User Bulletin

SUBJECT: AmpFℓSTR® Profiler Plus™ *ID* PCR Amplification Kit

Overview

This User Bulletin describes the AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit and experiments performed for its evaluation.

In This User Bulletin

This User Bulletin contains the following topics:

About This User Bulletin

The *AmpFℓSTR Profiler Plus ID PCR Amplification Kit User Bulletin* provides supporting documentation for the AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit. This user bulletin contains sections describing the following:

- Background information on the AmpFℓSTR Profiler Plus *ID* kit
- Experiments and Results

The AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit and the AmpFℓSTR® Profiler Plus™ PCR Amplification Kit use the same protocols. This User Bulletin does not contain the general protocols necessary for amplification or fragment analysis on the ABI PRISM® instruments. The *AmpFℓSTR Profiler Plus PCR Amplification Kit User's Manual* (P/N 4303501) should be referred to for use with the AmpFℓSTR Profiler Plus *ID* kit.

Chapters in the *AmpFℓSTR Profiler Plus PCR Amplification Kit User's Manual* that are especially useful for the Profiler Plus *ID* kit are as follows:

- AmpFℓSTR Profiler Plus Kit Amplification (Chapter 5)
- ABI PRISM® 377 DNA Sequencer Protocols (Chapter 7)
- ABI PRISM® 310 Genetic Analyzer Protocol (Chapter 8)
- Results and Interpretation (Chapter 9)
- Automated Genotyping (Chapter 10)
- Population Genetics (Chapter 13)

Product Overview

Purpose

The AmpFℓSTR Profiler Plus PCR Amplification Kit has proven to be extremely useful in applications for human identification. The AmpFℓSTR Profiler Plus PCR Amplification Kit co-amplifies the following nine short tandem repeat loci: D3S1358, D5S818, D7S820, D8S1179, D13S317, D18S51, D21S11, FGA, and vWA.

Previously, Budowle (Budowle *et.al.*, 2000) reported an excess of homozygosity at the D8S1179 locus in a population of Chamorro and Filipinos from Guam. A single G-to-A transition (point mutation) was observed in all null alleles at the D8S1179 reverse primer-binding site.

An additional D8S1179 reverse primer with a single nucleotide difference specific for the transition was included in the primer set of the newly developed AmpFℓSTR® Identifiler™ PCR Amplification Kit to address this mutation. The new AmpFℓSTR Profiler Plus *ID* kit contains the same primers and uses the same reaction conditions and thermal cycling parameters as the AmpFℓSTR Profiler Plus kit with the addition of this second D8S1179 reverse primer. The addition of the primer allows for the amplification of those D8S1179 alleles in samples containing this mutation without altering the overall performance of the AmpFℓSTR Profiler Plus *ID* kit (See Figure 1 and Figure 2 on page 4). In Figure 2, the DNA fragments are labeled with 5-FAM™ dye (blue), JOE™ dye (green), and NED™ dye (yellow, depicted in black). The GeneScan™-500 Size Standard is labeled with ROX™ dye (red).

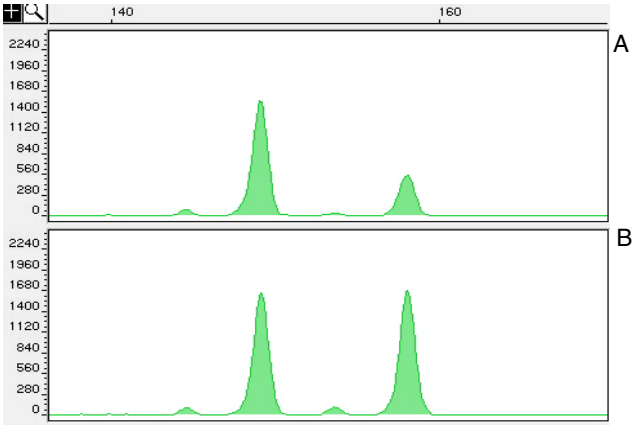

Figure 1 Profile of a DNA sample (heterozygous with one mutant allele) amplified without (A) and with (B) the D8S1179 unlabeled primer along with the standard Profiler Plus kit primers

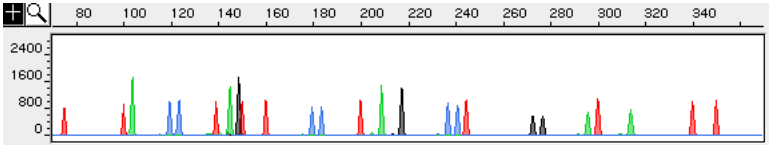

Figure 2 GeneScan® software electropherogram showing the AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit results for nine STR loci and the Amelogenin locus analyzed on the ABI PRISM 310 Genetic Analyzer

Safety

Documentation User Attention Words

Five user attention words appear in the text of all Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below.

Note Calls attention to useful information.

IMPORTANT Indicates information that is necessary for proper instrument operation.

⚠ CAUTION Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

⚠ WARNING Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

⚠ DANGER Indicates an imminently hazardous situation which, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

⚠ WARNING CHEMICAL HAZARD. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (*e.g.*, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.

Ordering MSDSs

You can order free additional copies of MSDSs for chemicals manufactured or distributed by Applied Biosystems using the contact information below..

To order documents by automated telephone service:

1	From the U.S. or Canada, dial **1.800.487.6809**, or from outside the U.S. and Canada, dial **1.858.712.0317**.
2	Follow the voice instructions to order documents (for delivery by fax). **Note** There is a limit of five documents per fax request.

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In the U.S.	Dial **1.800.345.5224**, and press **1**.
In Canada	♦ To order in English, dial **1.800.668.6913** and press **1**, then **2**, then **1** ♦ To order in French, dial **1.800.668.6913** and press **2**, then **2**, then **1**
From any other country	See the specific region under "To Contact Technical Support by Telephone or Fax (Outside North America)".

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Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, click **Documents on Demand**, then click **MSDS.**
3	Click **MSDS Index**, search through the list for the chemical of interest to you, then click on the MSDS document number for that chemical to open a pdf of the MSDS.

For chemicals not manufactured or distributed by Applied Biosystems, call the chemical manufacturer.

AmpFℓSTR Profiler Plus *ID* Kit Loci

The AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit co-amplifies the repeat regions of the following nine short tandem repeat loci: D3S1358 (Li *et al*., 1993), D5S818 (Hudson *et al*., 1995), D7S820 (Green *et al*., 1991), D8S1179 (Oldroyd *et al*., 1995), D13S317 (Hudson et al., 1995), D18S51 (Urquhart *et al*., 1995), and D21S11 (Sharma and Litt, 1992), FGA (Mills *et al*., 1992), and vWA (Kimpton *et al*., 1992).

A segment of the X-Y homologous gene Amelogenin is also amplified. Amplifying a segment of the Amelogenin gene with a single primer pair can be used for gender identification because different length products from the X and Y chromosomes are generated (Sullivan *et al*., 1993).

One primer of each locus-specific primer pair is labeled with either the 5-FAM, JOE, or NED dyes. These dyes are detected as blue, green, and yellow, respectively, on the ABI PRISM instruments. The loci amplified by these primers are summarized in the table below. Additionally, the AmpFℓSTR® Profiler Plus™ 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 listed in the table, as well.

AmpFℓSTR Profiler Plus *ID* Loci and Allele Information

Locus Designation	Chromosome Location	Dye Label	AmpFℓSTR Profiler Plus Allelic Ladder Alleles	Control DNA 9947A Genotype
D3S1358	3p	5-FAM	12, 13, 14, 15, 16, 17, 18, 19	14, 15
vWA	12p12-pter	5-FAM	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21	17, 18
FGA	4q28	5-FAM	18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30	23, 24
Amelogenin	X: p22.1–22.3 Y: p11.2	JOE	X, Y	X
D8S1179	8	JOE	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	13[a]
D21S11	21	JOE	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, 38	30[b]

AmpFℓSTR Profiler Plus *ID* Loci and Allele Information *(continued)*

Locus Designation	Chromosome Location	Dye Label	AmpFℓSTR Profiler Plus Allelic Ladder Alleles	Control DNA 9947A Genotype
D18S51	18q21.3	JOE	9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26	15, 19
D5S818	5q21–31	NED	7, 8, 9, 10, 11, 12, 13, 14, 15, 16	11[c]
D13S317	13q22–31	NED	8, 9, 10, 11, 12, 13, 14, 15	11[d]
D7S820	7q11.21–22	NED	6, 7, 8, 9, 10, 11, 12, 13, 14, 15	10, 11

a. For CODIS purposes, profile reported as 13, 13.

b. For CODIS purposes, profile reported as 30, 30.

c. For CODIS purposes, profile reported as 11, 11.

d. For CODIS purposes, profile reported as 11, 11.

Kit Components

The AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit (P/N 4330284) contains the PCR reagents necessary to co-amplify the ten AmpFℓSTR Profiler Plus loci. The kit components are shown in the table below.

AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit Components

Kit Component	Volume	Description
AmpFℓSTR® PCR Reaction Mix	1.1 mL/tube	Two tubes of PCR Reaction Mix containing $MgCl_2$, dATP, dGTP, dCTP, dTTP, bovine serum albumin, and 0.05% sodium azide in buffer and salt.
AmpFℓSTR® Profiler Plus™ *ID* Primer Set	1.1 mL	One tube containing locus specific 5-FAM, JOE and NED dye-labeled and unlabeled primers in buffer that amplify the STR loci D3S1358, D5S818, D7S820, D8S1179, D13S317, D18S51, D21S11, FGA, vWA, and the gender marker, Amelogenin.
AmpliTaq Gold® DNA Polymerase	0.05 mL/tube	Two tubes of enzyme with an activity of 5 U/µL.
AmpFℓSTR® Control DNA 9947A	0.3 mL	One tube containing 0.10 ng/µL human cell line DNA in 0.05% sodium azide and buffer. Refer to pages 7 and 8 for profile.
Mineral Oil	5 mL	Supplied in dropper bottle.

AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit Components *(continued)*

Kit Component	Volume	Description
AmpFℓSTR Profiler Plus Allelic Ladder	50 µL	One tube of AmpFℓSTR Profiler Plus Allelic Ladder. See the table on pages 7 and 8 for a list of alleles included in the allelic ladder.

Kit Storage

The table below lists the storage temperature for the kit components.

IMPORTANT The fluorescent dyes attached to the primers are light-sensitive. Protect the AmpFℓSTR Profiler Plus *ID* Primer Set from light when not in use. Amplified DNA, AmpFℓSTR Profiler Plus Allelic Ladder and GeneScan™-500 ROX Size Standard should also be protected from light.

Component	Storage Temperature
AmpFℓSTR PCR Reaction Mix	2 to 8 °C
AmpFℓSTR Profiler Plus *ID* Primer Set	
AmpFℓSTR Control DNA 9947A	
AmpFℓSTR Profiler Plus Allelic Ladder	
AmpliTaq Gold DNA Polymerase	– 15 to –25 °C

AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit Performance Characteristics

The AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit reagents and protocols have been optimized to give the sensitivity and specificity necessary for forensic analysis. Two nanograms of the AmpFℓSTR Control DNA 9947A provided in the kit will reliably type when the protocols described in the *AmpFℓSTR Profiler Plus PCR Amplification Kit User's Manual* (P/N 4303501) are followed. The recommended range of input sample DNA is 1.0–2.5 ng. Samples containing less than one nanogram of human DNA have successfully been typed using the AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit.

PCR amplification component concentrations and thermal cycler parameters have been determined to produce specific amplification of the AmpFℓSTR Profiler Plus *ID* PCR Amplification loci. The STR loci in the AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit are specific for primate DNA. The primers used to amplify the Amelogenin locus are known to amplify a ~103-bp monomorphic band in some animals (see page 12-9 of the *AmpFℓSTR Profiler Plus PCR Amplification Kit User's Manual).*

A Certificate of Analysis is available upon request. The certificate confirms that the specific combination of components that comprise a given kit lot number perform together to meet the stated performance.

How to Run Your Samples

Protocols to Use

The AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit is designed to follow the same protocols as the AmpFℓSTR Profiler Plus PCR Amplification Kit. For detailed protocols, please refer to the appropriate chapters of the *AmpFℓSTR Profiler Plus PCR Amplification Kit User's Manual* (P/N 4303501).

Protocol	Refer to...
Amplification	Chapter 5, "Amplification"
ABI PRISM 377 DNA Sequencer	Chapter 7, "ABI PRISM 377 DNA Sequencer Protocols"
ABI PRISM 310 Genetic Analyzer	Chapter 8, "ABI PRISM 310 Genetic Analyzer Protocol"

Allelic Ladder

Each of the 5-FAM, JOE, and NED-labeled alleles which make up the AmpFℓSTR Profiler Plus Allelic Ladder are combined into a single tube. The AmpFℓSTR Profiler Plus Allelic Ladder is used for both the AmpFℓSTR Profiler Plus kit and the AmpFℓSTR Profiler Plus *ID* kit.

The AmpFℓSTR Profiler Plus Allelic Ladder consists of one 25-µL tube containing the following alleles: D3S1358 alleles 12 to 19; FGA alleles 18 to 30, and 26.2; vWA alleles 11 to 21; Amelogenin alleles X and Y; D8S1179 alleles 8 to 19; D18S51 alleles 9 to 26, 10.2, 13.2, and 14.2; D21S11 alleles 24 to 38, 24.2, 28.2, 29.2, 30.2, 31.2,32.2, 33.2, 34.2, and 35.2; D5S818 alleles 7 to 16; D7S820 alleles 6 to 15; and D13S317 alleles 8 to 15.

Please note the following changes to your *AmpFℓSTR Profiler Plus PCR Amplification Kit User's Manual* with respect to the allelic ladder preparation:

- Page 1-7: Description of AmpFℓSTR Allelic Ladders in Table 1-2 should state a single kit component named "AmpFℓSTR Profiler Plus Allelic Ladder." The AmpFℓSTR Profiler Plus Allelic Ladder is composed of the AmpFℓSTR Blue™, the AmpFℓSTR Green™ II, and AmpFℓSTR Yellow™ Allelic Ladders as currently defined. (This change should also be noted in the AmpFℓSTR Profiler Plus PCR Amplification Kit Product Insert).
- Page 7-14: Under "Preparing Samples and AmpFℓSTR Profiler Plus Allelic Ladder," steps 3 and 4 should be omitted. Step 8 remains unchanged.

- Page 8-11: Under “Preparing Samples and AmpFℓSTR Profiler Plus Allelic Ladder,” Steps 3 and 4 should be omitted. Step 6 on page 8-12 remains unchanged.
- Page 9-8: The sub-sections entitled “Loading Allelic Ladder onto the ABI PRISM 310 Genetic Analyzer” and “Loading Allelic Ladder onto the ABI PRISM 377 DNA Sequencer and ABI PRISM 377 DNA Sequencer with XL Upgrade,” refer to combining the AmpFℓSTR Blue, AmpFℓSTR Green II, and AmpFℓSTR Yellow Allelic Ladders into a single tube to prepare the AmpFℓSTR Profiler Plus Allelic Ladder. This reference is now irrelevant since the AmpFℓSTR Profiler Plus Allelic Ladder is ready to use as shipped in the AmpFℓSTR Profiler Plus and AmpFℓSTR Profiler Plus *ID* kits.

Getting Started

In order to get started:

- Confirm that the Filter Set F module files are installed on the Macintosh computer connected to the ABI PRISM 377 DNA Sequencer or the ABI PRISM 310 Genetic Analyzer. The Filter Set F module files must be located in the Modules folder, located in the ABI PRISM 377 or ABI PRISM 310 folder. See Chapter 6, "Multicomponent Analysis" in the *AmpFℓSTR Profiler Plus PCR Amplification Kit User's Manual* for more information on Filter Set F module files.
- Make a matrix file using the 5-FAM, JOE, NED, and ROX matrix standard samples and the Filter Set F module files. See Chapter 6, "Multicomponent Analysis" in the *AmpFℓSTR Profiler Plus PCR Amplification Kit User's Manual* for protocols on how to make a matrix file. Be sure to verify the accuracy of the matrix file.
- Determine the quantity of DNA in samples to be amplified. See Chapter 4, "DNA Quantitation" in the *AmpFℓSTR Profiler Plus PCR Amplification Kit User's Manual* for more details on DNA quantitation.
- Amplify DNA samples using the AmpFℓSTR Profiler Plus *ID* kit reagents. See Chapter 5, "Amplification" of the *AmpFℓSTR Profiler Plus PCR Amplification Kit User's Manual*. The recommended range of input DNA is 1.0–2.5 ng.
- Run AmpFℓSTR Profiler Plus *ID* PCR products on the ABI PRISM platforms.

IMPORTANT Use the GeneScan-500 ROX Internal Lane Size Standard.

- Analyze samples using GeneScan Analysis v2.1 or higher software. See Chapter 9, "Results and Interpretation" of the *AmpFℓSTR Profiler Plus PCR Amplification Kit User's Manual* (P/N 4304501) and the *GeneScan Analysis 3.1 User's Manual* (P/N 403001).
- Use the Genotyper software v2.5 or higher with AmpFℓSTR Profiler Plus kit template for automatic genotyping of samples (See Chapter 9, "Results and Interpretation" in the *AmpFℓSTR Profiler Plus PCR Amplification Kit User's Manual*).

Experiments Performed Using the AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit

Importance of Validation

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

Experiments

The AmpFℓSTR Profiler Plus PCR Amplification Kit was validated according to TWGDAM guidelines (Frank *et al.*, 2001; Holt *et al.*, 2001; Moretti *et al.*, 2001). The experiments for the AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit were performed according to the DNA Advisory Board (DAB) standards which were effective October 1, 1998. Experiments to evaluate the performance of AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit were performed relevant to the addition of the D8S1179 degenerate primer.

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, Applied Biosystems has conducted experiments that 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 section will discuss many of the experiments performed by Applied Biosystems and examples of results obtained. Conditions were chosen which 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. However, each laboratory using the AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit should perform their own appropriate validation studies.

Developmental Validation

8.1.1 Developmental Validation

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

Critical reagent concentrations and reaction conditions were tested (*e.g.*, Magnesium Chloride concentration, annealing temperature) relevant to the addition of the D8S1179 degenerate primer to produce reliable, locus-specific amplification and appropriate sensitivity have been determined.

PCR Components

The concentration of the D8S1179 degenerate primer of the AmpFℓSTR Profiler Plus *ID* Primer Set was examined. The concentration for the D8S1179 degenerate primer was established to be in the window that meets the reproducible performance characteristics of specificity and sensitivity. After establishing the optimum unlabeled D8S1179 degenerate primer concentration, all experiments were performed at that concentration. Varying Magnesium Chloride concentrations were also tested to determine the optimum concentration (see Figure 3 on page 16).

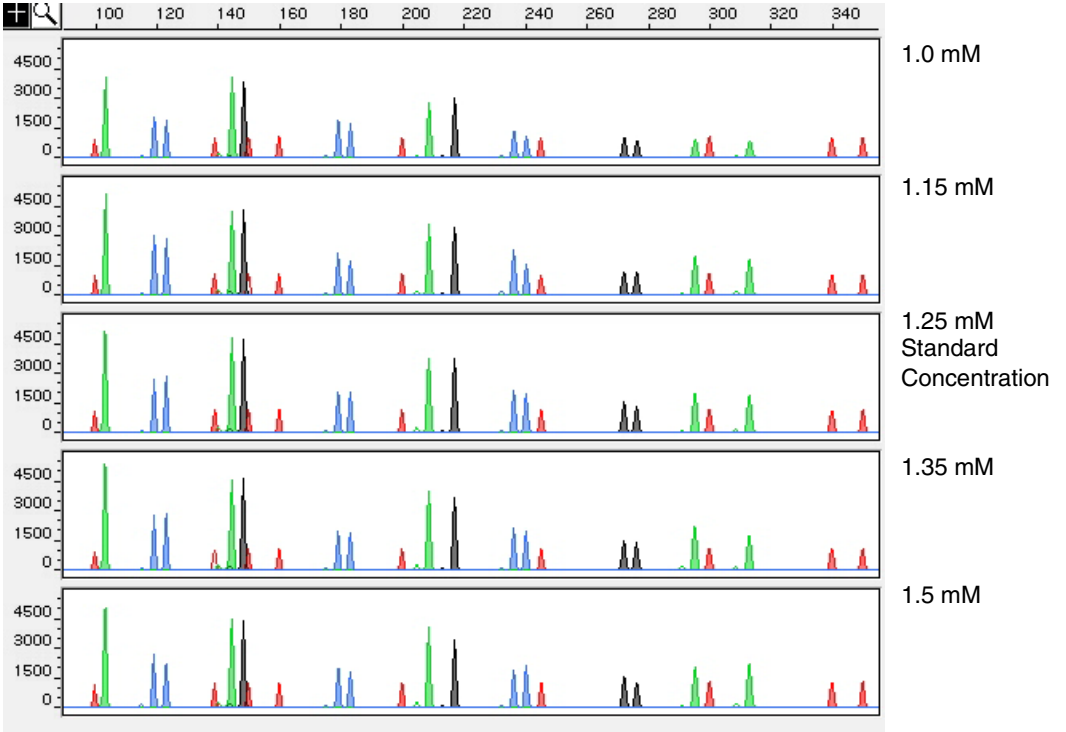

Figure 3 A 2 ng amplification of AmpFℓSTR Control DNA 9947A varying the Magnesium Chloride concentration, analyzed on the ABI PRISM 310 Genetic Analyzer

Thermal Cycler Parameters

Thermal cycling parameters were established for amplification of the AmpFℓSTR Profiler Plus *ID* kit on the GeneAmp® PCR System 9700 run in 9600 emulation mode. Varying annealing temperature windows were tested to verify that a ±2.0 °C window produced a specific PCR product with the desired sensitivity of at least 2 ng of AmpFℓSTR Control DNA 9947A.

The effects of annealing temperatures on the amplification of AmpFℓSTR Profiler Plus *ID* kit loci were examined using AmpFℓSTR Control DNA 9947A and two DNA samples with one mutant D8S1179 allele.

The annealing temperatures tested were 55, 57, 59, 61, and 63 °C (see Figure 4 on page 17) for 1-minute hold times in the GeneAmp PCR

System 9700. The PCR products were analyzed using the ABI PRISM 310 Genetic Analyzer.

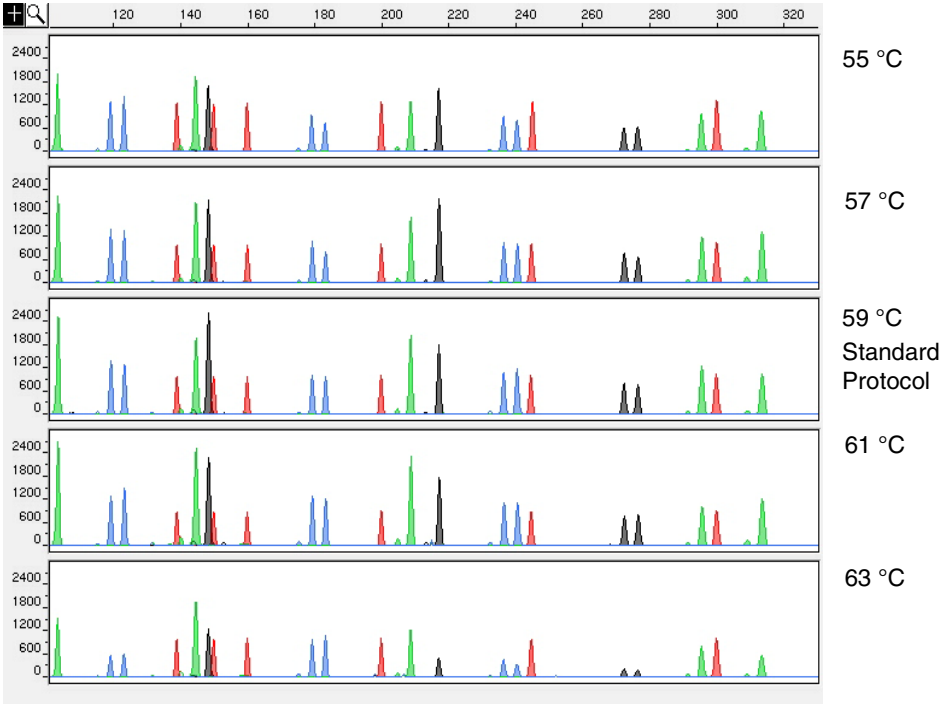

Figure 4 An amplification of 2 ng AmpFℓSTR Control DNA 9947A, amplified with the Profiler Plus *ID* kit while varying the annealing temperature, analyzed on the ABI PRISM 310 Genetic Analyzer

Species Specificity

8.1.2.2 Species Specificity

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

The AmpFℓSTR Profiler Plus *ID* 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 AmpFℓSTR Profiler Plus *ID* 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).The following experiments were conducted to investigate interpretation of AmpFℓSTR Profiler Plus *ID* kit results from nonhuman DNA sources.

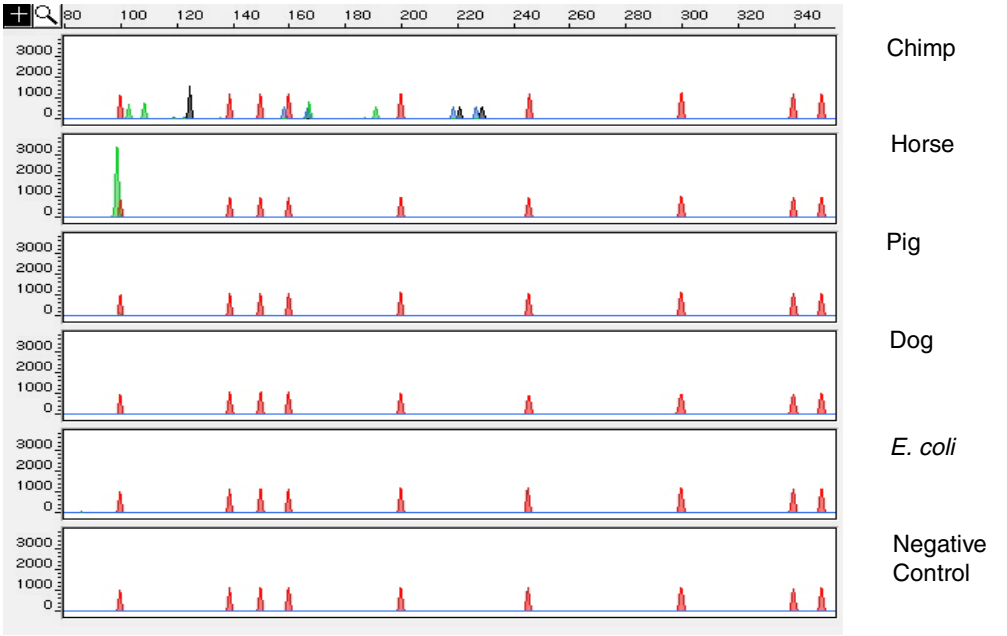

Figure 5 Representative electropherograms of a primate, non-primates, a microorganism, and a negative control are shown. All samples were analyzed on an ABI PRISM 310 Genetic Analyzer. The peaks depicted in red are the GeneScan-500 ROX size standard.

The extracted DNA samples were amplified with the AmpFℓSTR Profiler Plus *ID* kit reactions and analyzed using the ABI PRISM 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— *Escherichia*, and *Saccharomyces* (1–2.5 ng).

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

The microorganisms, chicken, cow, cat and mouse did not yield detectable product. Horse, dog, and pig produced a fragment near the Amelogenin locus in JOE dye (see Figure 5 on page 18).

Sensitivity

8.1.2.2 Sensitivity

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

The AmpFℓSTR Profiler Plus PCR Amplification Kit has been optimized to amplify and type approximately 1.0 to 2.5 ng of sample DNA reliably. The PCR cycle number and amplification conditions have been specified to produce low peak heights for a sample containing 35 pg of human genomic DNA. Low peak heights should be interpreted with caution. Thus, the overall sensitivity of the assay has been adjusted to avoid or minimize stochastic effects.

Importance of Quantitation

The amount of input DNA added to the AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit should be between 1.0 and 2.5 ng. The DNA sample should be quantitated prior to amplification using a system such as the QuantiBlot® Human DNA Quantitation Kit (P/N N808-0114). The final DNA concentration should be in the range of 0.05–0.125 ng/µL so that 1.0–2.5 ng of DNA will be added to the PCR reaction in a volume of 20 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 35-pg human genomic DNA. Low peak heights should be interpreted with caution (see Figure 6 on page 21).

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.

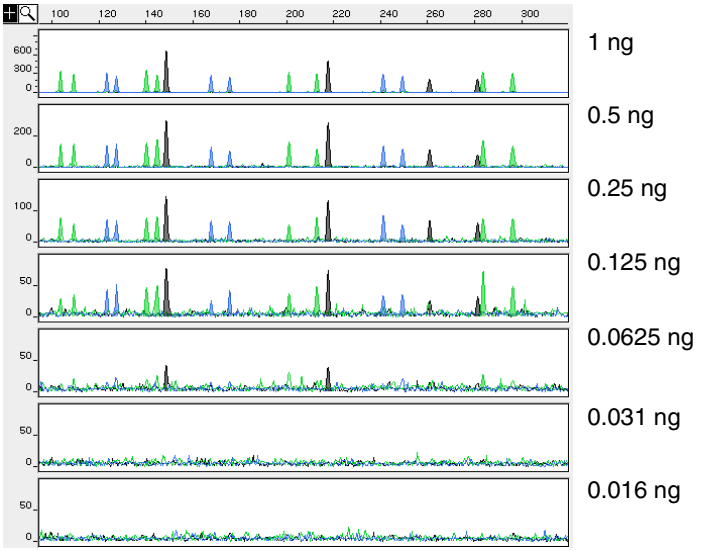

Figure 6 Effect of amplifying various amounts of DNA ranging from 16 pg to 1 ng. Note that the y axis scale differs in many of these panels.

Stability

8.1.2.2 Stability

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

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. Since 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. See the *AmpFℓSTR Profiler Plus PCR Amplification Kit User's Manual* for experiments pertaining to stability studies.

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 manual 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 AmpFℓSTR Profiler Plus *ID* 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 8 µg per 50-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).

See the *AmpFℓSTR Profiler Plus PCR Amplification User's Manual* for the effects of hematin on the amplification results obtained.

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.

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 AmpFℓSTR Profiler Plus *ID* 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.

Mixture Studies

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 during data analysis and interpretation. 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.

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 and were electrophoresed and detected using an ABI PRISM 310 Genetic Analyzer.

The results of the mixed DNA samples are shown in Figure 7 on page 25, where sample A and sample B were mixed according to the ratios provided.

The profiles of the samples in Figure 7 are the following:

Allele	Profile	
	Sample A	Sample B
Amelogenin	X,Y	X
D3S1358	15, 16	15, 18
D5S818	11	11, 13
D7S820	7, 12	9, 10
D8S1179	12, 13	13
D13S317	11	11
D18S51	12, 15	17, 19
D21S11	28, 31	30, 32.2
FGA	24, 26	23.2, 24
vWA	14, 16	17, 19

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.

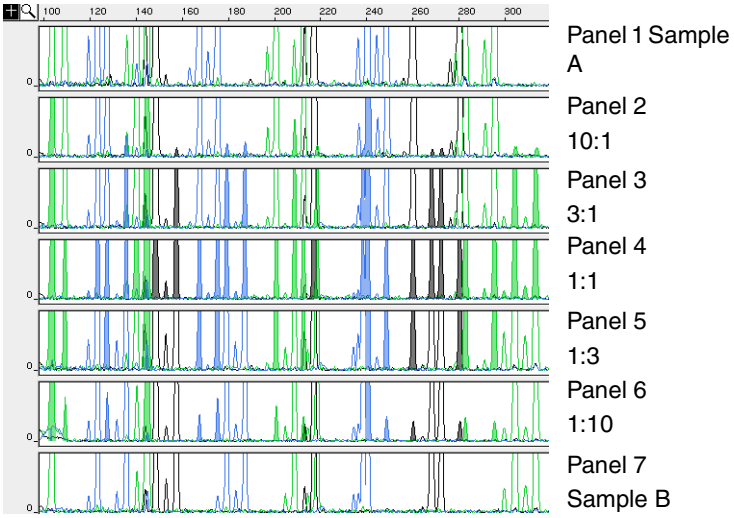

Figure 7 Results of the two DNA samples mixed together at defined ratios and amplified with the AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit. Sample A and Sample B are a male and female sample, respectively. The ratios of Sample A to Sample B (A:B ratios) shown are 10:1, 3:1, 1:1, 1:3, and 1:10, 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 AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit has been optimized to amplify and type approximately 1 to 2.5 ng of sample DNA reliably.

PCR Cycle Number and Amplification Conditions

The PCR cycle number and amplification conditions have been specified to produce low peak heights for a sample containing 35 pg human genomic DNA. Thus, the overall sensitivity of the assay has been adjusted to avoid or minimize stochastic effects. Applied Biosystems has successfully typed samples containing less than 1 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

8.1.2.3 Population Data

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

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 AmpFℓSTR Profiler Plus PCR Amplification Kit was used to generate the population data provided in the *AmpFℓSTR Profiler Plus PCR Amplification User's Manual* to include 195 African American and 200 Caucasians.

Homozygous samples at the D8S1179 locus were reamplified using the AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit to confirm the homozygosity at this locus. Of the 68% African American and the 60% Caucasian homozygous samples at the D8S1179 locus available for re-testing, all samples typed as homozygotes. None of these samples were found to be heterozygous using the AmpFℓSTR Profiler Plus *ID* PCR Amplification Kit. Refer to Chapter 13, "Population Genetics", Table 13-1 in the *AmpFℓSTR Profiler Plus PCR Amplification Kit User's Manual* for allele frequencies in the African American and Caucasian populations (Holt *et al*., 2001).

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