loci are inherited according to Mendelian rules, as has been reported in the literature (Nakahori *et al.*,1991; Edwards *et al.*,1992; Kimpton *et al.*,1992; Mills *et al.*,1992; Sharma and Litt, 1992; Li *et al.*,1993; Straub *et al.*,1993).

### Mapping

The Identifiler™ Kit loci Amelogenin, CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX and vWA 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).

## Species specificity

### DAB 8.1.2.2 Species Specificity

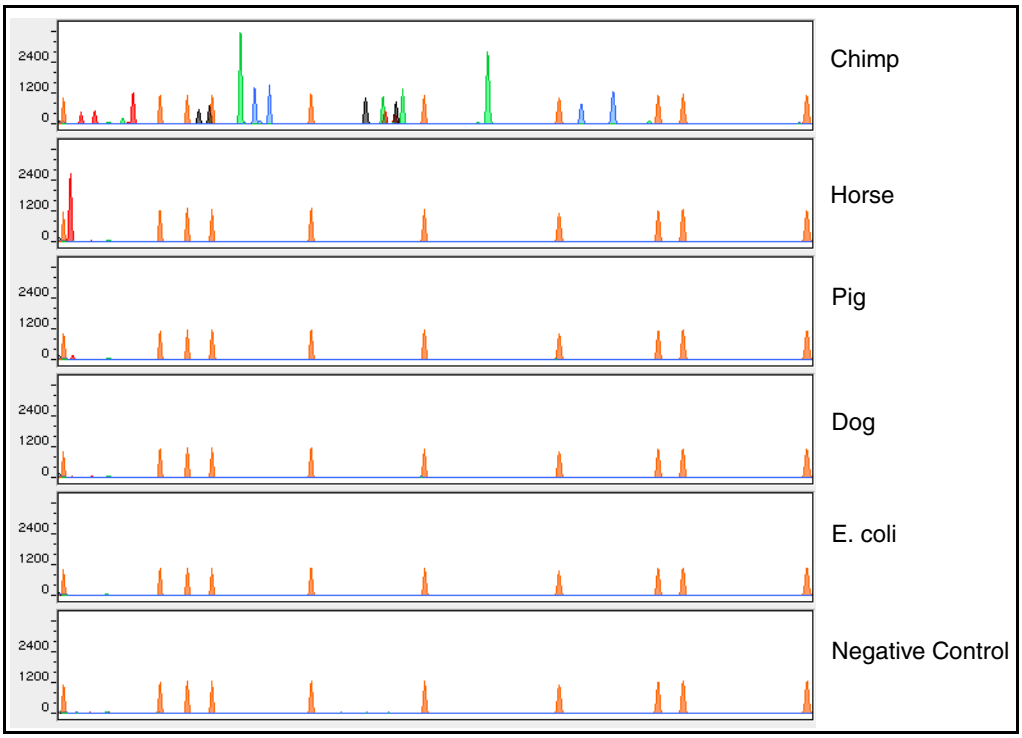
*"Species specificity, sensitivity, stability and mixture studies are conducted."* (DAB, 1998).

The Identifiler™ Kit provides the required degree of specificity such that it is specific to primates. Other species do not amplify for the loci tested, with the exception of the Amelogenin locus.

#### Nonhuman Studies

Nonhuman DNA may be present in forensic casework samples. The Identifiler™ Kit provides the required degree of specificity such that it is specific to primates for the species tested (with the exception of the Amelogenin locus) (Figure 15).

**Figure 15** Representative electropherograms of a primate, non-primates, a microorganism, and a negative control are shown. All samples were analyzed on an 310 Genetic Analyzer. The peaks shown in orange are the GeneScan™ 500 LIZ™ Size Standard.




The following experiments were conducted to investigate interpretation of Identifiler™ Kit results from nonhuman DNA sources.

The extracted DNA samples were amplified in Identifiler™ Kit reactions and analyzed using the 310 Genetic Analyzer.

- **Primates** – Gorilla, chimpanzee, orangutan, and macaque (1.0 ng each).
- **Non-primates** – Mouse, dog, pig, cat, horse, chicken and cow (2.5 ng each).
- **Bacteria and yeast** – *Brochothrix*, *Escherichia*, *Neisseria*, *Pseudomonas*, *Bacillus*, *Staphylococcus* (approximately 5 ng each), and *Saccharomyces* (1 ng).

The primate DNA samples all amplified, producing fragments within the 100–400 base pair region (Lazaruk, *et al.*, 2001; Wallin *et al.*,1998).

The microorganisms, chicken, cow, cat and mouse did not yield detectable product. Horse, dog, and pig produced a 103-bp fragment near the Amelogenin locus in PET™ dye.

# Sensitivity

## DAB 8.1.2.2 Sensitivity

*"Species specificity, sensitivity, stability and mixture studies are conducted."* (DAB, 1998).

## Effect of DNA quantity on results and importance of quantitation

The amount of input DNA added to the Identifiler™ Kit should be between 0.5 and 1.25 ng (Figure 16 on page 86). The DNA sample should be quantitated prior to amplification using a system such as the Quantifiler™ Human DNA Quantitation Kit (Part No. 4343895). The final DNA concentration should be in the range of 0.05–0.125 ng/µL so that 0.5–1.25 ng of DNA will be added to the PCR reaction in a volume of 10 µL. If the sample contains degraded DNA, amplification of additional DNA may be beneficial.

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

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

  Off-scale data is a problem for two reasons:

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

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

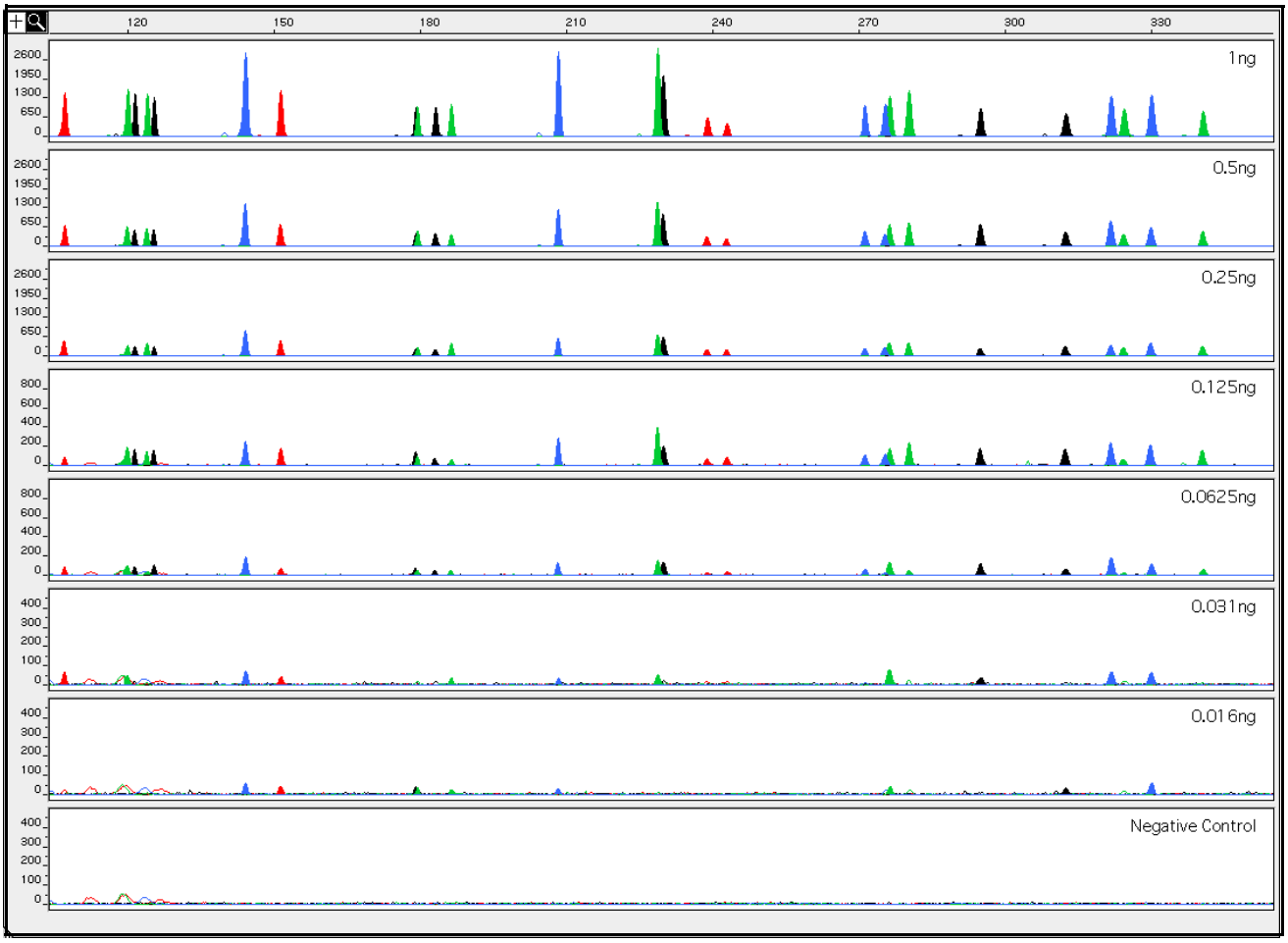
- Incomplete A nucleotide addition.

The sample can be re-amplified using less DNA.

When the total number of allele copies added to the PCR is extremely low, unbalanced amplification of the two alleles of a heterozygous individual may occur (Walsh *et al.*,1992; Wallin *et al.*,1998) due to stochastic fluctuation in the ratio of the two different alleles (Sensabaugh *et al.*,1991). The PCR cycle number and amplification conditions have been specified to produce low peak heights for a sample containing 20 pg human genomic DNA. Low peak heights should be interpreted with caution.

Individual laboratories may find it useful to determine an appropriate minimum peak height threshold based on their own results and instruments using low amounts of input DNA.

**Figure 16** Effect of amplifying various amounts of DNA ranging from 16 pg to 1 ng. Note that the y-axis scale is magnified for the lower amounts of DNA. Data analyzed using the 310 Genetic Analyzer




# Stability

## DAB 8.1.2.2 Stability

*"Species specificity, sensitivity, stability and mixture studies are conducted."* (DAB, 1998).

## Lack of amplification of some loci

As with any multi-locus system, the possibility exists that not every locus will amplify. This is most often observed when the DNA sample contains PCR inhibitors or when the DNA sample has been severely degraded. Because each locus is an independent marker whose results are not based upon information provided by the other markers, results generally can still be obtained from the loci that do amplify.

## Differential and preferential amplification

Differential amplification can be defined as the difference in the degree of amplification of each locus within a co-amplified system, such that one or more loci may amplify to a greater extent compared to the other loci. Preferential amplification is used in this guide to describe differences in the amplification efficiency of two alleles at a single locus.

Preferential amplification of alleles in systems that distinguish alleles based on length polymorphisms is most likely to be observed when the alleles differ significantly in base pair size. Since most Identifiler™ Kit loci have small size ranges, the potential for preferential amplification of alleles is low.

## Effect of inhibitors

Heme compounds have been identified as PCR inhibitors in DNA samples extracted from bloodstains (DeFranchis *et al.*,1988; Akane *et al.*, 1994). It is believed that the inhibitor is co-extracted and co-purified with the DNA and subsequently interferes with PCR by inhibiting polymerase activity.

Bovine serum albumin (BSA) can prevent or minimize the inhibition of PCR, most likely by binding to the inhibitor (Comey *et al.*, 1994). Since the presence of BSA can improve the amplification of DNA from blood-containing samples, BSA has been included in the AmpFℓSTR™ PCR Reaction Mix at 4 µg per 25-µL amplification. BSA has also been identified as an aid in overcoming inhibition from samples containing dyes, such as in denim (Comey *et al.*, 1994).

To examine the effects of hematin on the amplification results obtained by the Identifiler™ Kit, DNA samples were amplified using the Identifiler™ Kit reagents (including the BSA-containing PCR reaction mix) in the presence of varying concentrations of purified hematin. The concentrations of hematin used were 0 µM, 10 µM, 12 µM, 14 µM, 16 µM, 18 µM, and 20 µM. When the amount of hematin was increased to a concentration that started to inhibit the PCR, CSF1PO and D2S1338 were the first loci to exhibit decreased amplification, followed by D16S539 and D18S51.

Differential amplification was observed in the presence of increasing amounts of hematin. Moreover, as the concentration of hematin was increased, the overall yield of products was reduced particularly for the larger loci.

**Figure 17** DNA amplified with the Identifiler™ Kit in the presence of varying concentrations of hematin: 0, 10 µM, 12 µM, 14 µM, 16 µM, 18 µM, and 20 µM, analyzed on the 310 Genetic Analyzer


## Degraded DNA

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

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

Four nanograms of degraded DNA (or 1 ng undegraded DNA) was amplified using the Identifiler™ Kit (all 16 primer pairs together). As the DNA became increasingly degraded the loci became undetectable according to size. Preferential amplification was not observed. The loci failed to robustly amplify in the order of decreasing size as the extent of degradation progressed: CSF1PO and D2S1338 were the first loci to exhibit decreased amplification, followed by D16S539 and D18S51 and so forth. A similar result at each time point was obtained whether the DNA samples were amplified for each locus alone or co-amplified with the Identifiler™ Kit (Figure 18 on page 89).

**Figure 18** Multiplex amplifications of a DNA sample in the absence of DNase I and the sample incubated for 30 sec, 1 min, 4 min, and 8 min with DNase I, analyzed using the 310 Genetic Analyzer

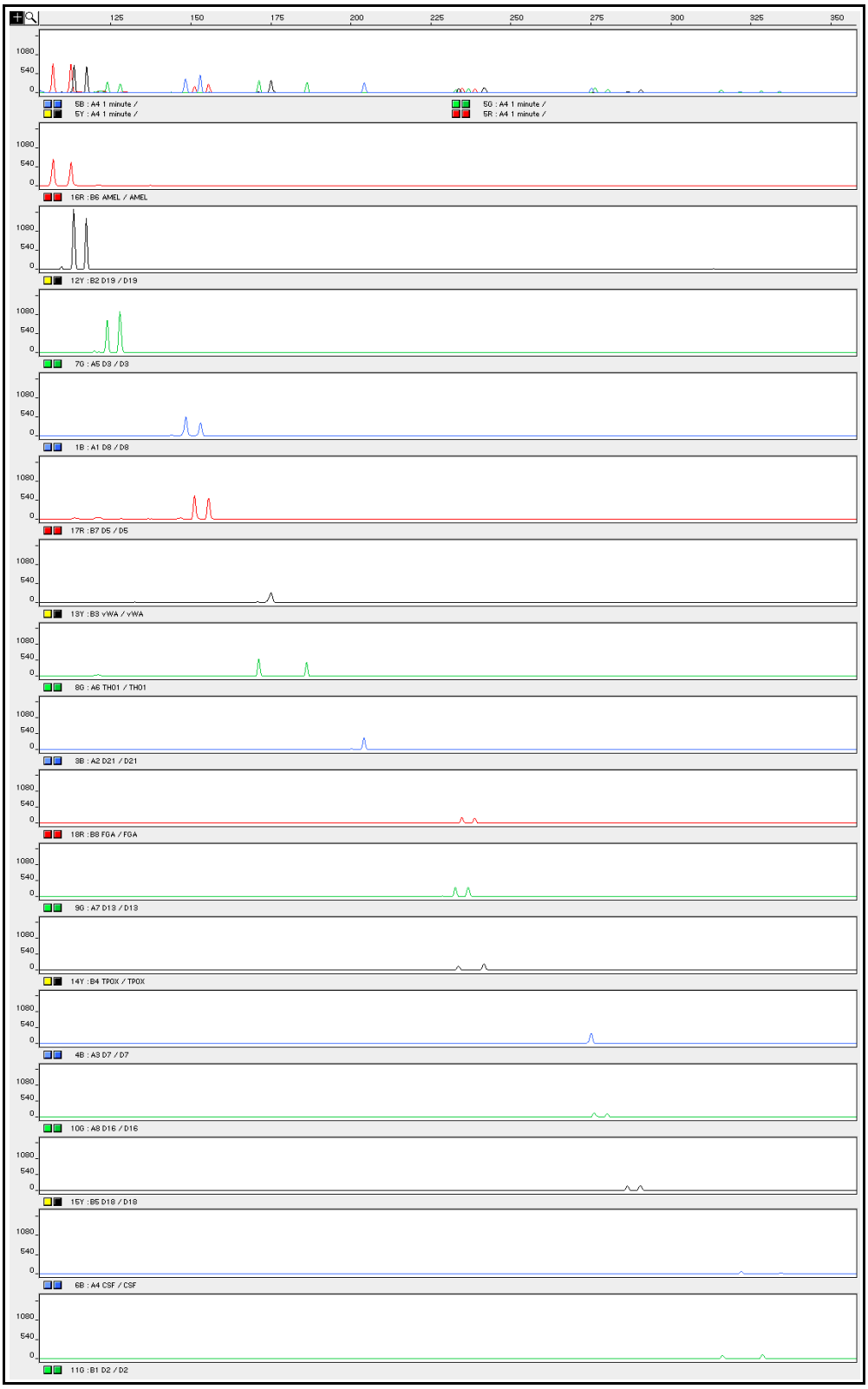

## Multiplex amplifications

DNA samples were amplified in 16 separate reactions containing primers for only one Identifiler™ Kit locus (singleplex) and a reaction containing all primers for the Identifiler™ Kit loci (multiplex). DNA used as PCR template consisted of a sample that had been degraded for 1 min with DNase I.

Amplified samples were analyzed using the 310 Genetic Analyzer. Similar results were obtained (genotype and peak height) whether the DNA samples were amplified for each locus alone or co-amplified in the Identifiler™ Kit reaction(Figure 19 on page 90).

When degraded DNA is suspected to have compromised amplification of one or more loci, the molecular weight of the DNA can be assessed by agarose gel analysis. If the DNA is degraded to an average of 400 base pairs in size or less, adding more DNA template to the Identifiler™ Kit amplification reaction may help produce a typeable signal for the loci. Adding more DNA to the amplification may provide more of the necessary size template for amplification.

**Figure 19** Multiplex and singleplex amplifications of a DNA sample incubated for 1 min with DNase I, analyzed on the 310 Genetic Analyzer



# Mixture studies

## DAB 8.1.2.2 Mixture Studies

*"Species specificity, sensitivity, stability and mixture studies are conducted."* (DAB, 1998).

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

## Mixed specimen studies

Evidence samples that contain body fluids and/or tissues originating from more than one individual are an integral component of forensic casework. Therefore it is essential to ensure that the DNA typing system is able to detect DNA mixtures. In the case of STRs, stutter peaks may be informative in the interpretation of mixed samples. Furthermore, alleles amplified with the Identifiler™ Kit have similar peak height values for a heterozygous genotype within a locus. This balance can be used as an aid in detecting and interpreting mixtures.

### Detection of mixed samples

Each of the following can aid in determining whether a sample is a mixture:

- The presence of more than two alleles at a locus.
- The presence of a peak at a stutter position that is significantly greater in percentage than what is typically observed in a single-source sample.
- Significantly imbalanced alleles for a heterozygous genotype.
  The peak height ratio is defined as the height of the lower peak (in RFU) divided by the height of the higher peak (in RFU), expressed as a percentage. Mean, median, and minimum and maximum peak height ratios observed for alleles in the Identifiler™ Kit loci in unmixed population database samples are listed in Table 4.

**Table 4** Peak height ratios

| Allele | Number of Observations (n) | Mean† | Median† | Minimum† | Maximum† |
|---|---|---|---|---|---|
| CSF1PO | 84 | 86 | 88 | 63.6 | 99.8 |
| D2S1338 | 93 | 84 | 86 | 42.8 | 99.7 |
| D3S1358 | 91 | 88 | 90 | 64.3 | 99.7 |
| D5S818 | 82 | 89 | 91 | 64.9 | 99.7 |
| D7S820 | 96 | 89 | 90 | 66.2 | 99.5 |
| D8S1179 | 89 | 90 | 93 | 57.5 | 99.8 |
| D13S317 | 96 | 87 | 87 | 63.3 | 100.0 |
| D16S539 | 92 | 88 | 91 | 61.5 | 99.9 |
| D18S51 | 99 | 82 | 83 | 56.3 | 99.9 |
| D19S433 | 98 | 88 | 92 | 48.8 | 100.0 |
| D21S11 | 92 | 88 | 89 | 66.4 | 99.6 |
| FGA | 94 | 85 | 87 | 60.9 | 99.5 |

| Allele | Number of Observations (n) | Mean† | Median† | Minimum† | Maximum† |
|---|---|---|---|---|---|
| TH01 | 99 | 86 | 88 | 48.8 | 99.9 |
| TPOX | 87 | 87 | 92 | 55.9 | 99.8 |
| vWA | 101 | 86 | 88 | 62.8 | 99.1 |

† Peak height ratios were determined for those heterozygous samples with peak heights > 200 RFU.

For all 15 loci, the mean peak height ratios indicate that the two alleles of a heterozygous individual are generally very well balanced.

If an unusually low peak height ratio is observed for one locus and there are no other indications that the sample is a mixture, the sample may be reamplified and reanalyzed to determine if the imbalance is reproducible. Possible causes of imbalance at a locus are degraded DNA, presence of inhibitors, extremely low amounts of input DNA, or the presence of an allele containing a rare sequence that does not amplify as efficiently as the other allele.

### Resolution of genotypes in mixed samples

A sample containing DNA from two sources can be comprised (at a single locus) of any of the seven genotype combinations listed below.

- Heterozygote + heterozygote, no overlapping alleles (four peaks)
- Heterozygote + heterozygote, one overlapping allele (three peaks)
- Heterozygote + heterozygote, two overlapping alleles (two peaks)
- Heterozygote + homozygote, no overlapping alleles (three peaks)
- Heterozygote + homozygote, overlapping allele (two peaks)
- Homozygote + homozygote, no overlapping alleles (two peaks)
- Homozygote + homozygote, overlapping allele (one peak)

Specific genotype combinations and input DNA ratios of the samples contained in a mixture determine whether it is possible to resolve the genotypes of the major and minor component(s) at a single locus.

The ability to obtain and compare quantitative values for the different allele peak heights on Applied Biosystems instruments provides additional valuable data to aid in resolving mixed genotypes. This quantitative value is much less subjective than comparing relative intensities of bands on a stained gel.

Ultimately, the likelihood that any sample is a mixture must be determined by the analyst in the context of each particular case, including the information provided from known reference sample(s).

### Limit of detection of the minor component

Mixtures of two DNA samples were examined at various ratios (1:1 to 1:10). The total amount of genomic input DNA mixed at each ratio was 1 ng.

The samples were amplified in a GeneAmp™ PCR System 9700 with a silver or gold-plated silver block and were electrophoresed and detected using a 310 Genetic Analyzer.

The results of the mixed DNA samples are shown in Figure 20, where sample A and sample B were mixed according to the ratios provided.

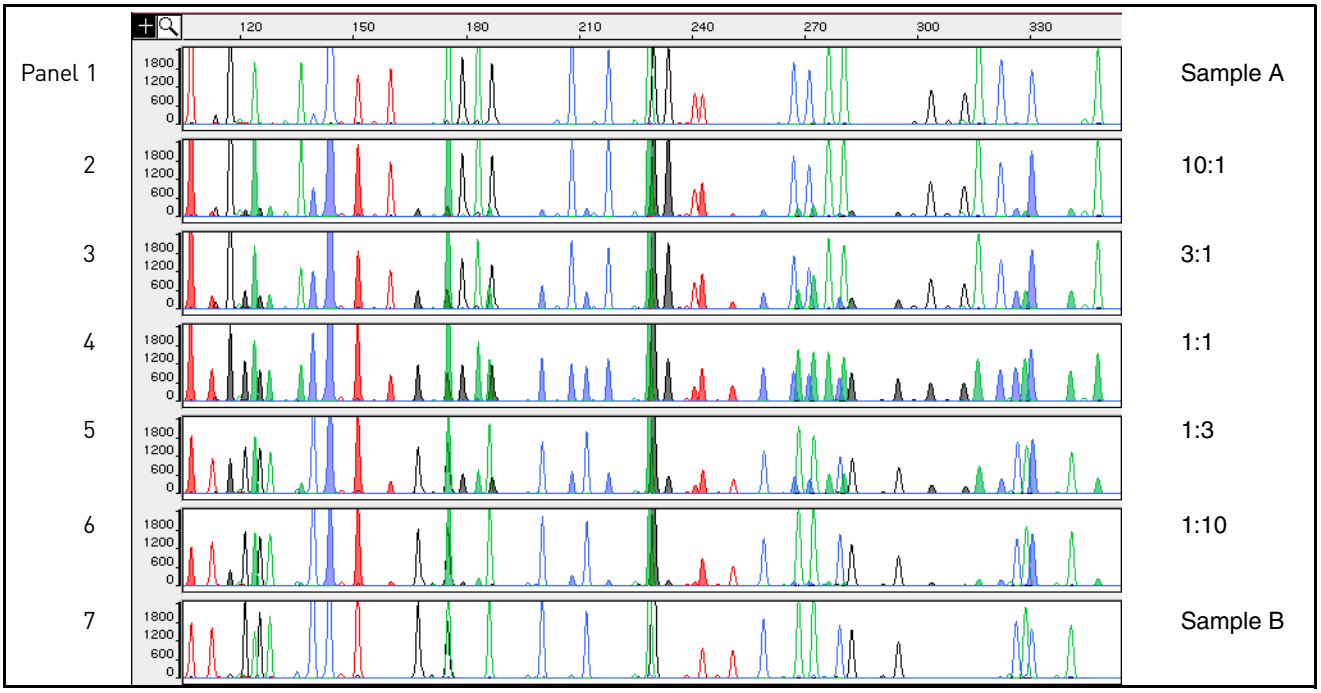
The profiles of the samples in Figure 20 are listed in Table 5.

**Table 5** Mixture profiles

| Allele | Profile | |
|---|---|---|
| | Sample A | Sample B |
| Amelogenin | X | X, Y |
| CSF1PO | 10, 12 | 11,12 |
| D2S1338 | 17, 25 | 20, 23 |
| D3S1358 | 15, 18 | 15,16 |
| D5S818 | 11, 13 | 11 |
| D7S820 | 9, 10 | 7,12 |
| D8S1179 | 13 | 12,13 |
| D13S317 | 11 | 11 |
| D16S539 | 11, 12 | 9, 10 |
| D18S51 | 17, 19 | 12, 15 |
| D19S433 | 13 | 14,15 |
| D21S11 | 30, 30.2 | 28, 31 |
| FGA | 23.2, 24 | 24, 26 |
| TH01 | 7, 9 | 7, 9.3 |
| TPOX | 8, 9 | 8 |
| vWA | 17, 19 | 14,16 |

For these 1-ng total DNA mixture studies, the limit of detection is when the minor component is present at approximately one-tenth of the concentration of the major component and a threshold of 50 RFU. The limit of detection for the minor component is influenced by the combination of genotypes in the mixture.

**Figure 20** Results of the two DNA samples mixed together at defined ratios and amplified with the Identifiler™ Kit. Sample A and Sample B are a female and male sample, respectively. The ratios of Sample A to Sample B (A:B ratios) shown are 10:1, 3:1, 1:1, 1:3, and 1:10, respectively. The alleles attributable to the minor component, even when the major component shares an allele, are highlighted in panels 2, 3, 5, and 6. All alleles are highlighted in panel 4.




# Data interpretation

## Minimum sample requirement

The Identifiler™ Kit has been optimized to reliably amplify and type approximately 0.5–1.25 ng of sample DNA.

The PCR cycle number and amplification conditions have been specified to produce low peak heights for a sample containing 20 pg human genomic DNA. Thus, the overall sensitivity of the assay has been adjusted to avoid or minimize stochastic effects. We have successfully typed samples containing less than 0.5 ng DNA.

**Note:** Individual laboratories may find it useful to determine an appropriate minimum peak height threshold based on their own results/instruments using low amounts of input DNA.

# Population data

## DAB 8.1.2.3 Population Data

*"Population distribution data are documented and available."* (DAB, 1998).

## DAB 8.1.2.3.1 Population Distribution Data

*"The population distribution data would include the allele and genotype distributions for the locus or loci obtained from relevant populations. Where appropriate, databases should be tested for independence expectations."* (DAB, 1998).

## Overview

To interpret the significance of a match between genetically typed samples, it is necessary to 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 suspect's reference sample, then the suspect is "excluded" as the donor of the biological evidence tested. An exclusion is independent of the frequency of the two genotypes in the population.

If the suspect and evidence samples have the same genotype, 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 population(s).

## Population samples used in these studies

The Identifiler™ Kit, prior to the addition of the D8S1179 degenerate primer, was used to generate the population data provided in this section. Samples were collected from individuals throughout the United States with no geographical preference.

| Population | Number of samples | Samples provided by |
|---|---|---|
| African-American | 357 | Kentucky State Police and the Federal Bureau of Investigation |
| U.S. Caucasian | 349 | |
| U.S. Hispanic | 290 | Minnesota Bureau of Criminal Apprehension/Memorial Blood Center of Minneapolis |
| Native American | 191 | |

## Allele frequencies

Table 6 shows the Identifiler™ Kit allele frequencies in four populations, listed as percentages.

**Table 6** Identifiler™ Kit allele frequencies

| Allele | African-American (n = 357) | U.S. Caucasian (n = 349) | U.S. Hispanic (n = 290) | Native American (n = 191) |
|---|---|---|---|---|
| **CSF1PO** | | | | |
| 6 | † | † | † | † |
| 7 | 4.62 | 0.14† | 0.34† | † |
| 8 | 7.56 | 0.29† | 0.17† | 0.52† |
| 9 | 3.78 | 1.72 | 0.86† | 8.38 |
| 10 | 27.87 | 24.21 | 23.10 | 30.89 |
| 11 | 20.59 | 31.81 | 28.28 | 21.99 |
| 11.3 | 0.14† | † | † | † |
| 12 | 29.13 | 32.81 | 39.66 | 32.72 |
| 13 | 5.32 | 7.31 | 6.38 | 4.71 |
| 14 | 0.98 | 1.43 | 0.86† | 0.79† |
| 15 | † | 0.29† | 0.34† | † |
| **D2S1338** | | | | |
| 15 | 0.14† | † | † | † |
| 16 | 5.32 | 4.73 | 2.41 | 2.62 |

| Allele | African-American (n = 357) | U.S. Caucasian (n = 349) | U.S. Hispanic (n = 290) | Native American (n = 191) |
|---|---|---|---|---|
| 17 | 10.78 | 17.34 | 21.21 | 9.95 |
| 18 | 5.60 | 6.30 | 4.14 | 7.07 |
| 19 | 14.15 | 13.75 | 22.76 | 29.58 |
| 20 | 6.02 | 14.61 | 13.79 | 9.69 |
| 21 | 14.01 | 2.58 | 2.59 | 2.36 |
| 22 | 13.17 | 4.01 | 7.41 | 15.18 |
| 23 | 10.78 | 11.46 | 11.38 | 11.78 |
| 24 | 9.80 | 11.75 | 8.45 | 7.85 |
| 25 | 8.12 | 10.60 | 5.17 | 3.14 |
| 26 | 1.96 | 2.72 | 0.69† | 0.79† |
| 27 | 0.14† | 0.14† | † | † |
| 28 | † | † | † | † |

| Allele | African-American (n = 357) | U.S. Caucasian (n = 349) | U.S. Hispanic (n = 290) | Native American (n = 191) |
|---|---|---|---|---|
| **D3S1358** | | | | |
| <11 | 0.42† | 0.14† | † | † |
| 11 | † | † | † | 0.26† |
| 12 | 0.56† | † | 0.17† | † |
| 13 | 0.70† | 0.29† | 0.17† | † |
| 14 | 12.04 | 15.76 | 7.41 | 6.81 |
| 15 | 30.53 | 25.36 | 39.14 | 40.84 |
| 15.2 | 0.14† | † | † | † |
| 16 | 28.57 | 22.78 | 26.72 | 32.98 |
| 17 | 19.47 | 18.19 | 16.03 | 9.95 |
| 18 | 6.72 | 16.48 | 8.97 | 8.38 |
| 19 | 0.84 | 1.00 | 1.03 | 0.79† |
| 20 | † | † | 0.34† | † |
| **D5S818** | | | | |
| 7 | 0.14† | † | 6.72 | 15.71 |
| 8 | 5.46 | † | 0.69† | † |
| 9 | 1.68 | 4.15 | 5.17 | 6.02 |
| 10 | 6.72 | 5.44 | 5.17 | 4.19 |
| 11 | 25.49 | 39.26 | 39.14 | 41.10 |
| 12 | 36.41 | 35.24 | 29.31 | 23.30 |
| 13 | 21.57 | 15.47 | 12.59 | 9.42 |
| 14 | 2.38 | 0.14† | 0.69† | 0.26† |
| 15 | † | 0.29† | 0.18† | † |
| 16 | † | † | 0.17† | † |
| 17 | 0.14† | † | 0.17† | † |

| Allele | African-American (n = 357) | U.S. Caucasian (n = 349) | U.S. Hispanic (n = 290) | Native American (n = 191) |
|---|---|---|---|---|
| **D7S820** | | | | |
| 6 | † | 0.14† | 0.17† | † |
| 7 | 0.42† | 1.29 | 1.72 | 0.52† |
| 8 | 18.77 | 16.48 | 11.72 | 13.09 |
| 9 | 13.73 | 17.62 | 6.21 | 8.12 |
| 10 | 34.45 | 27.22 | 27.41 | 21.99 |
| 11 | 19.89 | 18.05 | 28.79 | 28.80 |
| 12 | 10.78 | 14.76 | 20.17 | 24.08 |
| 13 | 1.54 | 3.72 | 3.45 | 3.40 |
| 14 | 0.42† | 0.72 | 0.34† | † |
| 15 | † | † | † | † |
| **D8S1179** | | | | |
| 8 | 0.42† | 2.29 | 0.34† | 0.52† |
| 9 | 0.42† | 1.15 | 0.34† | 0.26† |
| 10 | 2.38 | 9.74 | 8.45 | 4.71 |
| 11 | 3.92 | 6.02 | 5.86 | 3.40 |
| 12 | 13.31 | 14.04 | 12.07 | 11.52 |
| 13 | 23.25 | 32.52 | 32.93 | 37.43 |
| 14 | 30.11 | 21.35 | 26.21 | 30.63 |
| 15 | 20.17 | 9.89 | 10.86 | 9.42 |
| 16 | 4.62 | 2.72 | 2.41 | 1.57 |
| 17 | 1.12 | 0.29† | 0.52† | 0.52† |
| 18 | 0.28† | † | † | † |
| 19 | † | † | † | † |
| **D13S317** | | | | |
| 8 | 3.08 | 12.18 | 9.66 | 4.97 |
| 9 | 2.52 | 7.74 | 21.72 | 17.80 |
| 10 | 3.78 | 4.44 | 9.14 | 13.61 |
| 11 | 24.51 | 29.80 | 23.10 | 24.35 |
| 12 | 46.22 | 30.80 | 20.86 | 23.04 |
| 13 | 15.41 | 11.17 | 10.17 | 7.85 |
| 14 | 4.34 | 3.72 | 5.34 | 8.12 |
| 15 | 0.14† | 0.14† | † | 0.26† |

| Allele | African-American (n = 357) | U.S. Caucasian (n = 349) | U.S. Hispanic (n = 290) | Native American (n = 191) |
|---|---|---|---|---|
| **D16S539** | | | | |
| 5 | † | † | † | † |
| 8 | 3.22 | 1.72 | 1.72 | 0.79† |
| 9 | 19.05 | 10.46 | 9.31 | 12.30 |
| 10 | 10.92 | 5.59 | 15.69 | 15.45 |
| 11 | 31.51 | 31.95 | 30.17 | 30.89 |
| 12 | 18.77 | 30.23 | 29.48 | 27.75 |
| 13 | 14.85 | 16.76 | 11.55 | 10.73 |
| 14 | 1.54 | 3.01 | 2.07 | 2.09 |
| 15 | 0.14† | 0.29† | † | † |
| **D18S51** | | | | |
| 7 | † | † | † | † |
| 9 | 0.14† | † | † | † |
| 10 | 0.28† | 0.86 | 0.52† | 0.79† |
| 10.2 | 0.14† | † | † | † |
| 11 | 0.28† | 1.15 | 1.21 | † |
| 12 | 7.00 | 13.90 | 10.34 | 14.92 |
| 13 | 4.34 | 12.18 | 14.48 | 9.16 |
| 13.2 | 0.42† | † | † | † |
| 14 | 6.86 | 16.76 | 15.52 | 26.96 |
| 14.2 | 0.28† | † | † | † |
| 15 | 19.47 | 13.61 | 16.55 | 12.04 |
| 16 | 16.53 | 13.61 | 11.72 | 10.73 |
| 17 | 18.21 | 12.32 | 14.14 | 14.66 |
| 18 | 11.90 | 7.74 | 6.72 | 2.62 |
| 19 | 6.02 | 4.44 | 4.14 | 3.93 |
| 20 | 4.90 | 1.72 | 2.24 | 1.83 |
| 21 | 2.10 | 1.00 | 1.03 | 1.31 |
| 22 | 0.70† | 0.43† | 0.52† | 0.79† |
| 23 | 0.42† | 0.14† | 0.52† | 0.26† |
| 24 | † | 0.14† | 0.17† | † |
| 25 | † | † | 0.17† | † |
| 26 | † | † | † | † |
| 27 | † | † | † | † |

| Allele | African-American (n = 357) | U.S. Caucasian (n = 349) | U.S. Hispanic (n = 290) | Native American (n = 191) |
|---|---|---|---|---|
| **D19S433** | | | | |
| 9 | † | 0.14† | 0.17† | † |
| 10 | 1.54 | † | † | † |
| 11 | 7.14 | 0.72 | 0.52† | 0.52† |
| 11.2 | 0.14† | † | 0.17† | † |
| 12 | 10.78 | 7.74 | 6.21 | 3.14 |
| 12.2 | 6.30 | 0.57† | 1.90 | † |
| 13 | 29.83 | 28.94 | 16.03 | 17.80 |
| 13.2 | 5.74 | 1.72 | 8.62 | 15.45 |
| 14 | 21.01 | 34.10 | 31.72 | 24.87 |
| 14.2 | 4.20 | 0.86 | 5.00 | 3.66 |
| 15 | 4.76 | 15.76 | 13.45 | 13.35 |
| 15.2 | 3.36 | 2.72 | 8.79 | 10.73 |
| 16 | 2.38 | 4.15 | 4.31 | 3.93 |
| 16.2 | 2.38 | 1.72 | 2.93 | 1.83 |
| 17 | † | 0.29† | 0.17† | 0.79† |
| 17.2 | 0.28† | 0.29† | † | 2.88 |
| 18.2 | 0.14† | 0.29† | † | 1.05† |

| Allele | African-American (n = 357) | U.S. Caucasian (n = 349) | U.S. Hispanic (n = 290) | Native American (n = 191) |
|---|---|---|---|---|
| **D21S11** | | | | |
| 24 | † | † | † | † |
| 24.2 | 0.14† | 0.43† | 0.17† | † |
| 24.3 | 0.28† | † | † | † |
| 25 | † | † | † | † |
| 25.2 | † | 0.14† | 0.17† | † |
| 26 | 0.14† | 0.14† | 0.17† | † |
| 27 | 5.04 | 4.58 | 1.21 | 0.52† |
| 28 | 22.97 | 16.76 | 9.14 | 6.28 |
| 28.2 | † | † | † | † |
| 29 | 19.33 | 20.49 | 21.21 | 16.75 |
| 29.2 | 0.14† | † | 0.52† | 0.26† |
| 29.3 | 0.14† | † | † | † |
| 30 | 17.23 | 25.21 | 29.31 | 34.29 |
| 30.2 | 1.40 | 3.30 | 2.93 | 1.83 |
| 31 | 7.98 | 7.16 | 6.72 | 5.76 |
| 31.2 | 7.98 | 9.46 | 8.62 | 18.85 |
| 32 | 1.12 | 1.43 | 1.55 | 0.79† |
| 32.2 | 5.88 | 7.16 | 12.93 | 9.69 |
| 33 | 0.56† | † | † | 0.52† |
| 33.2 | 3.78 | 3.30 | 4.14 | 3.66 |
| 34 | 1.26 | † | † | † |
| 34.1 | 0.14† | † | † | † |
| 34.2 | 0.14† | 0.29† | 0.86† | 0.79† |
| 35 | 2.94 | † | 0.34† | † |
| 35.1 | 0.14† | † | † | † |
| 35.2 | † | 0.14† | † | † |
| 36 | 0.84 | † | † | † |
| 37 | 0.28† | † | † | † |
| 38 | 0.14† | † | † | † |

| Allele | African-American (n = 357) | U.S. Caucasian (n = 349) | U.S. Hispanic (n = 290) | Native American (n = 191) |
|---|---|---|---|---|
| **FGA** | | | | |
| 16 | † | 0.14† | † | † |
| 16.1 | 0.14† | † | † | † |
| 17 | † | 0.29† | 0.17† | † |
| 17.2 | 0.14† | † | † | † |
| 18 | 0.70† | 2.72 | 0.52† | 1.31 |
| 18.2 | 1.40 | † | † | † |
| 19 | 6.72 | 6.16 | 7.07 | 10.21 |
| 19.2 | 0.28† | † | † | † |
| 20 | 7.00 | 13.90 | 7.41 | 12.30 |
| 20.2 | † | 0.14† | † | † |
| 21 | 12.89 | 16.91 | 14.66 | 12.83 |
| 21.2 | † | 0.29† | 0.17† | † |
| 22 | 21.57 | 16.91 | 17.24 | 10.47 |
| 22.2 | 0.28† | 1.29 | 0.34† | 0.26† |
| 22.3 | 0.14† | † | † | † |
| 23 | 14.99 | 15.19 | 11.90 | 15.97 |
| 23.2 | 0.14† | † | 0.86† | 0.26† |
| 24 | 17.51 | 13.75 | 15.34 | 15.71 |
| 24.2 | † | 0.14† | 0.17† | † |
| 25 | 7.98 | 8.60 | 14.14 | 14.14 |
| 26 | 3.50 | 2.72 | 6.90 | 4.45 |
| 26.2 | † | † | † | 0.52 |
| 27 | 1.82 | 0.72 | 2.41 | 0.79† |
| 28 | 1.40 | 0.14† | 0.69† | 0.52† |
| 29 | 0.56† | † | † | † |
| 30 | † | † | † | † |
| 30.2 | 0.14† | † | † | † |
| 31.2 | † | † | † | † |
| 32.2 | † | † | † | † |
| 33.2 | † | † | † | † |
| 34.2 | 0.14† | † | † | † |
| 42.2 | † | † | † | † |
| 43.2 | † | † | † | † |
| 44.2 | 0.28† | † | † | † |
| 45.2 | † | † | † | 0.26† |
| 46.2 | 0.14† | † | † | † |

| Allele | African-American (n = 357) | U.S. Caucasian (n = 349) | U.S. Hispanic (n = 290) | Native American (n = 191) |
|---|---|---|---|---|
| **FGA (continued)** | | | | |
| 47.2 | † | † | † | † |
| 48.2 | 0.14† | † | † | † |
| 50.2 | † | † | † | † |
| 51.2 | † | † | † | † |
| **TH01** | | | | |
| 4 | † | † | † | † |
| 5 | 0.28† | 0.43† | 0.17† | † |
| 6 | 11.06 | 20.49 | 22.76 | 20.68 |
| 7 | 42.86 | 21.78 | 33.62 | 43.98 |
| 8 | 20.73 | 11.46 | 8.45 | 5.24 |
| 8.3 | † | 0.14† | † | † |
| 9 | 12.32 | 16.19 | 14.14 | 6.28 |
| 9.3 | 11.62 | 29.08 | 20.34 | 23.56 |
| 10 | 0.98 | 0.43† | 0.52† | 0.26† |
| 11 | † | † | † | † |
| 13.3 | 0.14† | † | † | † |
| **TPOX** | | | | |
| 6 | 6.72 | 0.14† | 0.34† | † |
| 7 | 2.24 | † | 0.34† | 0.26† |
| 8 | 36.13 | 53.30 | 49.66 | 37.96 |
| 9 | 21.15 | 11.60 | 7.24 | 4.19 |
| 10 | 9.24 | 4.30 | 4.66 | 3.40 |
| 11 | 21.43 | 25.93 | 27.24 | 39.27 |
| 12 | 3.08 | 4.73 | 10.52 | 14.92 |
| 13 | † | † | † | † |

| Allele | African-American (n = 357) | U.S. Caucasian (n = 349) | U.S. Hispanic (n = 290) | Native American (n = 191) |
|---|---|---|---|---|
| **vWA** | | | | |
| 11 | 0.28† | † | 0.17† | † |
| 12 | † | † | † | 0.26† |
| 13 | 1.26 | 0.43† | † | 0.26† |
| 14 | 7.14 | 8.31 | 6.90 | 4.45 |
| 15 | 20.03 | 11.32 | 10.00 | 7.07 |
| 16 | 26.75 | 23.35 | 34.31 | 32.98 |
| 17 | 20.59 | 24.50 | 21.55 | 33.51 |
| 18 | 14.71 | 22.49 | 18.45 | 15.45 |
| 19 | 6.72 | 8.31 | 7.07 | 4.71 |
| 20 | 1.96 | 1.15 | 1.38 | 1.05† |
| 21 | 0.28† | † | 0.17† | 0.26† |
| 22 | 0.28† | † | † | † |
| 23 | † | † | † | † |
| 24 | † | 0.14† | † | † |

† A minimum allele frequency (0.7% for the African-American database, 0.7% for the U.S. Caucasian database, 0.9% for the U.S. Hispanic database, and 1.3% for the Native American database) is suggested by the National Research Council in forensic calculations.

## Analyzing the four databases

Analysis across the four databases of 2274 total chromosomes per locus revealed the following number of different alleles: 10 CSF1PO alleles, 13 D2S1338 alleles, at least 12 D3S1358 alleles, 11 D5S818 alleles, 9 D7S820 alleles, 11 D8S1179 alleles, 8 D13S317 alleles, 8 D16S539 alleles, 20 D18S51 alleles, 17 D19S433 alleles, 26 D21S11 alleles, 31 FGA alleles, 9 TH01 alleles, 7 TPOX alleles, and 13 vWA alleles.

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

## Low-frequency alleles

Some alleles of the Identifiler™ Kit loci occur at a low frequency. For these alleles, a minimum frequency (5/ 2*n*, where *n* = the number of individuals in the database) was assigned for the Identifiler™ Kit African-American, U.S. Caucasian, U.S. Hispanic, and Native American databases, as suggested in the 1996 report of the Committee on DNA Forensic Science (National Research Council, 1996). These databases are summarized in Table 6 on page 95 through page 104. The minimum reportable genotype frequency at each locus is as follows: $1.19 \times 10^{-4}$ for the African-American database; $1.19 \times 10^{-4}$ for the U.S. Caucasian database; $1.70 \times 10^{-4}$ for the U.S. Hispanic database; and $2.97 \times 10^{-4}$ for the Native American database [$p2 + p(1–p)\ \theta$, where $\theta = 0.01$]. Hence, the minimum combined multilocus genotype frequency at 15 loci is as follows: $1.36 \times 10^{-59}$ for the African-American database; $1.36 \times 10^{-59}$ for the U.S. Caucasian database; $2.86 \times 10^{-57}$ for the U.S. Hispanic database; and $1.23 \times 10^{-53}$ for the Native American database.

# Mutation rate

## Estimating germline mutations

Estimation of spontaneous or induced germline mutation at genetic loci may be achieved through comparison of the genotypes of offspring to those of their parents. From such comparisons the number of observed mutations are counted directly.

In previous studies, genotypes of ten STR loci amplified by the AmpFℓSTR™ SGM™ Plus PCR Amplification Kit were determined for a total of 146 parent-offspring allelic transfers (meioses) at the Forensic Science Service, Birmingham, England. One length-based STR mutation was observed at the D18S11 locus; mutation was not detected at any of the other nine STR loci. The D18S11 mutation was represented by an increase of one 4-bp repeat unit, a 17 allele was inherited as an 18 (single-step mutation). The maternal/paternal source of this mutation could not be distinguished.

## Additional mutation studies

Additional studies (Edwards *et al.*,1991; Edwards *et al.*,1992; Weber and Wong, 1993; Hammond *et al.*,1994; Brinkmann *et al.*,1995; Chakraborty *et al.*,1996; Chakraborty *et al.*,1997; Brinkmann *et al.*,1998; Momhinweg *et al.*,1998; Szibor *et al.*,1998) of direct mutation rate counts produced:

- Larger sample sizes for some of the Identifiler™ Kit loci.
- Methods for modifications of these mutation rates (to infer mutation rates indirectly for those loci where these rates are not large enough to be measured directly and/or to account for those events undetectable as Mendelian errors).

# Probability of identity

Table 7 shows the Probability of Identity ($P_I$) values of the Identifiler™ Kit loci individually and combined.

**Table 7** Probability of Identity values for the Identifiler™ Kit STR loci

| Locus | African-American | U.S. Caucasian | U.S. Hispanic | Native American |
|---|---|---|---|---|
| CSF1PO | 0.079 | 0.132 | 0.141 | 0.123 |
| D2S1338 | 0.023 | 0.027 | 0.038 | 0.043 |
| D3S1358 | 0.097 | 0.076 | 0.112 | 0.158 |
| D5S818 | 0.104 | 0.147 | 0.115 | 0.110 |
| D7S820 | 0.085 | 0.063 | 0.083 | 0.081 |
| D8S1179 | 0.074 | 0.064 | 0.089 | 0.104 |
| D13S317 | 0.132 | 0.079 | 0.056 | 0.056 |
| D16S539 | 0.077 | 0.097 | 0.090 | 0.082 |
| D18S51 | 0.033 | 0.031 | 0.031 | 0.046 |
| D19S433 | 0.042 | 0.087 | 0.049 | 0.044 |
| D21S11 | 0.037 | 0.044 | 0.047 | 0.074 |
| FGA | 0.034 | 0.035 | 0.032 | 0.031 |
| TH01 | 0.109 | 0.079 | 0.097 | 0.134 |
| TPOX | 0.089 | 0.188 | 0.168 | 0.159 |

| Locus | African-American | U.S. Caucasian | U.S. Hispanic | Native American |
|---|---|---|---|---|
| vWA | 0.066 | 0.066 | 0.080 | 0.103 |
| Combined | $1.31 \times 10^{-18}$ | $5.01 \times 10^{-18}$ | $7.65 \times 10^{-18}$ | $3.62 \times 10^{-17}$ |

The $P_I$ value is the probability that two individuals selected at random will have an identical Identifiler™ Kit genotype (Sensabaugh, 1982). The $P_I$ values for the populations described in this section are then approximately $1/7.64 \times 10^{17}$ (African-American), $1/2.00 \times 10^{17}$ (U.S. Caucasian), $1/1.31 \times 10^{17}$ (U.S. Hispanic), and $1/2.76 \times 10^{16}$ (Native American).

# Probability of paternity exclusion

Table 8 shows the Probability of Paternity Exclusion ($P_E$) values of the Identifiler™ Kit STR loci individually and combined.

**Table 8** Probability of Paternity Exclusion for the Identifiler™ Kit STR loci

| Locus | African-American | U.S. Caucasian | U.S. Hispanic | Native American |
|---|---|---|---|---|
| CSF1PO | 0.545 | 0.496 | 0.450 | 0.409 |
| D2S1338 | 0.748 | 0.725 | 0.671 | 0.399 |
| D3S1358 | 0.591 | 0.630 | 0.495 | 0.510 |
| D5S818 | 0.506 | 0.440 | 0.525 | 0.601 |
| D7S820 | 0.591 | 0.582 | 0.574 | 0.492 |
| D8S1179 | 0.580 | 0.680 | 0.599 | 0.601 |
| D13S317 | 0.383 | 0.487 | 0.638 | 0.370 |
| D16S539 | 0.649 | 0.566 | 0.567 | 0.428 |
| D18S51 | 0.760 | 0.731 | 0.767 | 0.329 |
| D19S433 | 0.601 | 0.531 | 0.678 | 0.360 |
| D21S11 | 0.737 | 0.708 | 0.586 | 0.399 |
| FGA | 0.760 | 0.766 | 0.739 | 0.309 |
| TH01 | 0.492 | 0.566 | 0.618 | 0.646 |
| TPOX | 0.521 | 0.329 | 0.392 | 0.687 |
| vWA | 0.709 | 0.625 | 0.555 | 0.528 |
| Combined | 0.9999996 | 0.9999992 | 0.9999990 | 0.9999527 |

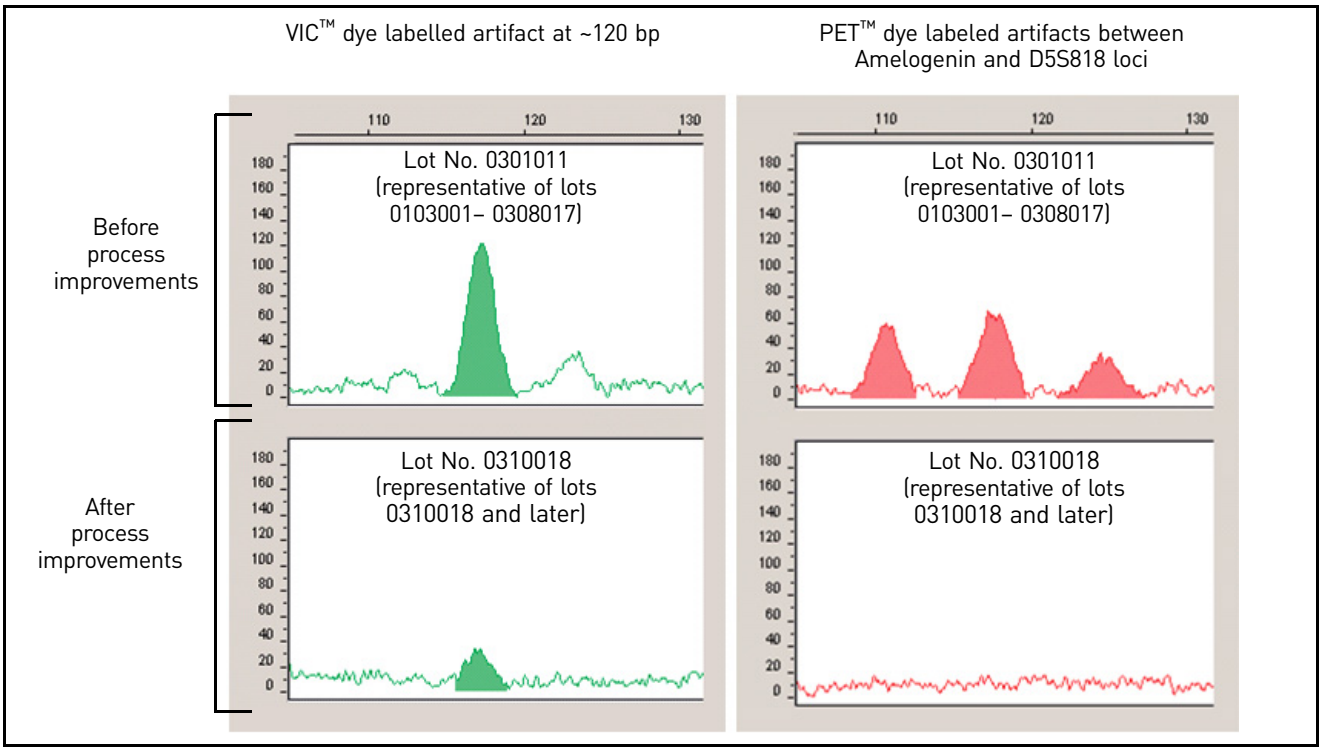
# Section 5.2 Performance Verification After Primer Manufacturing Process Improvements

As part of our continual efforts to improve the quality of our products, several improvements and updates have been made to the manufacturing process of the Identifiler™ Kit (Part No.4322288) since its introduction in 2001.

Effective from kit lot number 0310018, modifications were made to the manufacturing process of the Identifiler™ Kit to reduce the occurrence of artifacts in the PET™ dye and VIC™ dye channels that may interfere with the interpretation of casework samples.

We amplified negative control samples using lot number 0301011 and lot number 0310018 and generated data using the 310 Genetic Analyzer with the Windows™ NT OS using the G5 module. Results show that the VIC™ and PET™ labeled artifacts are greatly reduced in the after the manufacturing process improvements (Figure 21).

**Figure 21** Comparison of the observed VIC™ dye- and PET™ dye-labeled artifacts for negative control amplifications with Identifiler™ Kit lot numbers before and after kit lot number 0310018. The artifacts have been highlighted for illustrative purposes.




For more information, refer to *AmpFℓSTR™ Identifiler™ PCR Amplification Kit Human Identification Application Note* (Pub. no. 040302).

# Section 5.3 Performance Validation After Buffer and Enzyme Component Replacement

## Overview

As part of an ongoing program to exercise greater control over raw materials used in the AmpFℓSTR™ PCR Amplification Kits, manufacturing of the AmpliTaq Gold™ enzyme and 10× PCR Buffer II (Tris-KCl buffer) components is transitioning from Roche Molecular Systems to Life Technologies. Manufacturing of both components by Life Technologies will be conducted according to the same specifications used previously by Roche. The in-house components are established raw materials in our next generation kits (for example, the NGM™, NGM SElect™ and Identifiler™ Plus Kits).

## Experiments

We performed studies to compare the performance of the Identifiler™ Kit containing the in-house components (updated kit) with the performance of the original kit, focusing on studies most relevant to forensic DNA testing (see SWGDAM Guidelines effective January 1, 2011). These studies, while not exhaustive, are in our opinion appropriate for a manufacturer.

Our studies compared the performance of two Roche-manufactured enzyme and buffer lots (Control mixes) with three new lots of buffer and two new lots of enzyme manufactured by Life Technologies (Test mixes). Studies were performed using Test mixes containing both the enzyme and buffer manufactured by Life Technologies.

| Test Material | Control A mix | Control B mix | Test A mix | Test B mix | Test C mix |
|---|---|---|---|---|---|
| Buffer | Control Buffer Lot 1 | Control Buffer Lot 2 | Test Buffer Lot 1 | Test Buffer Lot 2 | Test Buffer Lot 3 |
| Enzyme | Control Enzyme Lot 1 | Control Enzyme Lot 2 | Test Enzyme Lot 1 | Control Enzyme Lot 2 | Test Enzyme Lot 2 |

Each of the five mixes listed above were used to conduct reproducibility, sensitivity, and inhibition studies. All amplifications were performed using a GeneAmp™ PCR System 9700 with either silver or gold-plated silver block using the recommended amplification conditions and cycle number for the Identifiler™ Kit. All data was run on an Applied Biosystems 3130*xl* Genetic Analyzer running Data Collection Software v3.0 and analyzed using GeneMapper™ *ID-X* Software. Subsequent data analysis was performed using Minitab™ Statistical Software. To minimize the effect of injection-to-injection variation on result interpretation, peaks heights for all studies were normalized using an in-house, multicolor reference standard.

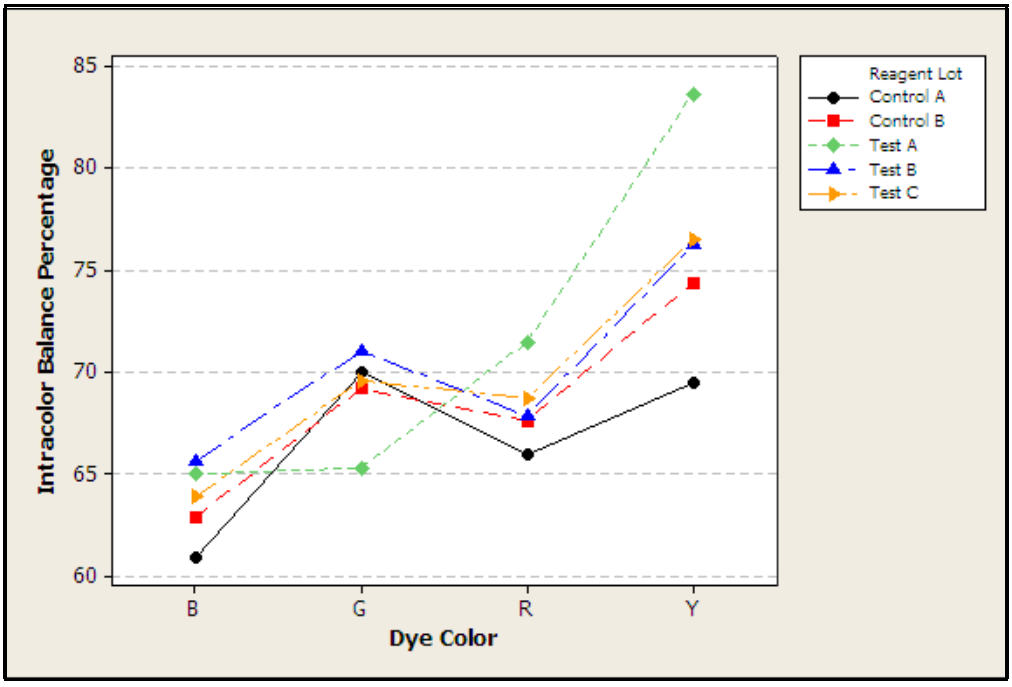
# Reproducibility study

For the reproducibility study, 12 replicates of control DNA 007 at 1 ng input and three negative control replicates were amplified. The results were evaluated for intracolor balance, stutter percentage, and the presence, signal intensity, and location of artifacts.

## Intracolor balance

No significant difference (<10% increase or decrease) in the level of intracolor balance was observed between the Test and Control mixes (Figure 22).

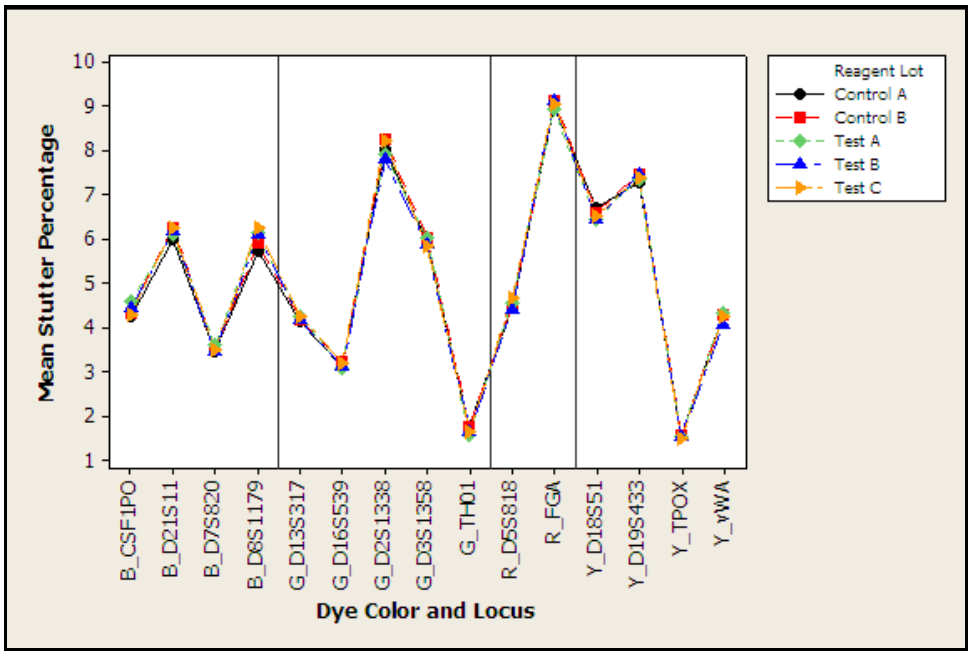
**Figure 22** Reproducibility study: intracolor balance



## Stutter percentages

Stutter percentage results for each marker were comparable across all Test and Control mixes (Figure 23).

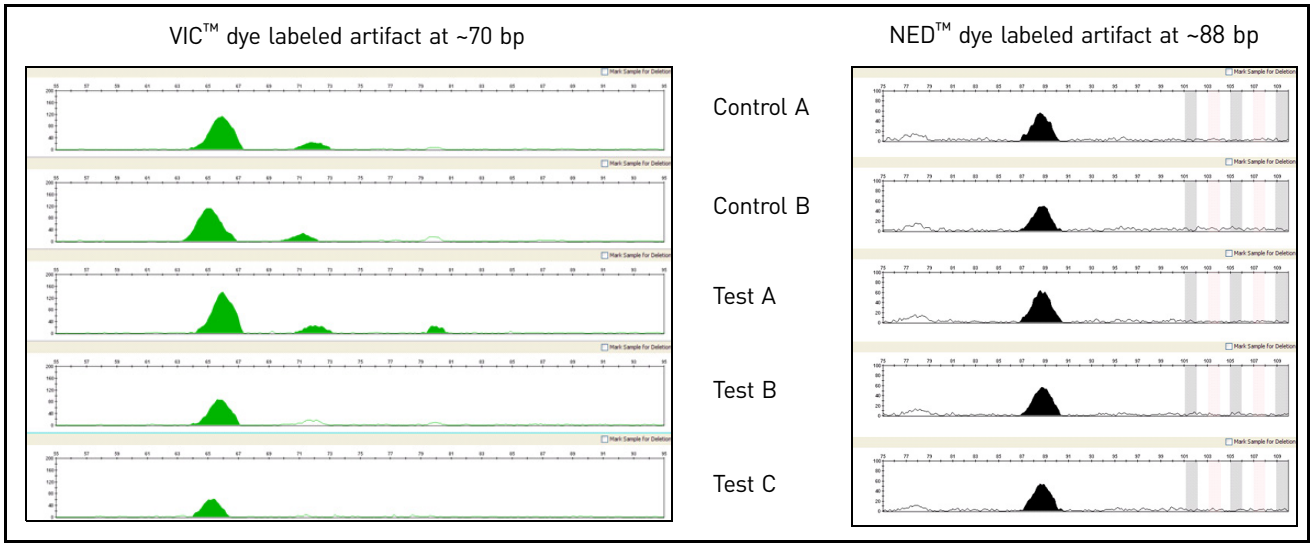
**Figure 23** Reproducibility study: mean stutter percentage




## Artifacts

Known artifacts observed showed the same morphology, signal intensity, and location in all Test and Control mixes and did not exceed 50 RFU (Figure 24). No new artifacts were observed in the Test mixes.

**Figure 24** Reproducibility study: known artifacts (Y-scale 100 RFU)



# Sensitivity study

For the sensitivity study, dilution series of three genomic DNA samples were amplified: 1 ng (three replicates each), 0.5 ng, 0.25 ng, and 0.125 ng (four replicates each). The results were evaluated for mean referenced peak height, degree of linearity between input DNA concentration and peak height, level of allelic dropout at 125 pg, and genotype concordance.

## Mean referenced peak height

Mean referenced peak height observations were consistent between all Test and Control mixes (Figure 25) demonstrating equivalent performance (Figure 26).

**Figure 25** Sensitivity study: mean referenced peak heights three genomic DNA samples

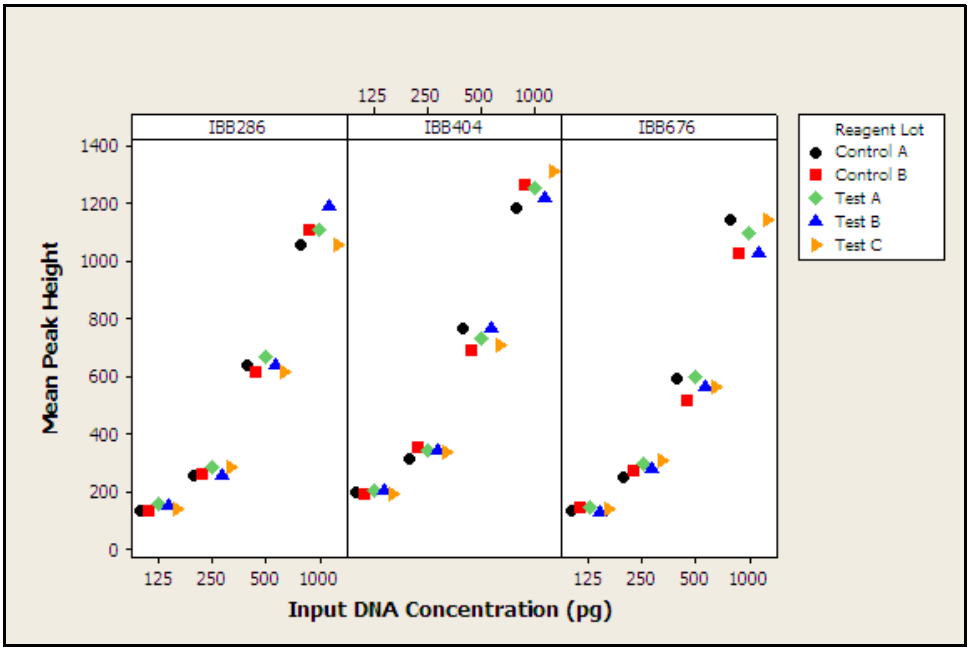
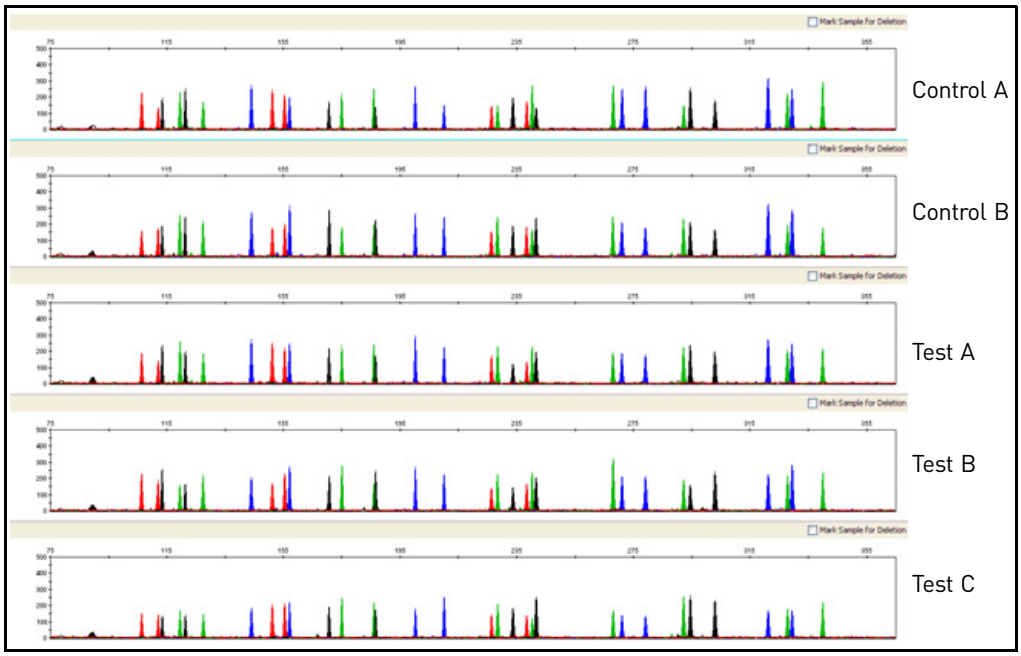


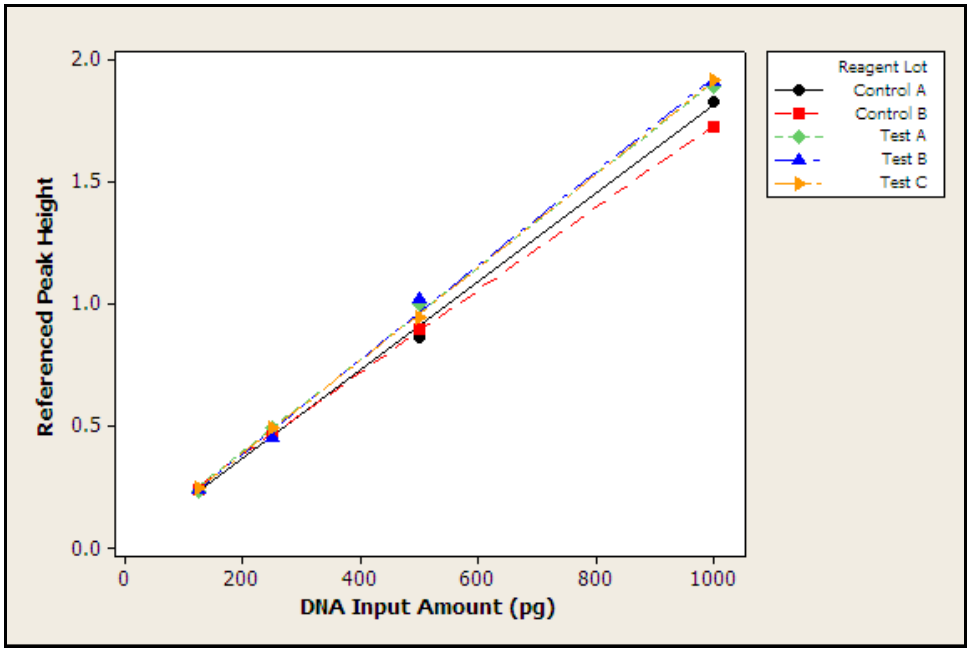
**Figure 26** Sensitivity study: representative electropherograms for Sample 2 amplified using 125 pg input DNA (Y-scale 500 RFU)




## DNA concentration and peak height

The calculated slope and $R^2$ values for each of the plotted curves were equivalent, showing comparable relationships between peak height and DNA input amount for the Test and Control mixes (Figure 27). In general, the Test mixes showed a slight increase in peak height compared to the Control mixes.

**Figure 27** Sensitivity study: linear regression plot of combined mean referenced peak height for three genomic DNA samples



## Allelic dropout

Allelic dropout was observed only for amplifications of 125 pg where dropout of a single allele was observed for Test A Sample 1 (Figure 28) and Control B Sample 3 (Figure 29). These results can be explained by stochastic variation and sampling from dilute DNA solutions. Allelic dropout results can therefore be considered equivalent between Test and Control mixes.

**Figure 28** Sensitivity study: electropherogram of 125 pg Sample 1 amplified with Test A mix. One allele at the FGA locus in the PET™ (Red) dye channel is below the analysis threshold of 50 RFU. (Y-scale 300 RFU)

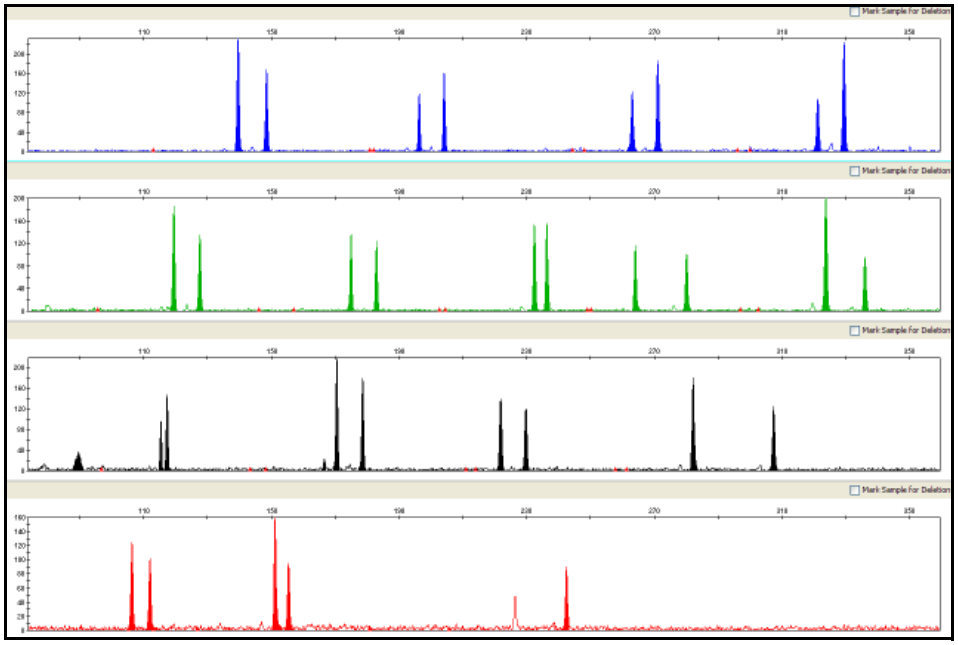
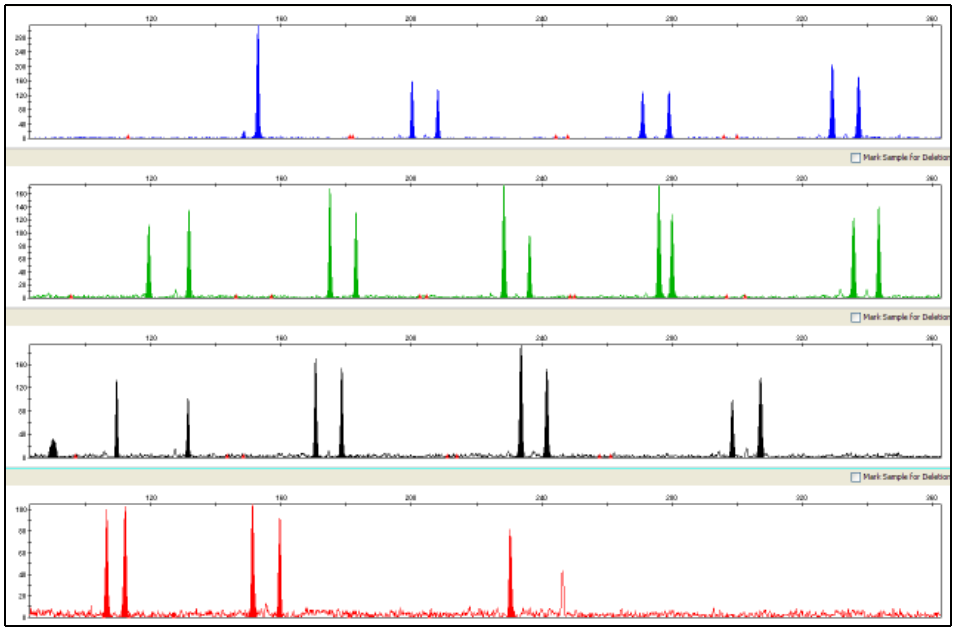


**Figure 29** Sensitivity study: electropherogram of 125 pg Sample 3 amplified with Control B mix. One allele at the FGA locus in the PET™ (Red) dye channel is below the analysis threshold of 50 RFU. (Y-scale 300 RFU)



### Genotype concordance

Genotypes for Test and Control mixes were 100% concordant (Table 9).

**Table 9** Sensitivity study: genotype concordance

| DNA Input Amount | Reagent Mix | Genotype Concordance |
|---|---|---|
| 125 pg | Test A | 100% |
| | Test B | 100% |
| | Test C | 100% |
| | Control A | 100% |
| | Control B | 100% |
| 250 pg | Test A | 100% |
| | Test B | 100% |
| | Test C | 100% |
| | Control A | 100% |
| | Control B | 100% |
| 500 pg | Test A | 100% |
| | Test B | 100% |
| | Test C | 100% |
| | Control A | 100% |
| | Control B | 100% |
| 1 ng | Test A | 100% |
| | Test B | 100% |
| | Test C | 100% |
| | Control A | 100% |
| | Control B | 100% |

## Inhibition study

An inhibition series of 1 ng control DNA 007 (consisting of uninhibited control, Humic Acid at a final concentration of 15.25 ng/µL, and Hematin at a final concentration of 34 µM in replicates of five) was amplified using each of Test and Control mixes. The amount of each inhibitor tested was titrated to cause an approximate 50% reduction in overall peak height of the samples. Results were evaluated for mean referenced peak height, minimum referenced peak height, intracolor balance, and levels of allelic dropout.

### Mean referenced peak height, minimum referenced peak height, and intracolor balance

Uninhibited Control DNA 007 Test and Control mixes displayed no significant difference in mean referenced peak height, minimum referenced peak height, and intracolor balance. For the Humic Acid-inhibited and Hematin-inhibited DNA, however, the Test mixes showed slightly improved performance compared to the Control mixes for mean referenced peak height, intracolor balance, and minimum referenced peak height (Figure 30, 31, and 32). All results obtained for all Test and Control mixes fall within the expected range of performance for the Identifiler™ Kit.

**Figure 30** Inhibition study: mean referenced peak height. Inhibitors: HA = Humic Acid, HE = Hematin, PRI = Pristine or Uninhibited DNA

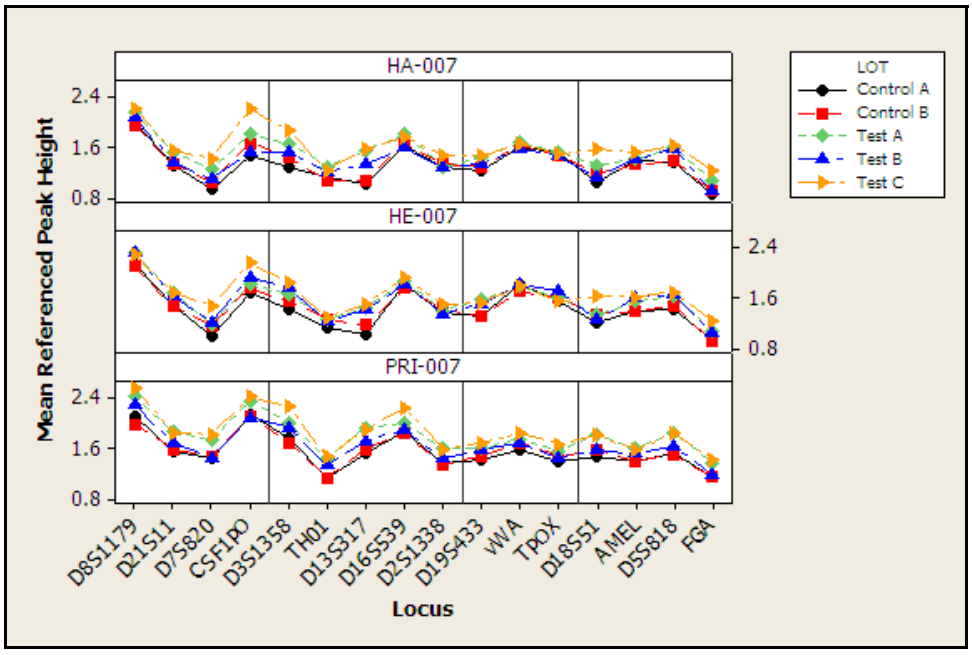



**Figure 31** Inhibition study: minimum referenced peak height. Inhibitors: HA = Humic Acid, HE = Hematin, PRI = Pristine or Uninhibited DNA

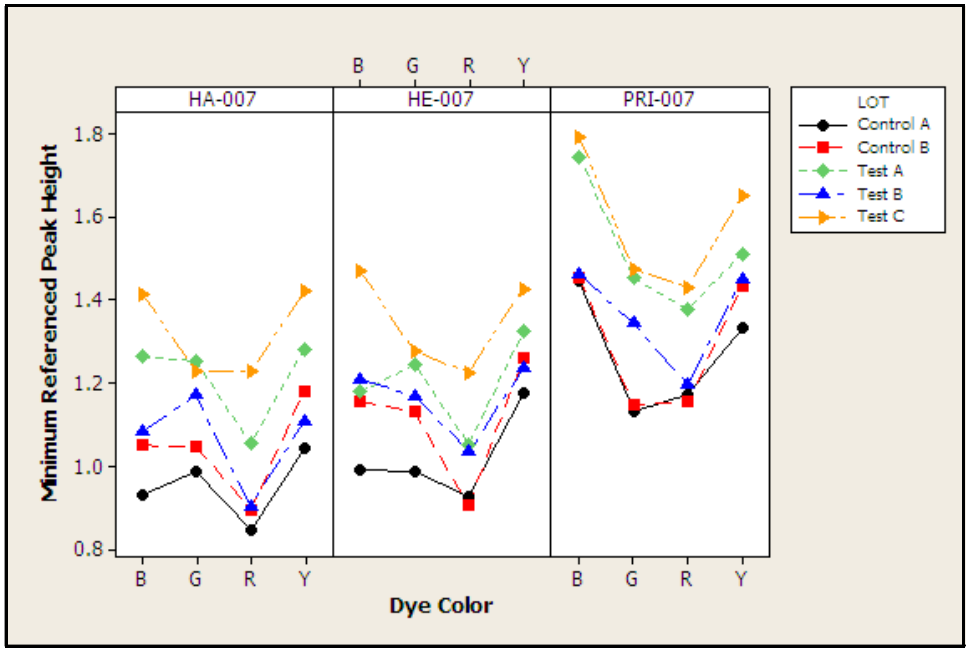
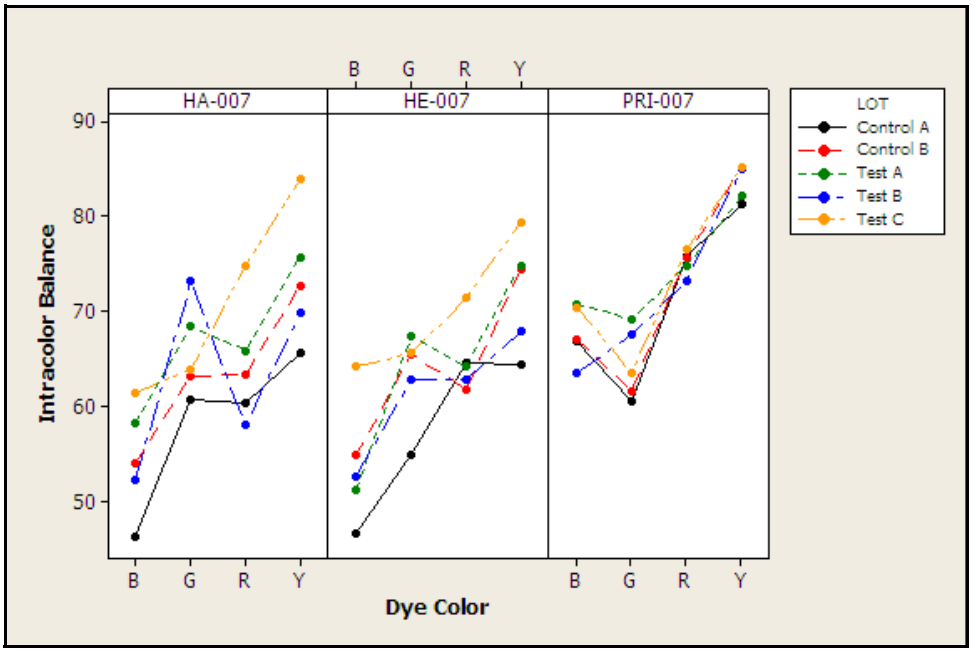


**Figure 32** Inhibition study: intracolor balance. (Y-axis intracolor balance percentage versus X-axis dye color. Inhibitors: HA = Humic Acid, HE = Hematin, PRI = Pristine or Uninhibited DNA




Representative electropherograms from the inhibition study are shown in Figure 33, 34, and 35.

**Figure 33** Inhibition study: representative electropherograms using uninhibited Control DNA 007 (Y-scale 4000 RFU)

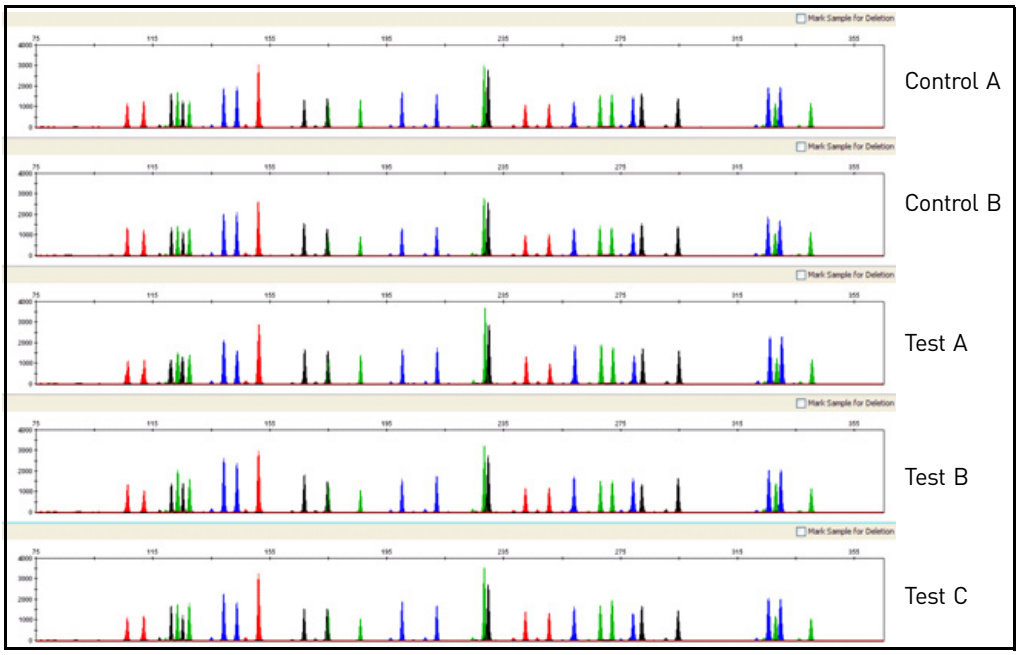


**Figure 34** Inhibition study: representative electropherograms using Control DNA 007 inhibited with 34 µM Hematin (Y-scale 4000 RFU)

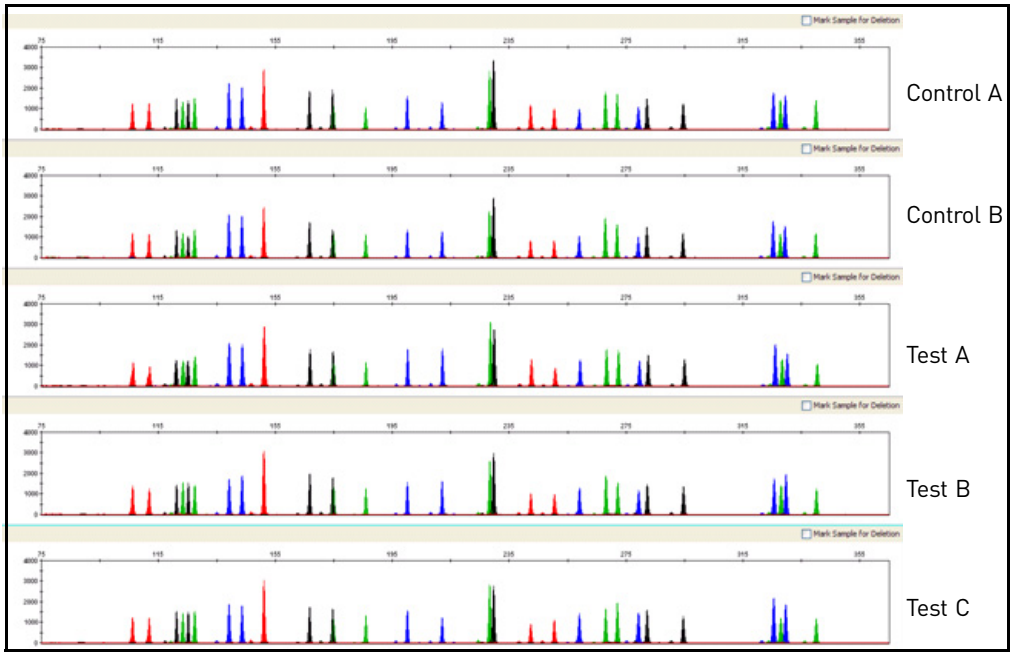
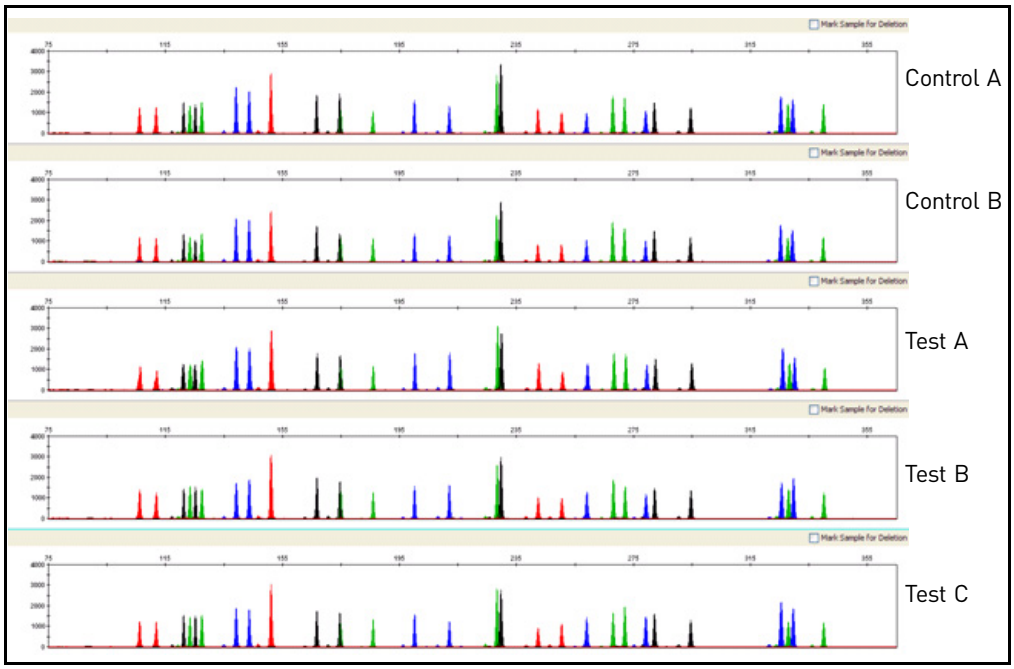



**Figure 35** Inhibition study: representative electropherograms using Control DNA 007 inhibited with15.25 ng/µL Humic Acid (Y-scale 4000 RFU)




## Allelic dropout

No allelic dropout events were seen for any Test or Control mixes tested on uninhibited Control DNA 007 and Control DNA 007 inhibited with Hematin or Humic Acid.

# Conclusions

Laboratories can expect to obtain equivalent quality profiles across a wide range of forensic samples when using the Identifiler™ Kit containing the AmpliTaq Gold™ enzyme and 10× PCR Buffer II manufactured by Life Technologies as compared to the original Identifiler™ Kit containing AmpliTaq Gold™ enzyme and 10× PCR Buffer II manufactured by Roche Molecular Systems.

# A Troubleshooting

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

**Table 10** Troubleshooting

| Observation | Possible causes | Recommended actions |
|---|---|---|
| Faint or no signal from both the AmpFℓSTR™ Control DNA 9947A and the DNA test samples at all loci | Incorrect volume or absence of PCR Master Mix or Identifiler™ Primer Set | Repeat amplification. |
| | No activation of AmpliTaq Gold™ DNA Polymerase | Repeat amplification, making sure to hold reactions initially at 95°C for 11 minutes. |
| | Master Mix not vortexed thoroughly before aliquoting | Vortex the Master Mix thoroughly. |
| | Identifiler™ Primer Set exposed to too much light | Store the Primer Set protected from light. |
| | GeneAmp™ PCR System malfunction | Refer to the thermal cycler user's manual and check instrument calibration. |
| | Use of incorrect thermal cycling parameters | Check the protocol for correct thermal cycling parameters. |
| | Tubes not seated tightly in the thermal cycler during amplification | Push reaction tubes firmly into contact with block after first cycle. Repeat test. |
| | Wrong PCR reaction tube | Use Applied Biosystems MicroAmp™ Reaction Tubes with Caps for the GeneAmp™ PCR System 9700. |
| | MicroAmp™ Base used with tray/ retainer set and tubes in GeneAmp™ 9700 | Remove MicroAmp™ Base from tray/retainer set and repeat test. |
| | Insufficient PCR product electrokinetically injected | Prepare PCR product as described in Chapter 3, "Perform Electrophoresis" on page 25. |
| | Degraded formamide | Check the storage of formamide; do not thaw and refreeze multiple times. Try Hi-Di™ Formamide. |

| Observation | Possible causes | Recommended actions |
|---|---|---|
| Positive signal from AmpFℓSTR™ Control DNA 9947A but partial or no signal from DNA test samples | Quantity of test DNA sample is below assay sensitivity | Quantitate DNA and add 1.0 ng of DNA. Repeat test. |
| | Test sample contains high concentration of PCR inhibitor (for example, heme compounds, certain dyes) | Quantitate DNA and add minimum necessary volume. Repeat test. |
| | | Wash the sample in a Centricon™-100 centrifugal filter unit. Repeat test. |
| | Test sample DNA is severely degraded | If possible, evaluate the quality of DNA sample by running an agarose gel. If DNA is degraded, reamplify with an increased amount of DNA or use the AmpFℓSTR™ MiniFiler™ Kit. |
| | Dilution of test sample DNA in water or wrong buffer (for example, TE formula with incorrect EDTA concentration) | Redilute DNA using low-TE Buffer (with 0.1 mM EDTA). |
| More than two allele present at a locus | Presence of exogenous DNA | Use appropriate techniques to avoid introducing foreign DNA during laboratory handling. |
| | Amplification of stutter product | See "Stutter products" on page 77. |
| | Mixed sample | |
| Some but not all loci visible on electropherogram of DNA test samples | Test-sample DNA is severely degraded | If possible, evaluate the quality of DNA sample by running an agarose gel. If DNA is degraded, reamplify with an increased amount of DNA or use the AmpFℓSTR™ MiniFiler™ Kit. |
| | Test sample contains high concentrations of a PCR inhibitor (for example, heme compounds, certain dyes) | Quantitate DNA and add minimum necessary volume. Repeat test. |
| | | Wash the sample in a Centricon™-100 centrifugal filter unit. Repeat test. |
| Poor peak height balance | Incorrect thermal cycler parameters | Check the protocol for correct thermal cycler parameters. |
| | GeneAmp™ PCR System 9700 with Aluminum 96-Well block or third-party thermal cyclers | Use Applied Biosystems GeneAmp™ PCR System 9700 with silver or gold-plated silver blocks only, or the Veriti™ 96-Well Thermal Cycler. |

# B Ordering Information

## Equipment and materials not included

Table 11 and Table 12 list required and optional equipment and materials not supplied with the Identifiler™ Kit. Unless otherwise noted, many of the items are available from major laboratory suppliers (MLS).

**Table 11** Equipment

| Equipment | Source |
|---|---|
| 3100/3100-*Avant* Genetic Analyzer | Contact your local Life Technologies sales representative |
| Applied Biosystems 3130/3130*xl* Genetic Analyzer | |
| Applied Biosystems 3500/3500xL Genetic Analyzer for Human Identification | |
| Applied Biosystems 310 Genetic Analyzer | |
| GeneAmp™ PCR System 9700 with the Silver 96-Well Block | N8050001 |
| GeneAmp™ PCR System 9700 with the gold-plated silver 96-well block | 4314878 |
| Veriti™ 96-Well Thermal Cycler | 4375786 |
| Silver 96-well sample block | N8050251 |
| Gold-plated silver 96-well sample block | 4314443 |
| Tabletop centrifuge with 96-well plate adapters (optional) | MLS |

**Table 12** User-supplied materials

| Item† | Source |
|---|---|
| AmpFℓSTR™ Identifiler™ PCR Amplification Kit | 4322288 |
| **3100 Analyzer materials** | |
| 96-well plate septa | 4315933 |
| Reservoir septa | 4315932 |
| 3100/3130*xl* Genetic Analyzer capillary array, 36-cm | 4315931 |
| POP-4™ polymer for 3100/3100-*Avant* Genetic Analyzers | 4316355 |
| 3100/3100-*Avant* Genetic Analyzer Autosampler Plate Kit, 96-well | 4316471 |
| GeneScan™ 500 LIZ™ Size Standard<br>*OR*<br>GeneScan™ 600 LIZ™ Size Standard v2.0 | 4322682<br>*OR*<br>4408399 |
| Running Buffer, 10X | 402824 |
| Hi-Di™ Formamide | 4311320 |

| Item† | Source |
|---|---|
| DS-33 Matrix Standard Kit (Dye Set G5) | 4345833 |
| 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 |
| For a complete list of parts and accessories for the 3100/3100-*Avant* instrument, refer to Appendix B of the *3100 Genetic Analyzer and 3100-Avant Genetic Analyzer User Reference Guide* (Pub. No. 4335393). | |
| **3130*xl* Analyzer materials** | |
| 96-well plate septa | 4315933 |
| Reservoir septa | 4315932 |
| 3100/3130*xl* Genetic Analyzer capillary array, 36-cm | 4315931 |
| POP-4™ polymer for 3130/3130*xl* Genetic Analyzers | 4352755 |
| 3100/3100-*Avant* Genetic Analyzer Autosampler Plate Kit, 96-well | 4316471 |
| GeneScan™ 500 LIZ™ Size Standard | 4322682 |
| *OR* | *OR* |
| GeneScan™ 600 LIZ™ Size Standard v2.0 | 4408399 |
| Running Buffer, 10X | 402824 |
| DS-33 Matrix Standard Kit (Dye Set G5) | 4345833 |
| MicroAmp™ Optical 96-well reaction plate | N8010560 |
| Hi-Di™ Formamide | 4311320 |
| For a complete list of parts and accessories for the 3130/3130*xl* instrument, refer to Appendix A of the *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* (Pub. No. 4352716). | |
| **3500/3500xL Analyzer materials** | |
| Anode buffer container (ABC) | 4393927 |
| Cathode buffer container (CBC) | 4408256 |
| POP-4™ polymer (960 samples) for 3500/3500xL Genetic Analyzers | 4393710 |
| POP-4™ polymer (384 samples) for 3500/3500xL Genetic Analyzers | 4393715 |
| 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 |
| GeneScan™ 600 LIZ™ Size Standard v2.0 | 4408399 |
| DS-33 Matrix Standard Kit (Dye Set G5) | 4345833 |
| For a complete list of parts and accessories for the 3500/3500xL instrument, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Pub. No. 4401661) | |

| Item† | Source |
|---|---|
| **310 Analyzer materials** | |
| 310 DNA Analyzer capillary array, 47-cm | 402839 |
| 0.5 mL sample tray | 5572 |
| 96-well tray adaptor (for 9700 thermal cycler trays) | 4305051 |
| GeneScan™ 500 LIZ™ Size Standard | 4322682 |
| *OR* | *OR* |
| GeneScan™ 600 LIZ™ Size Standard v2.0 | 4408399 |
| Running Buffer, 10X | 4335643 |
| Genetic analyzer septa retainer clips for 96-tube sample tray | 402866 |
| Genetic analysis sample tubes (0.5-mL) | 401957 |
| Septa for 0.5-mL sample tubes | 401956 |
| DS-33 Matrix Standard Set (6-FAM™, VIC™, NED™, PET™, and LIZ™ dyes) for 310/377 systems | 4318159 |
| MicroAmp™ 8-tube strip, 0.2-mL | N8010580 |
| MicroAmp™ 96-well base (holds 0.2-mL reaction tubes) | N8010531 |
| MicroAmp™ 96-well full plate cover | N8010550 |
| MicroAmp™ 96-well tray/retainer set | 403081 |
| POP-4™ polymer for the 310 Genetic Analyzer | 402838 |
| For a complete list of parts and accessories for the 310 instrument, refer to Appendix B of the *310 Genetic Analyzer User Guide* (Pub. No. 4317588). | |
| PCR Amplification | |
| MicroAmp™ 96-well tray | N8010541 |
| MicroAmp™ reaction tube with cap, 0.2-mL | N8010540 |
| MicroAmp™ 8-tube strip, 0.2-mL | N8010580 |
| MicroAmp™ 8-cap strip | N8010535 |
| MicroAmp™ 96-well tray/retainer set | 403081 |
| MicroAmp™ 96-well base | N8010531 |
| MicroAmp™ clear adhesive film | 4306311 |
| MicroAmp™ optical adhesive film | 4311971 |
| MicroAmp™ optical 96-well reaction plate | N8010560 |
| Other user-supplied materials | |
| Hi-Di™ Formamide, 25-mL | 4311320 |
| Aerosol resistant pipette tips | MLS |
| Microcentrifuge tubes | MLS |
| Pipettors | MLS |
| Tape, labeling | MLS |
| Tube, 50-mL Falcon | MLS |
| Tube decapper, autoclavable | MLS |
| Deionized water, PCR grade | MLS |

| Item† | Source |
|---|---|
| Tris-HCL, pH 8.0 | MLS |
| EDTA, 0.5 M | MLS |
| Vortex | MLS |

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

C

# PCR Work Areas



## Work area setup and lab design

Many resources are available for the appropriate design of a PCR laboratory. If you are using the AmpFℓSTR™ Identifiler™ PCR Amplification Kit for:

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

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

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

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

## PCR setup work area

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

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

- Tube decapper, autoclavable
- Vortex

# Amplified DNA work area

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

You can use the following systems:

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

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

- Veriti™ 96-Well Thermal Cycler

D

# Safety

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

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

# Chemical safety

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

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

## Specific chemical handling

| CAS | Chemical | Phrase |
|---|---|---|
| 26628-22-8 | Sodium Azide | Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. |

# Biological hazard safety

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

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

In the U.S.:

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

In the EU:

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

# Documentation and Support

## Related documentation

| Document title | Pub. No. |
|---|---|
| *AmpFℓSTR™* Identifiler™ PCR Amplification Kit*: Human Identification: Application Note* | 040302 |
| *3100/3100-Avant Data Collection v2.0 User Guide* | 4347102 |
| *3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0 User Bulletin* | 4350218 |
| *3100 Genetic Analyzer User Manual (Data Collection v1.1)* | 4315834 |
| *3100/3100-Avant Genetic Analyzers Protocols for Processing* AmpFℓSTR™ *PCR Amplification Kit PCR Products User Bulletin* | 4332345 |
| *Applied Biosystems 3130/3100xl Genetic Analyzers Using Data Collection Software v3.0 User Bulletin* | 4363787 |
| *Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide* | 4352715 |
| *Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* | 4352716 |
| *Applied Biosystems 3130/3130xl Genetic Analyzers Quick Reference Card* | 4362825 |
| *Applied Biosystems 3130/3130xl Genetic Analyzers AB Navigator Software Administrator Guide* | 4359472 |
| *Applied Biosystems 3130/3100xl DNA Analyzers User Guide* | 4331468 |
| *Applied Biosystems 3500/3500xL Genetic Analyzer Quick Reference Card* | 4401662 |
| Applied Biosystems 3500/3500xL Genetic Analyzer User Guide, Data Collection v1.0 | 4401661 |
| *Applied Biosystems 3500/3500xL Genetic Analyzer User Bulletin: Solutions to issues related to software, data, hardware, and consumables*<br>**Note:** Additional user bulletins may be available at **www.lifetechnologies.com** | 4445098 |
| *Applied Biosystems 3730/3730xl Genetic Analyzer Getting Started Guide* | 4359476 |
| *GeneAmp™ PCR System 9700 Base Module User's Manual* | N805-0200 |
| *Veriti™ 96-Well Thermal Cycler AmpFℓSTR™ Kit Validation User Bulletin* | 4440754 |
| Quantifiler™ *Kits:* Quantifiler™ *Human DNA Quantification Kit and* Quantifiler™ *Y Human Male DNA Quantification Kit User's Manual* | 4344790 |
| *PrepFiler™ Forensic DNA Extraction Kit User Guide* | 4390932 |
| *GeneMapper™ ID Software Version 3.1 Human Identification Analysis User Guide* | 4338775 |
| *GeneMapper™ ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* | 4335523 |
| *Installation Procedures and New Features for* GeneMapper™ *ID* Software *v3.2 User Bulletin* | 4352543 |
| *GeneMapper™ ID-X Software Version 1.0 Getting Started Guide* | 4375574 |
| *GeneMapper™ ID-X Software Version 1.0 Quick Reference Guide* | 4375670 |
| *GeneMapper™ ID-X Software Version 1.0 Reference Guide* | 4375671 |
| *GeneMapper™ ID-X Software Version 1.1 (Mixture Analysis) Getting Started Guide* | 4396773 |

| Document title | Pub. No. |
|---|---|
| *GeneMapper™ ID-X Software Version 1.1 (Mixture Analysis) Quick Reference Guide* | 4402094 |
| *GeneMapper™ ID-X Software Version 1.2 Reference Guide* | 4426481 |
| *GeneMapper™ ID-X Software Version 1.2 Quick Reference Guide* | 4426482 |

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

**Note:** To open the user documentation available from the Applied Biosystems web site, use the Adobe™ Acrobat™ Reader™ software available from **www.adobe.com**.Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

# Obtain SDSs

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

# Obtain support

For HID support:

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

For the latest services and support information for all locations, go to:

**www.lifetechnologies.com**

At the website, you can:

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

## Limited product warranty

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

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