AmpFℓSTR® Sinofiler™

PCR Amplification Kit

User Guide

Applied Biosystems

AmpFℓSTR® Sinofiler™

PCR Amplification Kit

User Guide

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Preface

How to Use This Guide

Purpose of This Guide

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

Pull-Out Chapters

This guide is designed to allow users to pull out chapters 2, 3, and 4. The pull-out chapters have title and back pages, which indicate the chapter number and title.

Text Conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: The Calibrate function is also available in the Control Console.

IMPORTANT! To verify your client connection to the database, you need a valid user ID and password.

Safety

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word–**IMPORTANT, CAUTION, WARNING, DANGER**–implies a particular level of observation or action, as defined below.

Definitions

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION – Indicates a potentially hazardous situation that, 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 that, if not avoided, could result in death or serious injury.

DANGER – Indicates an imminently hazardous situation that, 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.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- 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. (See **"About MSDSs"** on **page vii**.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, 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 (for example, 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 in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then click **MSDS Search**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest, then click **Search**.
3. Find the MSDS of interest, click the link or right-click the MSDS title, then select any of the following:
   - **Open** – To view the MSDS

- **Print Target** – To print the MSDS
- **Save Target As** – To download a PDF version of the MSDS

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical Waste Hazards

CAUTION **HAZARDOUS WASTE.** Refer to Material Safety Data Sheets and local regulations for handling and disposal.

WARNING **CHEMICAL WASTE HAZARD.** Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING **CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide 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.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, 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 (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological Hazard Safety

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:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **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

How to Obtain More Information

Related Documentation

To obtain any of the following documents, go to **www.appliedbiosystems.com**, then click the links for **Support ▸ Products & Services Literature**.

Document	Part Number
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
ABI PRISM® 3100/3100-Avant Data Collection v2.0 User Guide	4347102
ABI PRISM® 3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0 User Bulletin	4350218
ABI PRISM® 3100 Genetic Analyzer User Manual (Data Collection v1.1)	4315834
ABI PRISM® 3100-Avant Genetic Analyzer User Guide (Data Collection v1.0)	4333549
ABI PRISM® 3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products User Bulletin	4332345
ABI PRISM® 310 Genetic Analyzer User Guide (Windows NT)	4317588
Installation Procedures and New Features for GeneMapper® ID Software v3.2 User Bulletin	4352543
GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial	4335523
GeneMapper® ID Software Version 3.1 Human Identification Analysis: User Guide	4338775
Quantifiler® Kits: Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit User's Manual	4344790
GeneMapper® ID Software v3.2.1 Patch User Bulletin	4382255
AmpFℓSTR® Identifiler® PCR Amplification Kit User's Manual	4323291

Note: For additional documentation, see **"How to Obtain Support"** on **page xii**.

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is only for submitting comments and suggestions relating to documentation. To order documents, download PDF files, or for help with a technical question, go to **www.appliedbiosystems.com**, then click the link for **Support**. See **"How to Obtain Support"** below).

How to Obtain Support

For the latest services and support information for all locations, go to **www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

Overview 1

This chapter covers:

Product Overview

Purpose

The AmpFℓSTR® Sinofiler™ PCR Amplification Kit (Sinofiler kit) is a short tandem repeat (STR) multiplex assay that amplifies 15 autosomal STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, D5S818, D13S317, D16S539, D2S1338, D19S433, vWA, D12S391, D18S51, D6S1043, FGA) and the sex-determining marker, amelogenin, in a single PCR reaction.

Product Description

The Sinofiler 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:

- Applied Biosystems 3130/3130*xl* Genetic Analyzer
- ABI PRISM® 3100/3100-*Avant* Genetic Analyzer
- ABI PRISM® 310 Genetic Analyzer
- GeneAmp® PCR System 9600
- Silver 96-Well GeneAmp® PCR System 9700
- Gold-plated silver block GeneAmp® PCR System 9700

About the Primers

The AmpFℓSTR® Sinofiler™ kit employs the same primer sequences as used in the previous AmpFℓSTR® kits with the exception of D6S1043 and D12S391. Degenerate primers for the loci D8S1179, vWA, and D16S539 were added to the AmpFℓSTR® Sinofiler™ Primer Set to address mutations in the primer binding sites. The addition of the degenerate primers allows for the amplification of those alleles in samples containing the mutations without altering the overall performance of the AmpFℓSTR Sinofiler PCR Amplification Kit.

Non-nucleotide linkers are used in primer synthesis for the following loci: CSF1PO, D5S818, D13S317, D16S539, D2S1338, D12S391, D18S51, amelogenin, and D6S1043. 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 inter-locus 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

Table 1-1 shows the loci amplified, their chromosomal locations, and the corresponding fluorescent marker dyes. The AmpFℓSTR® Sinofiler™ Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the allelic ladder, and the genotype of the Control DNA 9947A are also listed in the table.

Table 1-1 AmpFℓSTR® Sinofiler™ PCR Amplification Kit loci and alleles

Locus Designation	Chromosome Location	Alleles Included in Sinofiler 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
D5S818	5q21-31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16		11
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
D12S391	12p13.2	14, 15, 16, 17, 18, 19, 19.3, 20, 21, 22, 23, 24, 25, 26, 27		18, 20
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

Table 1-1 AmpFℓSTR® Sinofiler™ PCR Amplification Kit loci and alleles *(continued)*

Locus Designation	Chromosome Location	Alleles Included in Sinofiler Allelic Ladder	Dye Label	Control DNA 9947A
Amelogenin	X: p22.1-22.3 Y: p11.2	X, Y	PET®	X
D6S1043	6q16.1	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 21.3, 22, 23, 24, 25		12, 18
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

Allelic Ladder

Figure 1-1 shows the allelic ladder for the AmpFℓSTR® Sinofiler™ kit. See "Allelic Ladder Requirements" on page 3-2 for information on ensuring accurate genotyping.

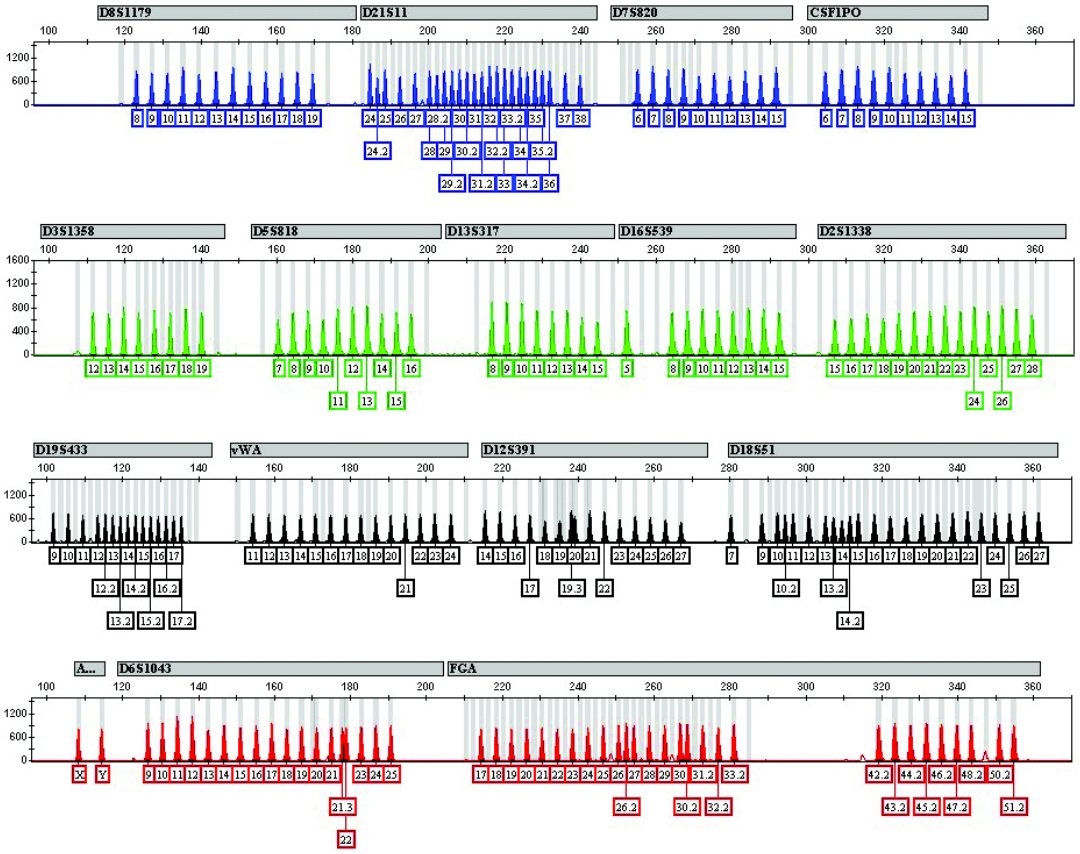
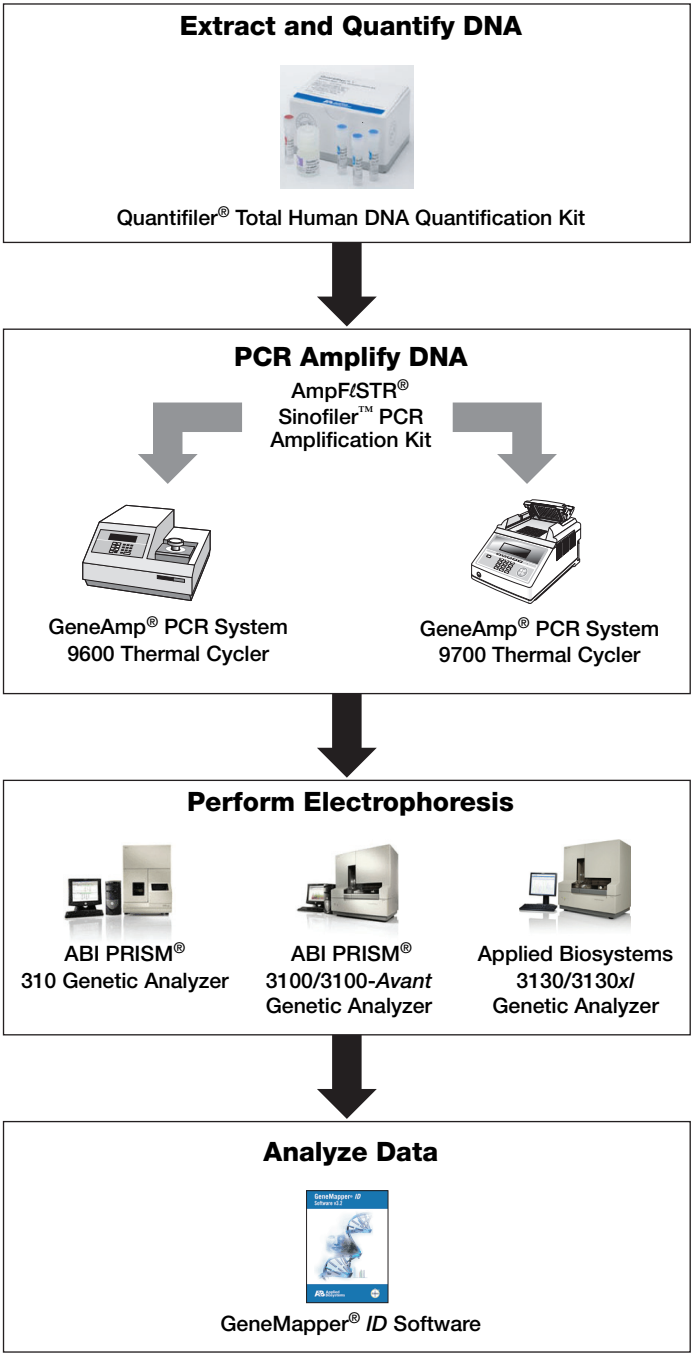


Figure 1-1 GeneMapper® *ID* Software plot of the AmpFℓSTR® Sinofiler™ Allelic Ladder

Workflow Overview



Instrument and Software Overview

This section provides information about the data collection and analysis software versions required to run the AmpFℓSTR® Sinofiler™ PCR Amplification Kit on specific instruments.

Data Collection and Analysis 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 analysis software.

Instrument and Software Compatibility

Instrument	Operating System	Data Collection Software	Analysis Software
3130/3130*xl*‡	Windows XP	3.0	GeneMapper® *ID* v3.2.1 and later
3100/3100-*Avant*	Windows NT®	1.1 (3100) 1.0 (3100-*Avant*)	GeneMapper *ID* v3.2.1 and later
	Windows 2000	2.0	GeneMapper *ID* v3.2.1 and later
310	Windows XP	3.1	GeneMapper *ID* v3.2.1 and later
	Windows NT and Windows 2000	3.0	GeneMapper *ID* v3.2.1 and later

‡ Applied Biosystems conducted validation studies for the Sinofiler kit using these configurations.

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 AmpFℓSTR Sinofiler PCR Amplification 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.

How Multicomponent Analysis Works

Each of these fluorescent dyes emits its maximum fluorescence at a different wavelength. During data collection on the Applied Biosystems and ABI PRISM® instruments, the fluorescence signals are separated by a 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 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 1-2). The goal of multicomponent analysis is to correct for spectral overlap.

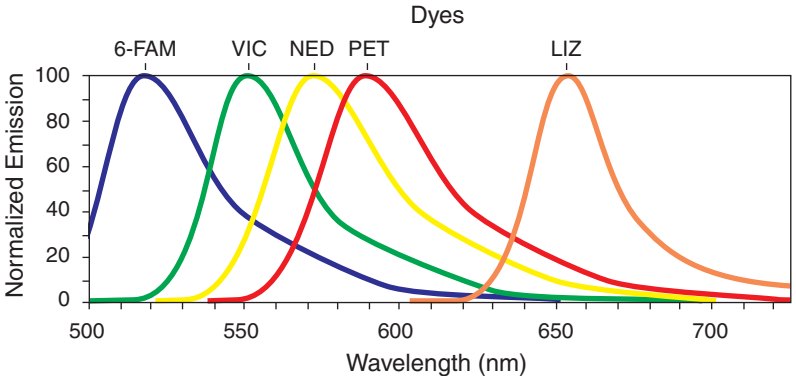


Figure 1-2 Emission spectra of the five dyes used in the AmpFℓSTR® Sinofiler™ PCR Amplification Kit

Materials and Equipment

Kit Contents

The AmpFℓSTR® Sinofiler™ kit contains sufficient quantities of the following reagents and the appropriate licenses to perform 200 25-µL amplifications:

Component	Description	Volume
AmpFℓSTR® PCR Reaction Mix	Two tubes containing $MgCl_2$, deoxynucleotide triphosphates, and bovine serum albumin in buffer with 0.05% sodium azide	1.1 mL/tube
AmpFℓSTR® Sinofiler™ Primer Set	Two tubes containing fluorescently-labeled primers and non-labeled primers	0.55 mL/tube
AmpFℓSTR® Control DNA 9947A	One tube containing 0.10 ng/µL human female cell line DNA in 0.05% sodium azide and buffer (refer to pages 1-3 and 1-4 for profile)	0.3 mL
AmpFℓSTR® Sinofiler™ Allelic Ladder	One tube of AmpFℓSTR® Sinofiler™ Allelic Ladder containing amplified alleles. See Table 1-1 on pages 1-3 and 1-4 for a list of alleles included in the allelic ladder	50 µL
AmpliTaq Gold® DNA Polymerase	Two tubes of enzyme with an activity of 5 U/µL	50 µL/tube

Kit Storage and Stability

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

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

Component	Storage Temperature
AmpFℓSTR® PCR Reaction Mix	–20 °C on receipt, 2 to 8 °C after initial use
AmpFℓSTR® Control DNA 9947A	
AmpFℓSTR® Sinofiler™ Allelic Ladder	
AmpliTaq Gold DNA Polymerase	–15 to –25 °C
AmpFℓSTR® Sinofiler™ Primer Set	

Standards for Samples

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

- **Control DNA 9947A** – A positive control for evaluating the efficiency of the amplification step and STR genotyping using the AmpFℓSTR Sinofiler Allelic Ladder.
- **GeneScan™ 500 LIZ® Size Standard** – Used for obtaining sizing results. It contains 16 single-stranded fragments of 35, 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490, and 500 nucleotides. This standard, which has been evaluated as an internal lane size standard, yields precise sizing results for AmpFℓSTR Sinofiler PCR products. Order the GeneScan 500 LIZ Size Standard (PN 4322682) separately.
- **AmpFℓSTR® Sinofiler™ Allelic Ladder** – Developed by Applied Biosystems for accurate characterization of the alleles amplified by the Sinofiler kit. The AmpFℓSTR Sinofiler Allelic Ladder contains most alleles reported for the 15 autosomal loci. Refer to "Loci Amplified by the Kit" on page 1-3 for a list of the alleles included in the Sinofiler kit.

Equipment and Materials Not Included

Tables 1-2 and 1-3 list required and optional equipment and materials not supplied with the Sinofiler kit. Unless otherwise noted, many of the items are available from major laboratory suppliers (MLS).

Table 1-2 Equipment

Equipment	Source
Applied Biosystems 3130/3100*xl* Genetic Analyzer	Contact your local Applied Biosystems sales representative
ABI PRISM® 3100/3100-*Avant* Genetic Analyzer	
ABI PRISM® 310 Genetic Analyzer	
GeneAmp® PCR System 9700 with the Silver 96-Well block	N8050001
GeneAmp® PCR System 9700 with the Gold-plated silver block	4314878
Silver 96-Well sample block	N8050251
Gold-plated Silver 96-Well sample block	4314443
Tabletop centrifuge with 96-well plate adapters (optional)	Major Laboratory Supplier (MLS)

Table 1-3 User-supplied materials‡

Item	Source
AmpFℓSTR® Sinofiler™ PCR Amplification Kit	4382306
3130/3100*xl* Analyzer materials	
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3130*xl*/3100 Genetic Analyzer Capillary Array, 36-cm	4315931
3130/3100-*Avant* Genetic Analyzer Capillary Array, 36-cm	4333464
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
Running Buffer, 10×	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* (PN 4352716).	

Table 1-3 User-supplied materials‡ *(continued)*

Item	Source
3100/3100-*Avant* Analyzer materials	
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3130*xl*/3100 Genetic Analyzer Capillary Array, 36-cm	4315931
3130/3100-*Avant* Genetic Analyzer Capillary Array, 36-cm	4333464
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	4322682
Running Buffer, 10×	402824
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 *ABI PRISM® 3100 Genetic Analyzer and 3100-Avant Genetic Analyzer User Reference Guide* (PN 4335393).	
310 Analyzer materials	
310 Genetic Analyzer Capillary, 47-cm	402839
0.5-mL Sample Tray	5572
96-Well Tray Adapter (for 9700 thermal cycler trays)	4305051
GeneScan™ 500 LIZ® Size Standard	4322682
Running Buffer, 10×	402824
Genetic Analyzer Septa Retainer Clips for 96-Tube Sample Tray	402866
Genetic Analysis Sample Tubes (0.5-mL)	401957

Table 1-3 User-supplied materials‡ *(continued)*

Item	Source
Septa for 0.5-mL Sample Tubes	401956
DS-33 Matrix Standard Set [6FAM™, VIC®, NED™, PET®, and LIZ® dyes] for ABI PRISM® 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 *ABI PRISM® 310 Genetic Analyzer User Guide* (PN 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-Caps Strip	N8010535
MicroAmp™ 96-Well Tray/Retainer Set	403081
MicroAmp™ 96-Well Base	N8010531
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

Table 1-3 User-supplied materials‡ *(continued)*

Item	Source
Tube, 50-mL Falcon	MLS
Tube decapper, autoclavable	MLS
Deionized water, PCR grade	MLS
Tris-HCL, pH 8.0	MLS
0.5-M EDTA	MLS
Vortex	MLS

‡ For the Material Safety Data Sheet (MSDS) of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the MSDS provided by the manufacturer, and observe all relevant precautions.

Chapter 2

PCR Amplification

PCR Amplification 2

This chapter covers:

PCR Work Areas

PCR Setup Work Area

IMPORTANT! The following 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! The following GeneAmp® PCR Systems should never leave the Amplified DNA Work Area.

- Silver 96-Well GeneAmp® PCR System 9700
- Gold-plated silver block GeneAmp® PCR System 9700
- GeneAmp® PCR System 9600

Required User-Supplied Materials and Reagents

Kit Contents and Storage

Each AmpFℓSTR® Sinofiler™ PCR Amplification Kit contains materials sufficient to perform 200 reactions at a 25-µL reaction volume. See "Kit Contents" on page 1-9 for details on Sinofiler kit contents.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set from light when not in use. Amplified DNA, AmpFℓSTR® Sinofiler™ Allelic Ladder, and GeneScan™ 500 LIZ® Size Standard should also be protected from light. Minimize freeze-thaw cycles.

User-Supplied Reagents

In addition to the Sinofiler 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 following table or order it from Teknova (Cat # T0223).

To prepare low TE buffer

1.	Mix together: • 10 mL of 1 M Tris-HCl, pH 8.0 • 0.2 mL of 0.5 M EDTA, pH 8.0 • 990 mL glass-distilled or deionized water **WARNING** **CHEMICAL HAZARD. EDTA.** Exposure causes eye irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Note:** Adjust the volumes accordingly for specific needs.
2.	Aliquot and autoclave the solutions.
3.	Store at room temperature.

Quantifying DNA

Importance of Quantitation

By quantifying the DNA in a sample, you determine if there is enough DNA for adequate amplification. You can determine the smallest volume necessary to obtain 0.50 to 1.25 ng of DNA. However, the maximum allowable addition of DNA is 10 µL.

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, resulting 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 for Quantifying DNA

Applied Biosystems provides several kits for accurately quantifying DNA in samples. See the reference cited in Table 2-1 on page 2-5 for details about these kits.

Table 2-1 Methods for quantifying DNA

Product	Description	References
Quantifiler® Y Human Male DNA Quantification Kit (PN 4343906) Quantifiler® Human DNA Quantification Kit (PN 4343895)	**Properties:** • Both Quantifiler® kits have high specificity for human DNA. The Quantifiler® Y kit is highly specific for human male DNA. • The kit detects single-stranded and degraded DNA. **How it works:** The DNA quantification assay combines two 5′ nuclease assays: • A target-specific (human DNA or human male DNA) assay, which consists of two primers for amplifying human or human male DNA and one TaqMan® MGB probe labeled with FAM™ dye for detecting the amplified sequence • An 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 DNA, and one TaqMan MGB probe labeled with VIC® dye for detecting the amplified IPC DNA	*Quantifiler® Human DNA Quantification Kits User's Manual* (PN 4344790)

Preparing the Reactions

Master Mix

Prepare the master mix by combining AmpFℓSTR® PCR Reaction Mix, AmpliTaq Gold® DNA Polymerase, and AmpFℓSTR® Sinofiler™ Primer Set reagents.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the AmpFℓSTR® Sinofiler Primer Set from light when not in use. Also protect the AmpFℓSTR® Sinofiler Allelic Ladder, GeneScan™ 500 LIZ® Size Standard, and amplified, fluorescently-labeled PCR products from light.

To prepare the master mix

1. Determine the total number of samples, including controls.

2. **IMPORTANT!** Vortex the following reagents for 5 sec:
   - AmpFℓSTR® PCR Reaction Mix
   - AmpliTaq Gold DNA Polymerase
   - AmpFℓSTR® Sinofiler Primer Set

   WARNING **CHEMICAL HAZARD. AmpliTaq Gold DNA Polymerase** may cause eye and skin irritation. It may cause discomfort if swallowed or inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eye wear, clothing, and gloves.

3. Spin the tubes briefly in a microcentrifuge to remove any liquid from the caps.

4. Select a clean, unused tube for the master mix.

If you are preparing...	Then use a...
≤84 samples and controls	1.5-mL microcentrifuge tube
85–110 samples and controls	2.0-mL microcentrifuge tube
>110 samples and controls	Tube that is appropriate

To prepare the master mix *(continued)*

5.	Calculate the required amount of components as shown: **Note:** The formulation in the list below provides a slight overfill to allow for volume lost in pipetting. Number of samples × 10.5 µL of AmpFℓSTR® PCR Reaction Mix Number of samples × 0.5 µL of AmpliTaq Gold DNA Polymerase Number of samples × 5.5 µL of AmpFℓSTR® Sinofiler Primer Set
6.	Dispense the appropriate volume of each of the components from step 5 into a microcentrifuge tube.
7.	Vortex the master mix at medium speed for 3 sec, then centrifuge the tube or plate briefly before opening the tubes.
8.	Dispense 15 µL of master mix into each reaction tube or plate well.

Preparing Sinofiler Kit Reactions

1. Prepare the DNA samples:

DNA Sample	To Prepare...
Negative Control	Add 10 µL of low TE buffer to the reaction tube or plate well.
Your Sample	Dilute a portion of your DNA sample with low TE buffer so that 0.50–1.25 ng of total DNA is in a final volume of 10 µL. Add your sample to the reaction tube or plate well.
Positive Control	Add 10 µL of control DNA 9947A (0.1 ng/µL) to the reaction tube or plate well.

Note: The final reaction volume should be 25 µL.

2. Centrifuge the plate at 3,000 rpm for about 20 seconds in a tabletop centrifuge with plate holders to remove bubbles.

3. Amplify the DNA in a GeneAmp® PCR System 9600 or a Silver 96-Well GeneAmp® PCR System 9700, or a Gold-plated silver block GeneAmp® PCR System 9700.

Performing PCR

To run PCR

1. Program the thermal cycling conditions.

   IMPORTANT! If using the Gold-plated Silver or Silver 96-Well GeneAmp PCR System 9700, select the **9600 Emulation Mode**.

Initial Incubation Step	Cycle (28 cycles)			Final Extension	Final Hold
	De-nature	Anneal	Extend		
HOLD	CYCLE			HOLD	HOLD
95 °C 11 min	94 °C 1 min	59 °C 1 min	72 °C 1 min	60 °C 60 min	4 °C ∞

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

   WARNING **PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the heated cover can be as high as 108 °C, and the temperature of the sample block can be as high as 100 °C. Keep hands away from the heated cover and sample block.

3. Start the run.

4. Store the amplified DNA.

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

   IMPORTANT! Protect the amplified products from light.

Amplification Using Bloodstained FTA Cards

FTA™-treated DNA collection cards can be useful for the collection, storage, and processing of biological samples. A small punch of the bloodstained card can be placed directly into an amplification tube, purified, and amplified without transferring the evidence. Applied Biosystems studies have indicated that a 1.2-mm bloodstained punch contains approximately 5–20 ng DNA. Accordingly, an appropriate cycle number for this high quantity of DNA is 25 cycles. It is recommended that each laboratory determine the cycle number based on individual validation studies.

In the example shown in Figure 2-1, a 1.2-mm punch of a bloodstained FTA card was purified using three washes with FTA Purification Reagent and two washes with 1✕ TE buffer. After drying at room temperature overnight, the punch was then amplified directly in the MicroAmp® tube for 25 cycles.

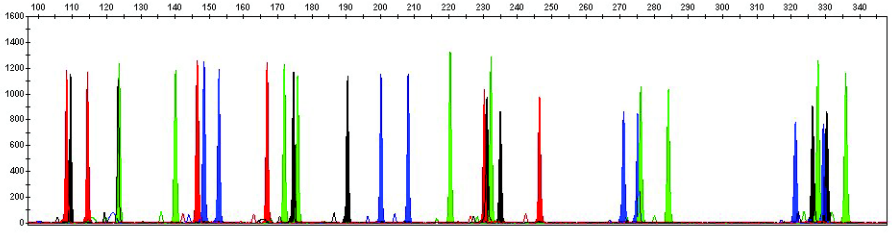


Figure 2-1 AmpFℓSTR® Sinofiler™ PCR Amplification Kit results from a 1.2-mm FTA bloodstain punch (25-cycle amplification), analyzed on the ABI PRISM® 3130*xl* Genetic Analyzer.

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Chapter 3

Electrophoresis

Performing Electrophoresis 3

This chapter covers:

Allelic Ladder Requirements

To accurately genotype samples, you must run an allelic ladder sample along with the unknown samples. For samples that are run on the:

- ABI PRISM® 310 Genetic Analyzer – Run at least one allelic ladder for every 10 sample injections.
- ABI PRISM® 3100 or Applied Biosystems 3130 series instruments – Run at least one allelic ladder per every set of 16 samples.
  - Applied Biosystems 3130*xl* or ABI PRISM® 3100 – One ladder per injection; one injection = 16 samples (15 samples + 1 allelic ladder)
  - Applied Biosystems 3130 or ABI PRISM® *Avant* – One ladder for every 4 injections; one injection = 4 samples

IMPORTANT! Variation in laboratory temperature can cause changes in fragment migration speed which can cause sizing variation. Applied Biosystems recommends 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.
- Slight procedural and reagent variations between single and multiple capillaries result in greater size variation than that found between samples injected in the same capillary in a single run.

Setting Up the 3100/3100-*Avant* or 3130/3130*xl* Instrument for Electrophoresis

Reagents and Parts

Table 1-3 on page 1-11 lists the required materials not supplied with the AmpFℓSTR® Sinofiler™ PCR Amplification Kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set from light when not in use. Amplified DNA, AmpFℓSTR® Sinofiler™ Allelic Ladder, and GeneScan™ 500 LIZ® Size Standard should also be protected from light. Minimize freeze-thaw cycles.

Electrophoresis Setup Software and Reference Documents

This table lists data collection software and the run modules that you can use to analyze Sinofiler kit products. For details on the procedures, refer to the documents listed in the table.

Operating System	Data Collection Software	Run Module	References
Windows XP	3.0 (3130/3130*xl* Analyzer)‡	• HIDFragmentAnalysis36_POP4_1 • 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* (PN 4363787)
Windows 2000	2.0	• HIDFragmentAnalysis36_POP4_1 • Dye Set G5	*ABI PRISM® 3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0, Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products User Bulletin* (PN 4350218)
Windows NT®	1.1 (3100 Analyzer)	Run Module: GeneScan36vb_DyeSetG5Module Analysis Module: GS500Analysis.gsp	*ABI PRISM® 3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products User Bulletin* (PN 4332345)
	1.0 (3100-*Avant* Analyzer)	Run Module: GeneScan36Avb_DyeSetG5Module Analysis Module: GS500Analysis.gsp	

‡ Applied Biosystems conducted validation studies for the Sinofiler kit using this configuration.

Preparing Samples for Electrophoresis on the 3100/3100-*Avant* or 3130/3130*xl* Instrument

Preparing the Samples

Prepare the samples for electrophoresis on the 3100/3100-*Avant* or 3130/3130*xl* immediately before loading.

To prepare samples for electrophoresis

1. Calculate the volume of Hi-Di™ Formamide and GeneScan™ 500 LIZ® Internal Size Standard needed to prepare the samples, using the table below.

Reagent	Volume Per Reaction (µL)
GeneScan™ 500 LIZ® Size Standard	0.3
Hi-Di™ Formamide	8.7

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/experiments.

WARNING CHEMICAL HAZARD. Hi-Di Formamide. Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

2. Pipette the required volumes of components into an appropriately sized polypropylene tube.

3. Vortex the tube, then centrifuge briefly.

To prepare samples for electrophoresis *(continued)*

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 briefly centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.
6.	Heat the reaction plate in a thermal cycler for 3 minutes at 95 °C. **WARNING** **PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the heated cover can be as high as 108 °C, and the temperature of the sample block can be as high as 100 °C. Keep hands away from the heated cover and sample block.
7.	Immediately place the plate on ice for 3 minutes.
8.	Prepare the plate assembly on the autosampler.
9.	Start the electrophoresis run.

Setting Up the 310 Instrument for Electrophoresis

Reagents and Parts

Table 1-3 on page 1-11 lists the required materials not supplied with the AmpFℓSTR® Sinofiler™ PCR Amplification Kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set from light when not in use. Amplified DNA, AmpFℓSTR® Sinofiler™ Allelic Ladder, and GeneScan™ 500 LIZ® Size Standard should also be protected from light. Minimize freeze-thaw cycles.

Electrophoresis Setup Software and Reference Documents

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

Operating System	Data Collection Software	Run Module	References
Windows XP	3.1‡	GS STR POP4 (1 mL) G5 v2.md5	*ABI PRISM® 310 Genetic Analyzer User's Manual (Windows)* (PN 4317588) *ABI PRISM® 310 Protocols for Processing AmpFℓSTR® PCR Amplification Kit Products with Microsoft Windows NT Operating System: User Bulletin* (PN 4341742)
Windows NT® and Windows 2000	3.0	GS STR POP4 (1 mL) G5 v2.md5	*ABI PRISM® 310 Genetic Analyzer User's Manual (Windows)* (PN 4317588) *ABI PRISM® 310 Protocols for Processing AmpFℓSTR® PCR Amplification Kit Products with Microsoft Windows NT Operating System: User Bulletin* (PN 4341742)

‡ Applied Biosystems conducted concordance studies for the Sinofiler kit using this configuration.

Preparing Samples for Electrophoresis on the 310 Instrument

Preparing the Samples

Prepare the samples for electrophoresis on the 310 instrument immediately before loading.

To prepare samples for electrophoresis

1. Calculate the volume of Hi-Di™ Formamide and GeneScan™ 500 LIZ® Internal Size Standard needed to prepare the samples, using the table below.

Reagent	Volume Per Reaction (µL)
GeneScan™ 500 LIZ® Size Standard	0.5
Hi-Di™ Formamide	24.5

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/experiments.

WARNING CHEMICAL HAZARD. Hi-Di Formamide. Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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 or 0.5-mL sample tube, add:
   - 25 µL of the formamide:size standard mixture
   - 1.5 µL of PCR product or Allelic Ladder

To prepare samples for electrophoresis *(continued)*

5.	Seal the tubes with appropriate septa, then briefly centrifuge the tubes 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. **WARNING PHYSICAL INJURY HAZARD.** During instrument operation, the temperature of the heated cover can be as high as 108 °C, and the temperature of the sample block can be as high as 100 °C. Keep hands away from the heated cover and sample block.
7.	Immediately place the tubes on ice for 3 minutes.
8.	Place the sample tray on the autosampler.
9.	Start the electrophoresis run.

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Chapter 4

Analyzing Data

Analyzing Data 4

This chapter covers:

Overview of GeneMapper® *ID* Software

GeneMapper® *ID* Software is an automated genotyping software for forensic, paternity, and database data analysis and other genotyping needs.

After electrophoresis, the data collection software stores information for each sample in a .fsa file. Using GeneMapper *ID* v3.2.1 and v3.3 software, you can then analyze and interpret the data from the .fsa files.

Instruments

Refer to "Instrument and Software Overview" on page 1-7 for a list of compatible instruments.

Before You Start

When using GeneMapper *ID* Software v3.2.1 and v3.3 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.
- Allelic bin definitions are stored in the AmpFℓSTR_Sinofiler panels in the Panel Manager.
- Lanes or 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 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.

Setting Up GeneMapper® *ID* Software for Analyzing AmpFℓSTR® Sinofiler™ Kit Data

Workflow

Before you can analyze sample (.fsa) files using GeneMapper *ID* Software v3.2.1 or v3.3 for the first time, you need to:

- Import panels and bins into the Panel Manager, as explained in "Importing Panels and Bins (3.2.1 only)" on page 4-4. (This section does not apply to GeneMapper *ID* Software v3.3, which automatically installs panels and bin sets during installation.)
- Import an analysis method, as explained in "Importing an HID Analysis Method (v3.2.1 and v3.3)" on page 4-9.
- Import a size standard, as explained in "Importing an HID Size Standard (v3.2.1 and v3.3)" on page 4-16.
- Define custom views of analysis tables (v.3.2.1 and v3.3).

  Refer to Chapter 1 of the *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (PN 4335523) for more information.
- Define custom views of plots (v.3.2.1 and v3.3).

  Refer to Chapter 1 of the *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (PN 4335523) for more information.
- If necessary, convert any GeneScan software sample files generated on the Macintosh® platform to the .fsa format using the Mac-to-Win AppleScript® software provided with GeneMapper *ID* software. Conversion is described in the GeneMapper® *ID Software Version 3.1 Human Identification Analysis User Guide* (PN 4338775).

For More Info

For quick set up instructions, refer to the *GeneMapper® ID Software Version 3.3 Getting Started Guide* (PN 4385329).

For details about GeneMapper *ID* features, refer to the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (PN 4338775) and the *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (PN 4335523). Also, refer to the *Installation Procedures and New Features for GeneMapper® ID Software v3.2 User Bulletin* (PN 4352543).

Importing Panels and Bins (3.2.1 only)

To import the Sinofiler kit panels and bin sets from the Applied Biosystems web site into the GeneMapper *ID* Software v3.2.1 database:

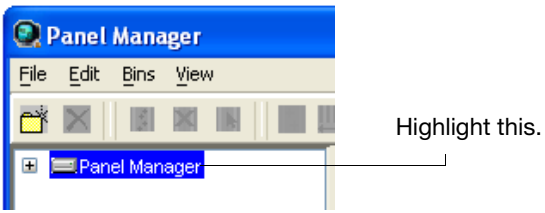
To import panels and bin sets

1.	Download and open the file containing panels and bins: a. Open an internet browser, then download the file GMID_Sinofiler_files.zip from www.appliedbiosystems.com/support/download/GeneMapper/GMID_Sinofiler_files.zip b. Unzip the file.
2.	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* (PN 4338775).
3.	Select **Tools ▸ Panel Manager**.

To import panels and bin sets *(continued)*

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

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

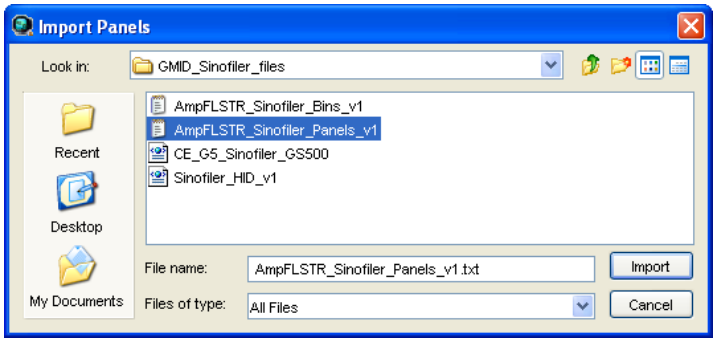


   b. Select **File** ▸ **Import Panels** to open the Import Panels dialog box.

   c. Navigate to, then open the GMID_Sinofiler_files folder that you unzipped in step 1.

5. Select **AmpFLSTR_Sinofiler_Panels_v1**, then click **Import.**

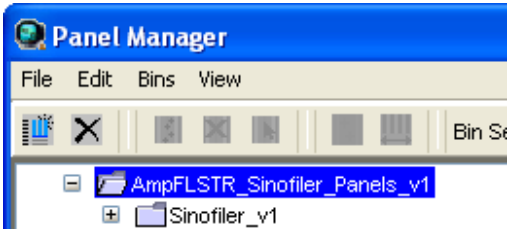
   Note: Importing this file creates a new folder in the navigation pane of the Panel Manager, AmpFLSTR_Sinofiler_Panels_v1. This folder contains the panels and associated markers.



To import panels and bin sets *(continued)*

6. Import AmpFLSTR_Sinofiler_Bins_v1:

   a. Select the **AmpFLSTR_Sinofiler_Panels_v1** folder in the navigation pane.

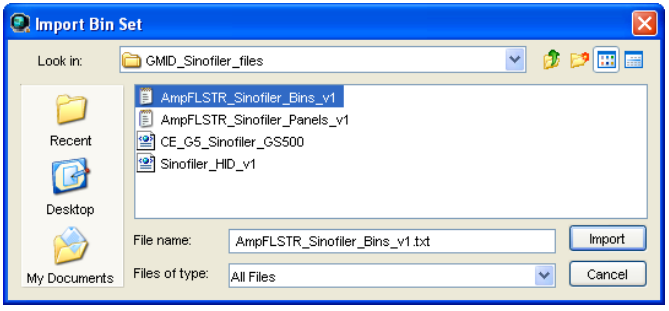


   b. Select **File** ▸ **Import Bin Set** to open the Import Bin Set dialog box.

   c. Navigate to, then open the GMID_Sinofiler_files folder.

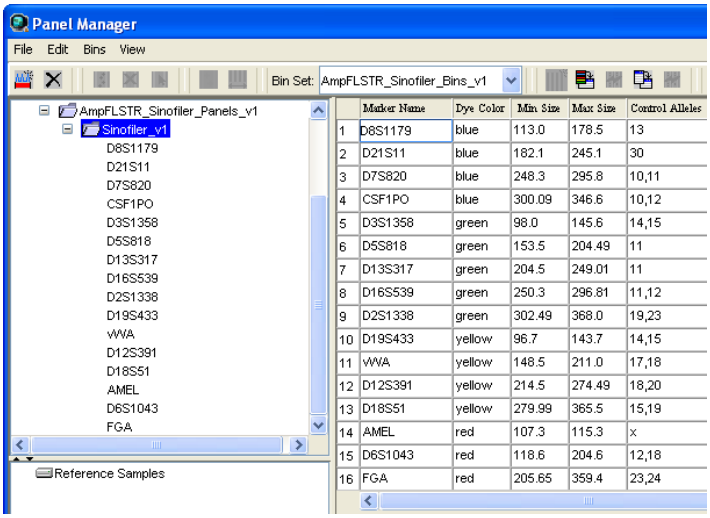
   d. Select **AmpFLSTR_Sinofiler_Bins_v1**, then click **Import**.

   Note: Importing this file associates the bin set with the panels in the AmpFLSTR_Sinofiler_Panels_v1 folder.



To import panels and bin sets *(continued)*

7. View the imported panels in the navigation pane:
   a. Double-click the **AmpFLSTR_Sinofiler_Panels_v1** folder to view the Sinofiler_v1 folder.
   b. Double-click the **Sinofiler_v1** 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	113.0	178.5	13
2	D21S11	blue	182.1	245.1	30
3	D7S820	blue	248.3	295.8	10,11
4	CSF1PO	blue	300.09	346.6	10,12
5	D3S1358	green	98.0	145.6	14,15
6	D5S818	green	153.5	204.49	11
7	D13S317	green	204.5	249.01	11
8	D16S539	green	250.3	296.81	11,12
9	D2S1338	green	302.49	368.0	19,23
10	D19S433	yellow	96.7	143.7	14,15
11	vWA	yellow	148.5	211.0	17,18
12	D12S391	yellow	214.5	274.49	18,20
13	D18S51	yellow	279.99	365.5	15,19
14	AMEL	red	107.3	115.3	X
15	D6S1043	red	118.6	204.6	12,18
16	FGA	red	205.65	359.4	23,24

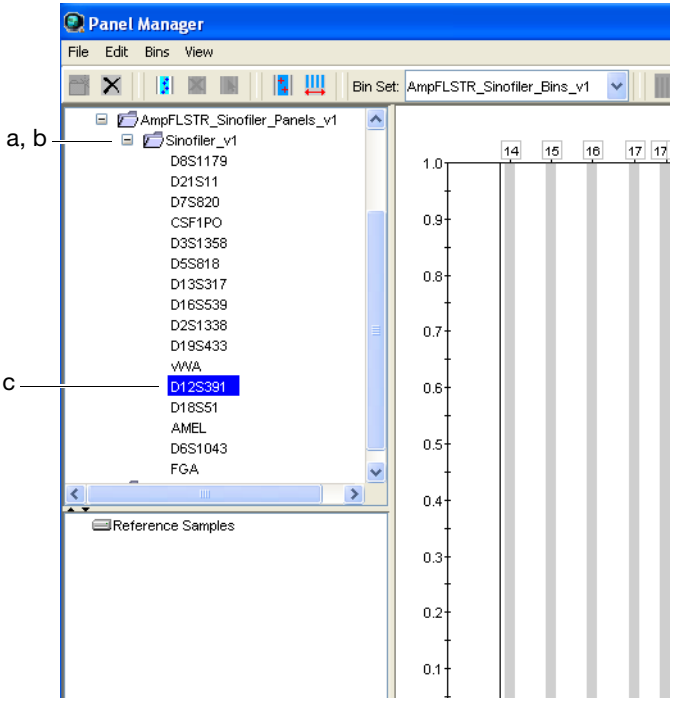
To import panels and bin sets *(continued)*

8. View the markers and display the Bin view in the navigation pane:

   a. Select the **Sinofiler_v1** folder to display its list of markers in the right pane.

   b. Double-click the **Sinofiler_v1** folder to display its list of markers below it.

   c. Select **D12S391** to display the Bin view for the marker in the right pane.



9. Click **Apply**, then **OK** to add the Sinofiler panel and bin set to the GeneMapper *ID* database.

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

Importing an HID Analysis Method (v3.2.1 and v3.3)

The analysis method for the AmpFℓSTR® Sinofiler™ PCR Amplification Kit uses the HID Advanced Mode Peak Detection Algorithm. This analysis method provides users with the same analysis parameters available in GeneScan® Software v3.7.1 for the Windows operating system.

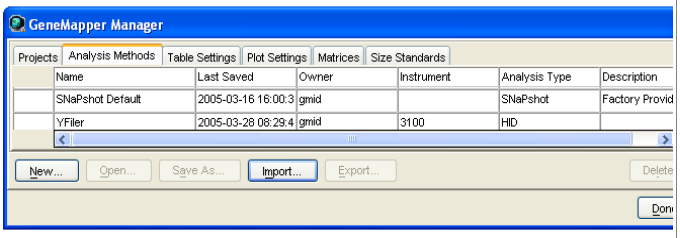
Note: The HID Advanced Mode analysis method below makes use of the AmpFLSTR_Sinofiler_Bins_v1 file described in Table 4-1 on page 4-12.

Use the following procedure to import the analysis method for the Sinofiler kit from the folder that you downloaded from the Applied Biosystems web site, into the GeneMapper *ID* software database. Refer to step 1 on page 4-4 for downloading instructions.

Note: Sinofiler_HID_v1_33 analysis method is provided as a default analysis method in GeneMapper *ID* software v3.3. By following the above procedure it is possible to manually import additional analysis methods besides the one supplied with GeneMapper *ID* software v3.3.

To import the HID Advanced Mode analysis method into GeneMapper *ID* software

1. Select **Tools** ▸ **GeneMapper Manager** to open the GeneMapper Manager.
2. Import an analysis method for HID_Advanced:
   a. Select the **Analysis Methods** tab, then click **Import**.



   b. Navigate to, then open the **GMID_Sinofiler_files** folder.

To import the HID Advanced Mode analysis method into GeneMapper *ID* software *(continued)*

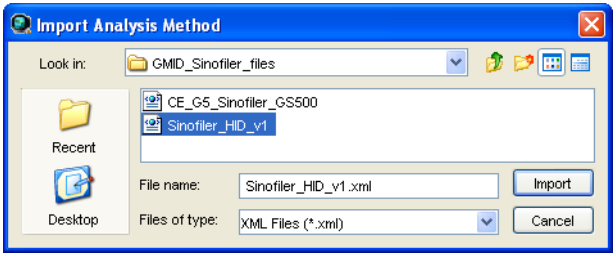
3.	Select **Sinofiler_HID_v1**, then click **Import** to import the Sinofiler_HID_v1 analysis method into the GeneMapper *ID* database. 
4.	To view the settings in the Sinofiler_HID_v1 analysis method: a. Select the **Analysis Methods** tab. b. Select **Sinofiler_HID_v1** in the Name column, then click **Open**. Table 4-1 on page 4-11 shows the settings for each tab of the Analysis Method Editor - HID.

Table 4-1 Sinofiler_HID_v1 Advanced Mode analysis method settings

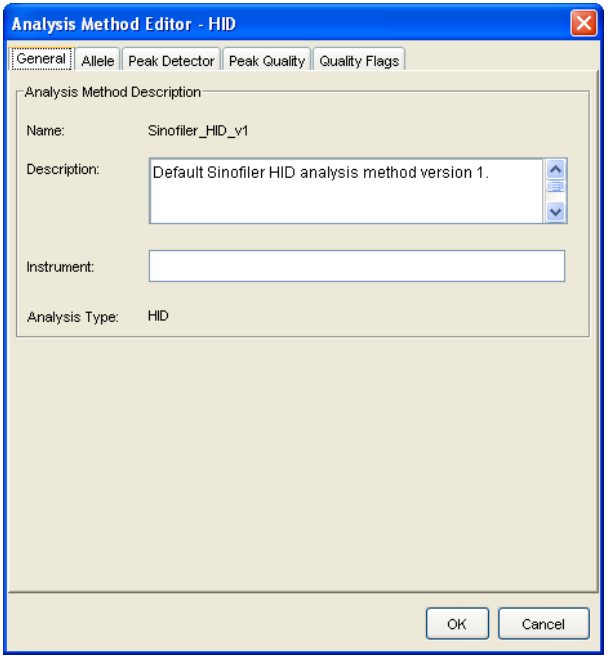
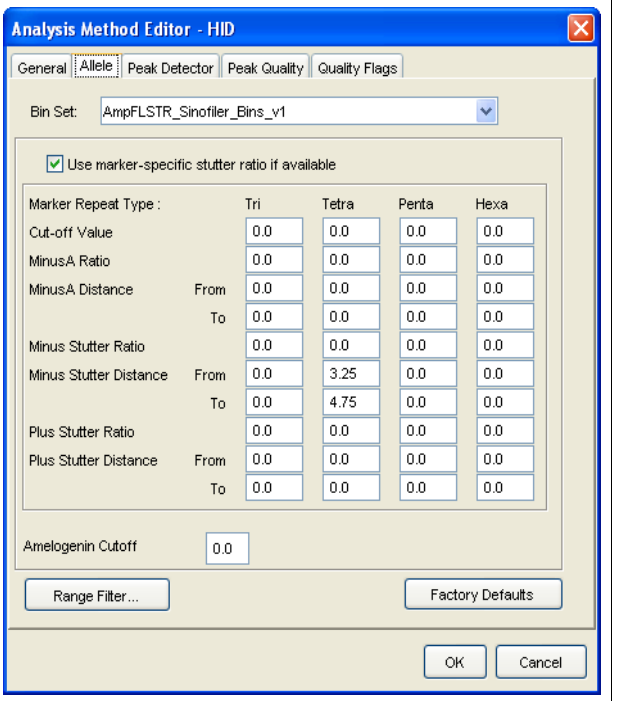
Tab	Settings
General	Name: Sinofiler_HID_v1 

Table 4-1 Sinofiler_HID_v1 Advanced Mode analysis method settings *(continued)*

Tab	Settings
Allele	

- GeneMapper® *ID* Software v3.2.1 and v3.3 allow 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.
- The “Use marker-specific stutter ratio if available” check box is selected by default. Consequently, the software applies the stutter ratio filters supplied in the AmpFLSTR_Sinofiler_Panels_v1 file.

Note: For more information about allele filters, refer to the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide,* Chapter 3 (PN 4338775) and the *Installation Procedures and New Features for GeneMapper® ID Software v3.2 User Bulletin* (PN 4352543).

Table 4-1 Sinofiler_HID_v1 Advanced Mode analysis method settings *(continued)*

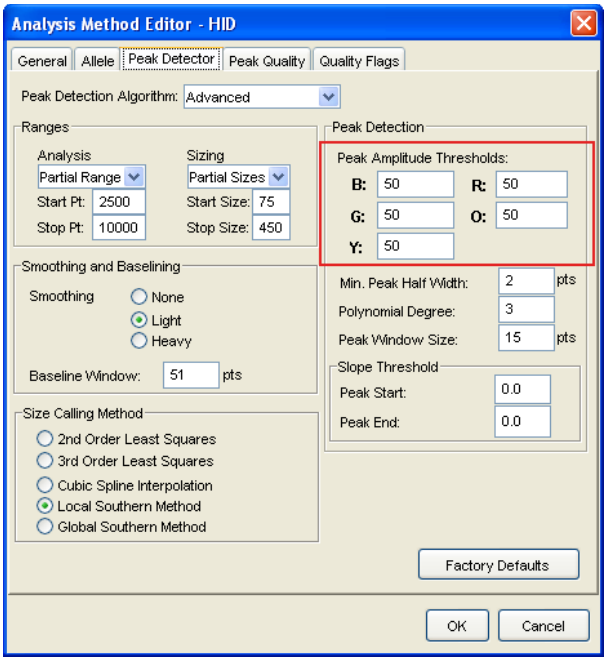
Tab	Settings
Peak Detector	**IMPORTANT!** Laboratories need to perform the appropriate internal validation studies to determine the peak amplitude threshold (highlighted in red below) that allows for reliable interpretation of Sinofiler data.  The software uses the peak detection parameters to specify the minimum peak height 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. **Note:** The analysis range is set by the user based on the locations of the primer peaks and the size standard peaks. **Note:** For information on peak detection algorithms, refer to the *GeneMapper ID Software v3.1 Human Identification Analysis User Guide* (PN 4338775), Appendix A, and the *Installation Procedures and New Features for GeneMapper ID Software v3.2 User Bulletin* (PN 4352543).

Table 4-1 Sinofiler_HID_v1 Advanced Mode analysis method settings *(continued)*

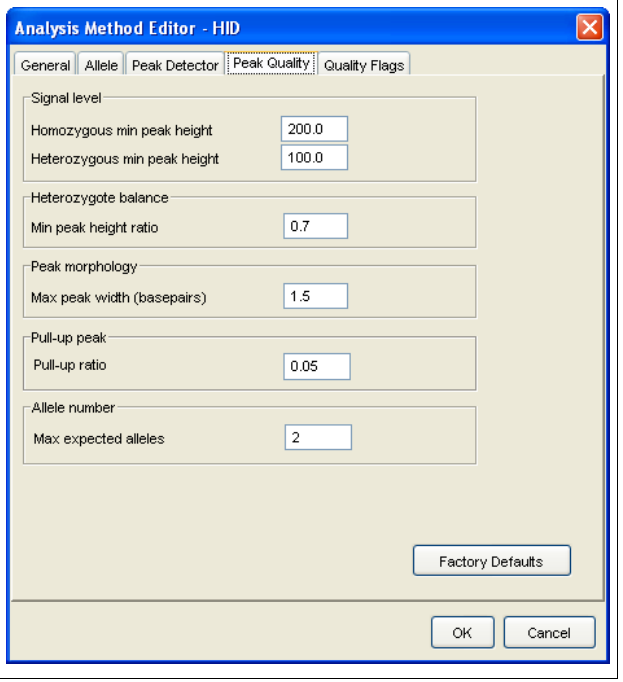
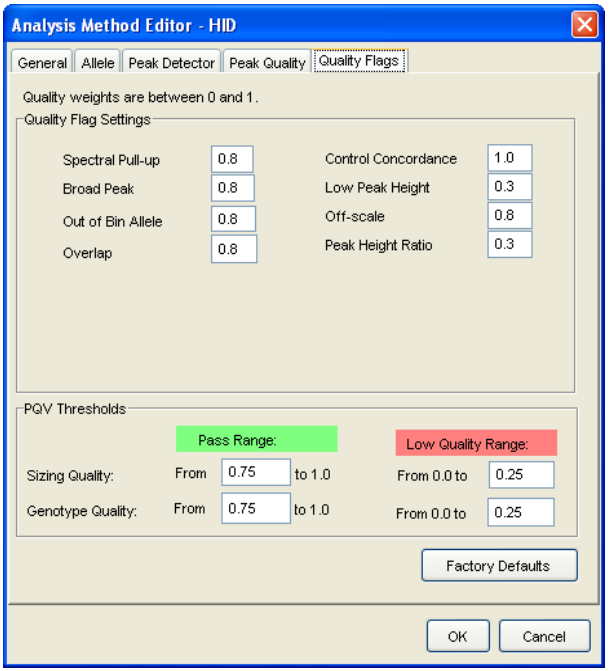
Tab	Settings
Peak Quality	

Table 4-1 Sinofiler_HID_v1 Advanced Mode analysis method settings *(continued)*

Tab	Settings
Quality Flags and PQV Thresholds	

Importing an HID Size Standard (v3.2.1 and v3.3)

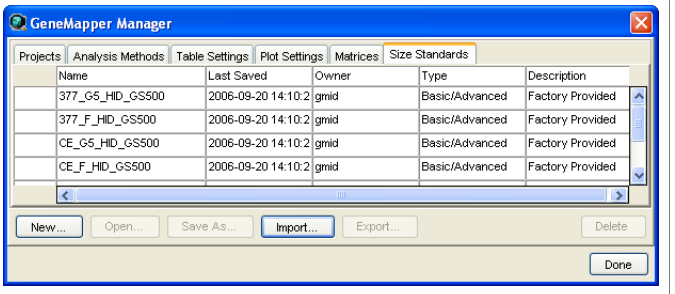
The size standard for the AmpFℓSTR® Sinofiler™ PCR Amplification Kit uses the following GS500 peaks in its sizing algorithm: 75, 100, 139, 150, 160, 200, 300, 350, 400, and 450.

Use the following procedure to import the size standard for the Sinofiler kit from the folder that you downloaded from the Applied Biosystems web site, into the GeneMapper *ID* software database. Refer to step 1 on page 4-4 for downloading instructions.

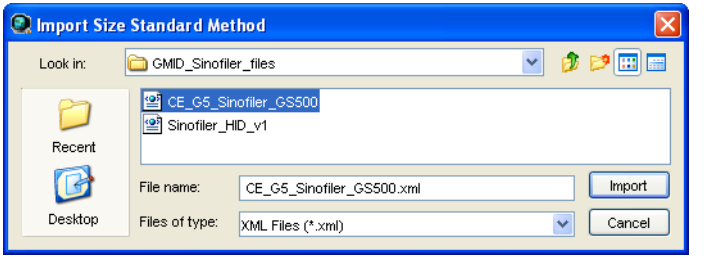
Note: CE_G5_Sinofiler_GS500 size standard is provided as a default size standard in GeneMapper *ID* software v3.3.

To import an HID Size Standard

1. Select **Tools ▸ GeneMapper Manager** to open the GeneMapper Manager.
2. Import a size standard:
   a. Select the **Size Standards** tab, then click **Import**.



   b. Navigate to, then open the **GMID_Sinofiler_files** folder.
3. Select **CE_G5_Sinofiler_GS500**, then click **Import** to import the Sinofiler_HID_v1 analysis method into the GeneMapper *ID* database.



Analyzing and Editing Sample Files with GeneMapper® *ID* Software

Analyzing a Project (v3.2.1 and v3.3)

To analyze a project

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	Advanced Analysis Method
Sample Type	Select the sample type.
Analysis Method (v3.2.1)	**Sinofiler_HID_v1**
Analysis Method (v3.3)	**Sinofiler_HID_v1_33**
Panel (v3.2.1)	**AmpFLSTR_Sinofiler_Panels_v1**
Panel (v3.3)	**AmpFLSTR_Sinofiler_Panels_v1_33**
Size Standard‡	**CE_G5_Sinofiler_GS500§,#**
Matrix	Select a matrix for 310 instruments only.

‡ For more information about how the Size Caller works, refer to the *ABI PRISM® GeneScan® Analysis Software for the Windows NT® Operating System Overview of the Analysis Parameters and Size Caller User Bulletin* (PN 4335617).

§ The following fragments are defined for the CE_G5_Sinofiler_GS500 size standard provided with the AmpFℓSTR® kits: 75, 100, 139, 150, 160, 200, 300, 350, 400, and 450. For additional information about size standards, refer to the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (PN 4338775), Appendix D.

Neither the 250-nt nor the 340-nt peak are included in the size-standard definition. These peaks can be used as an indicator of precision within a run.

To analyze a project *(continued)*

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 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.

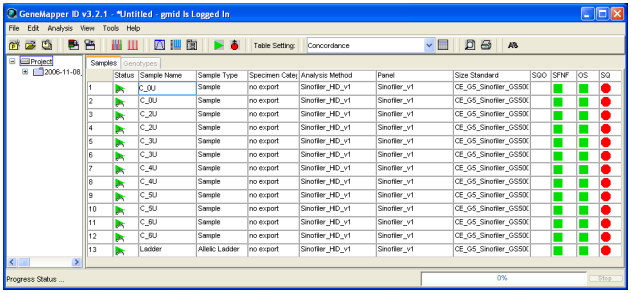


Figure 4-1 Project Window before analysis

For more information about any of these tasks, refer to the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (PN 4338775).

Examining and Editing a Project (v3.2.1 and v3.3)

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 about any of these tasks, refer to:

- *GeneMapper® ID Software Version 3.3 Getting Started Guide* (PN 4385329).
- *Installation Procedures and New Features for GeneMapper® ID Software Version v3.2 User Bulletin* (PN 4352543)
- *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (PN 4338775)
- *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (PN 4335523)

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03/2012

Experiments and Results 5

This chapter covers:

Overview

Experiments Using AmpFℓSTR® Sinofiler™ PCR Amplification Kit

This chapter provides results of the developmental validation experiments performed by Applied Biosystems using the AmpFℓSTR® Sinofiler™ PCR Amplification Kit (Sinofiler kit).

Importance of Validation

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

Experiment Conditions

Experiments to evaluate the performance of the AmpFℓSTR® Sinofiler™ PCR Amplification Kit were performed at Applied Biosystems. The experiments were performed 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.

Additional validation was performed according to the revised guidelines from the Scientific Working Group on DNA Analysis Methods (SWGDAM, July 10, 2003). Based on these guidelines, Applied Biosystems conducted experiments that comply with guidelines 1.0 and 2.0 and its associated subsections. This DNA methodology is not novel. (Moretti *et al.,* 2001; Frank *et al.,* 2001; Wallin *et al.,* 2002; and Holt *et al.,* 2000).

This chapter discusses many of the experiments performed by Applied Biosystems and provides examples of results obtained. Applied Biosystems chose conditions that produced maximum PCR product yield and that met reproducible performance standards. It is our opinion that while these experiments are not exhaustive, they are appropriate for a manufacturer. Each laboratory using the AmpFℓSTR® Sinofiler™ PCR Amplification Kit should perform internal validation studies.

Accuracy, Precision, and Reproducibility

SWGDAM Guideline 1.2.1

"Developmental validation is the demonstration of the accuracy, precision, and reproducibility of a procedure by the manufacturer, technical organization, academic institution, government laboratory, or other party." (SWGDAM, July 2003)

SWGDAM Guideline 2.9

"The extent to which a given set of measurements of the same sample agree with their mean and the extent to which these measurements match the actual values being measured should be determined." (SWGDAM, July 2003)

Accuracy

Laser-induced fluorescence detection of length polymorphism at short tandem repeat loci is not a novel methodology (Holt *et al.,* 2000; and Wallin *et al.,* 2002). However, accuracy and reproducibility of AmpFℓSTR® Sinofiler™ PCR Amplification Kit profiles have been determined from various sample types. Figures 5-1 illustrates the size differences that are typically observed between sample alleles and allelic ladder alleles on the Applied Biosystems 3130*xl* Genetic Analyzer with POP-4™ polymer. The x-axis in Figure 5-1 on page 5-4 represents the nominal nucleotide sizes for the AmpFℓSTR® Sinofiler™ Allelic Ladder. The dashed lines parallel to the x-axis represent the ±0.25-nt windows. The y-axis represents the deviation of each sample allele size from the corresponding allelic ladder allele size. All sample alleles are within ±0.5 nt from a corresponding allele in the allelic ladder.

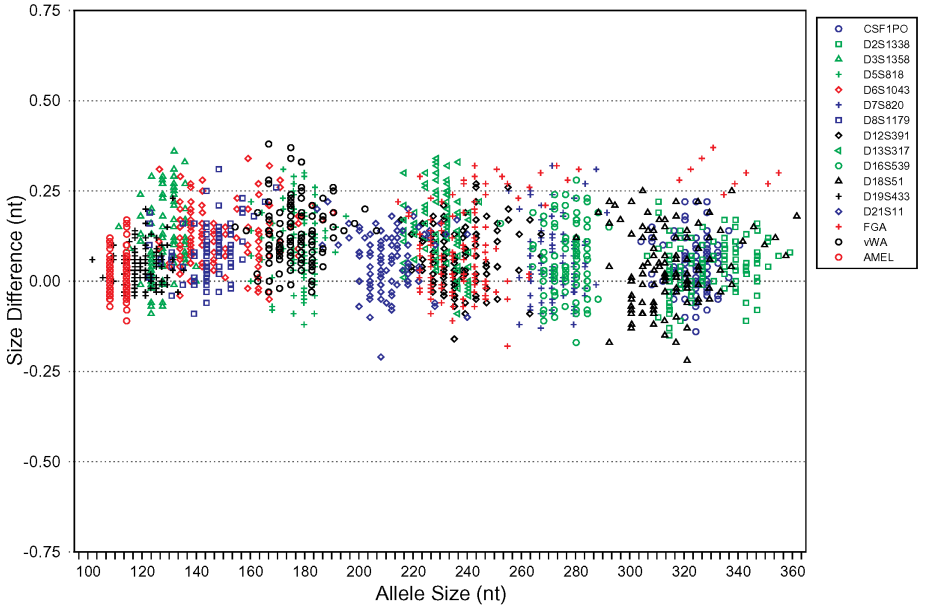

Figure 5-1 Size deviation of 54 samples analyzed on the Applied Biosystems 3130*xl* Genetic Analyzer

Precision and Size Windows

Sizing precision allows for determining accurate and reliable genotypes. Sizing precision was measured on the Applied Biosystems 3130*xl* Genetic Analyzer. The recommended method for genotyping is to employ a ±0.5-nt "window" around the size obtained for each allele in the AmpFℓSTR® Sinofiler™ Allelic Ladder. A ±0.5-nt window allows for the detection and correct assignment of alleles. Any sample allele that sizes outside the specified window could be:

- An "off-ladder" allele, that is, an allele of a size that is not represented in the AmpFℓSTR® Sinofiler™ Allelic Ladder

  or

- An allele that corresponds to an allelic ladder allele, but whose size is just outside a window because of measurement error

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

Table 5-1 on page 5-6 shows typical precision results obtained from five runs (16 capillaries/run) of the AmpFℓSTR® Sinofiler™ Allelic Ladder on the Applied Biosystems 3130*xl* Genetic Analyzer (36-cm capillary and POP-4™ polymer). The internal size standard that was used was GeneScan™ 500 LIZ® Size Standard. The results were obtained within a set of injections on a single capillary array.

Sample alleles may occasionally size outside of the ±0.5-nt window for a respective allelic ladder allele because of measurement error. The frequency of such an occurrence is lowest in detection systems having the smallest standard deviations in sizing. Figure 5-1 on page 5-4 illustrates the tight clustering of allele sizes obtained on the Applied Biosystems 3130*xl* Genetic Analyzer, where the standard deviation in sizing is typically less than 0.15 nt. The instance of a sample allele sizing outside the ±0.5-nt window because of measurement error is relatively rare when the standard deviation in sizing is approximately 0.15 nt or less (Smith, 1995).

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

The GeneMapper® *ID* software v3.2.1 and later automatically flag sample alleles that do not size within the prescribed window around an allelic ladder allele.

Although the precision within a gel or set of capillary injections is very good, the determined allele sizes vary between platforms. Cross-platform sizing differences occur from a number of factors, including type and concentration of polymer mixture, run temperature, and electrophoresis conditions. Variations in sizing can also occur between runs on the same instrument and between runs on different instruments because of these factors.

Applied Biosystems strongly recommends that the allele sizes obtained be compared to the sizes obtained for known alleles in the AmpFℓSTR® Sinofiler™ Allelic Ladder from the same run and then be converted to genotypes (as described in "Before You Start" on

page 4-2). Refer to Table 5-1 for the results of five runs of the AmpFℓSTR® Sinofiler™ Allelic Ladder. For more information on precision and genotyping, see Lazaruk *et al.,* 1998 and Mansfield *et al.*, 1998.

In Table 5-1, the mean size for all the alleles in each run (16 capillaries) was calculated. The mean range shown in the table is the lowest- and highest-mean size values of the five runs. Similarly, the standard deviation for the allele sizing was calculated for all the alleles in each run. The standard deviation range shown in Table 5-1 is the lowest and highest standard deviation values of the five runs.

Table 5-1 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR® Sinofiler™ Allelic Ladder

Applied Biosystems 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
Amelogenin		
X	108.2–108.38	0.056–0.076
Y	114.33–114.52	0.047–0.073
CSF1PO		
6	304.11–304.52	0.054–0.083
7	308.15–308.56	0.057–0.084
8	312.21–312.6	0.042–0.075
9	316.26–316.64	0.041–0.073
10	320.3–320.66	0.055–0.0768
11	324.34–324.69	0.051–0.077
12	328.37–328.71	0.035–0.078
13	332.42–332.72	0.045–0.072
14	336.45–336.74	0.04–0.073
15	340.47–340.74	0.037–0.081

Table 5-1 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR® Sinofiler™ Allelic Ladder *(continued)*

Applied Biosystems 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
D12S391		
8	215.54–215.79	0.05–0.066
9	219.5–219.77	0.054–0.071
10	223.5–223.79	0.047–0.067
11	227.42–227.71	0.045–0.062
12	231.38–231.68	0.046–0.066
13	235.33–235.63	0.042–0.067
14	238.35–238.67	0.053–0.075
15	239.3–239.63	0.054–0.07
D13S317		
8	216.67–217.02	0.06–0.07
9	220.64–221.01	0.07–0.087
10	224.64–225.01	0.07–0.095
11	228.63–229.01	0.058–0.087
12	232.7–233.11	0.05–0.08
13	236.6–237	0.074–0.087
14	240.54–240.95	0.067–0.088
15	244.53–244.97	0.063–0.093
D16S539		
5	252.29–252.66	0.068–0.085

Table 5-1 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR® Sinofiler™ Allelic Ladder *(continued)*

Applied Biosystems 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
8	264.25–264.65	0.065–0.093
9	268.24–268.66	0.065–0.093
10	272.23–272.65	0.068–0.082
11	276.24–276.65	0.064–0.085
12	280.25–280.67	0.063–0.103
13	284.26–284.68	0.062–0.093
14	288.28–288.7	0.063–0.089
15	292.29–292.72	0.058–0.095
D18S51		
7	280.24–280.62	0.066–0.092
9	288.35–288.77	0.061–0.087
10	292.41–292.83	0.066–0.101
10.2	294.41–294.83	0.055–0.099
11	296.49–296.91	0.064–0.099
12	300.56–301	0.053–0.101
13	304.68–305.1	0.056–0.105
13.2	306.7–307.12	0.046–0.112
14	308.79–309.19	0.064–0.101
14.2	310.82–311.23	0.062–0.106
15	312.89–313.31	0.071–0.104
16	317–317.41	0.068–0.105

Table 5-1 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR® Sinofiler™ Allelic Ladder *(continued)*

Applied Biosystems 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
17	321.11–321.49	0.063–0.111
18	325.2–325.58	0.066–0.104
19	329.29–329.66	0.062–0.11
20	333.4–333.74	0.059–0.11
21	337.49–337.81	0.067–0.107
22	341.65–341.96	0.05–0.09
23	345.63–345.92	0.059–0.091
24	349.7–349.98	0.054–0.086
25	353.65–353.9	0.063–0.086
26	357.6–357.82	0.048–0.082
27	361.54–361.76	0.042–0.073
D19S433		
15	101.68–101.76	0.042–0.055
16	105.56–105.64	0.039–0.054
17	109.48–109.54	0.039–0.05
18	113.4–113.47	0.034–0.052
19	115.39–115.44	0.043–0.051
20	117.35–117.4	0.037–0.044
21	119.35–119.41	0.031–0.047
22	121.32–121.36	0.035–0.048
23	123.32–123.37	0.035–0.051

Table 5-1 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR® Sinofiler™ Allelic Ladder *(continued)*

Applied Biosystems 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
24	125.3–125.35	0.027–0.048
25	127.33–127.38	0.031–0.044
26	129.33–129.36	0.035–0.042
27	131.36–131.39	0.033–0.044
28	133.36–133.41	0.04–0.055
D21S11		
24	184.54–184.72	0.049–0.069
24.2	186.53–186.71	0.039–0.061
25	188.49–188.67	0.039–0.067
26	192.42–192.59	0.041–0.066
27	196.38–196.54	0.036–0.047
28	200.23–200.39	0.042–0.051
28.2	202.21–202.38	0.036–0.049
29	204.2–204.36	0.042–0.046
29.2	206.24–206.42	0.041–0.049
30	208.21–208.38	0.034–0.053
30.2	210.18–210.37	0.028–0.049
31	212.22–212.38	0.036–0.052
31.2	214.18–214.37	0.042–0.061
32	216.19–216.38	0.037–0.052
32.2	218.17–218.38	0.035–0.055

Table 5-1 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR® Sinofiler™ Allelic Ladder *(continued)*

Applied Biosystems 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
33	220.18–220.4	0.04–0.057
33.2	222.12–222.34	0.038–0.048
34	224.25–224.47	0.048–0.058
34.2	226.16–226.39	0.042–0.056
35	228.24–228.47	0.046–0.053
35.2	230.15–230.39	0.04–0.064
36	232.14–232.39	0.044–0.053
37	236.21–236.46	0.043–0.054
38	240.15–240.38	0.049–0.059
D2S1338		
15	306.48–306.82	0.042–0.082
16	310.54–310.89	0.05–0.093
17	314.61–314.95	0.049–0.087
18	318.67–318.99	0.052–0.082
19	322.73–323.02	0.049–0.081
20	326.76–327.07	0.047–0.07
21	330.83–331.11	0.038–0.083
22	334.87–335.15	0.05–0.078
23	338.93–339.17	0.051–0.082
24	342.97–343.19	0.049–0.08
25	346.99–347.2	0.047–0.088

Table 5-1 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR® Sinofiler™ Allelic Ladder *(continued)*

Applied Biosystems 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
26	351–351.19	0.044–0.069
27	354.92–355.08	0.044–0.065
28	359.12–359.29	0.052–0.068
D3S1358		
12	111.45–111.62	0.047–0.071
13	115.54–115.73	0.051–0.072
14	119.53–119.7	0.044–0.08
15	123.44–123.6	0.056–0.076
16	127.63–127.8	0.047–0.078
17	131.83–132.01	0.042–0.076
18	135.93–136.11	0.047–0.068
19	140.03–140.22	0.045–0.067
D5S818		
7	160.14–160.51	0.062–0.097
8	164.11–164.46	0.066–0.123
9	168.05–168.42	0.064–0.111
10	172.01–172.36	0.065–0.113
11	175.91–176.28	0.055–0.111
12	179.81–180.17	0.072–0.11
13	183.68–184.04	0.064–0.103
14	187.55–187.89	0.067–0.108

Table 5-1 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR® Sinofiler™ Allelic Ladder *(continued)*

Applied Biosystems 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
15	191.39–191.73	0.065–0.08
16	195.23–195.55	0.071–0.084
D6S1043		
9	126.37–126.61	0.069–0.096
10	130.22–130.47	0.064–0.091
11	134.13–134.38	0.071–0.084
12	138.08–138.33	0.065–0.088
13	142.26–142.55	0.058–0.087
14	146.48–146.79	0.062–0.098
15	150.8–151.12	0.068–0.101
16	154.98–155.34	0.064–0.102
17	159.04–159.44	0.074–0.117
18	163.01–163.42	0.061–0.134
19	166.96–167.36	0.066–0.132
20	170.87–171.28	0.081–0.143
21	174.78–175.19	0.075–0.121
21.3	177.66–178.07	0.073–0.12
22	178.67–179.09	0.078–0.121
23	182.61–183.03	0.082–0.116
24	186.48–186.9	0.074–0.125
25	190.42–190.83	0.077–0.105

Table 5-1 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR® Sinofiler™ Allelic Ladder *(continued)*

Applied Biosystems 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
D7S820		
6	255.26–255.68	0.053–0.082
7	259.27–259.71	0.066–0.084
8	263.32–263.74	0.058–0.089
9	267.34–267.79	0.055–0.085
10	271.36–271.82	0.06–0.101
11	275.4–275.86	0.066–0.098
12	279.43–279.9	0.063–0.09
13	283.48–283.95	0.063–0.092
14	287.52–288	0.065–0.098
15	291.57–292.06	0.068–0.106
D8S1179		
8	122.88–123.03	0.051–0.065
9	126.94–127.1	0.047–0.06
10	131.05–131.2	0.053–0.071
11	135.17–135.32	0.046–0.072
12	139.37–139.53	0.049–0.071
13	143.95–144.16	0.049–0.067
14	148.43–148.66	0.044–0.067
15	152.8–153.04	0.053–0.07
16	157.05–157.34	0.058–0.086

Table 5-1 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR® Sinofiler™ Allelic Ladder *(continued)*

Applied Biosystems 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
17	161.23–161.53	0.058–0.112
18	165.33–165.64	0.059–0.098
19	169.38–169.71	0.054–0.112
FGA		
17	214.44–214.82	0.068–0.094
18	218.47–218.87	0.076–0.101
19	222.5–222.92	0.067–0.099
20	226.53–226.95	0.069–0.102
21	230.57–231	0.084–0.094
22	234.61–235.07	0.067–0.103
23	238.65–239.11	0.074–0.102
24	242.69–243.16	0.073–0.114
25	246.74–247.23	0.078–0.119
26	250.79–251.29	0.079–0.109
26.2	252.8–253.32	0.07–0.121
27	254.8–255.32	0.072–0.114
28	258.85–259.38	0.075–0.118
29	262.92–263.46	0.081–0.117
30	267.01–267.56	0.081–0.119
30.2	268.84–269.39	0.08–0.13
31.2	272.91–273.48	0.081–0.13

Table 5-1 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR® Sinofiler™ Allelic Ladder *(continued)*

Applied Biosystems 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
32.2	276.99–277.56	0.09–0.14
33.2	281.06–281.64	0.079–0.134
42.2	318.17–318.74	0.089–0.141
43.2	322.29–322.85	0.091–0.145
44.2	326.41–326.95	0.083–0.148
45.2	330.53–331.07	0.089–0.136
46.2	334.53–335.06	0.078–0.13
47.2	338.62–339.12	0.084–0.144
48.2	342.77–343.24	0.083–0.147
50.2	350.88–351.3	0.07–0.121
51.2	354.83–355.24	0.074–0.125
vWA		
11	154.27–154.51	0.059–0.07
12	158.44–158.7	0.052–0.087
13	162.57–162.84	0.047–0.1
14	166.8–167.09	0.057–0.099
15	170.72–171.01	0.053–0.101
16	174.75–175.04	0.059–0.098
17	178.73–179.02	0.05–0.098
18	182.68–182.96	0.057–0.094
19	186.64–186.9	0.039–0.081

Table 5-1 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR® Sinofiler™ Allelic Ladder *(continued)*

Applied Biosystems 3130*xl* Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
20	190.56–190.84	0.064–0.075
21	194.46–194.71	0.053–0.085
22	198.35–198.59	0.053–0.068
23	202.2–202.45	0.055–0.076
24	206.51–206.79	0.052–0.069

Extra Peaks in the Electropherogram

Causes of Extra Peaks

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

Stutter Products

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

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. Peak heights were measured for amplified samples (n = 840) at the loci used in the AmpFℓSTR® Sinofiler™ PCR Amplification Kit. All data were generated on the Applied Biosystems 3130*xl* Genetic Analyzer.

Some conclusions from these measurements and observations are:

- For each Sinofiler kit locus, the percent stutter generally increases with allele length, as shown in Figure 5-2 to Figure 5-5 on pages 5-19 through 5-22.
- Smaller alleles display a lower level of stutter relative to the longer alleles within each locus.
- Each allele within a locus displays a percent stutter that is consistent.
- The highest observed percent stutter for each locus is included as the filtering step in GeneMapper® *ID* software v3.2.1 and later. These values are shown in Table 5-2 on page 5-23. 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 5-37.

- The measurement of percent stutter for peaks that are off-scale may be unusually high.

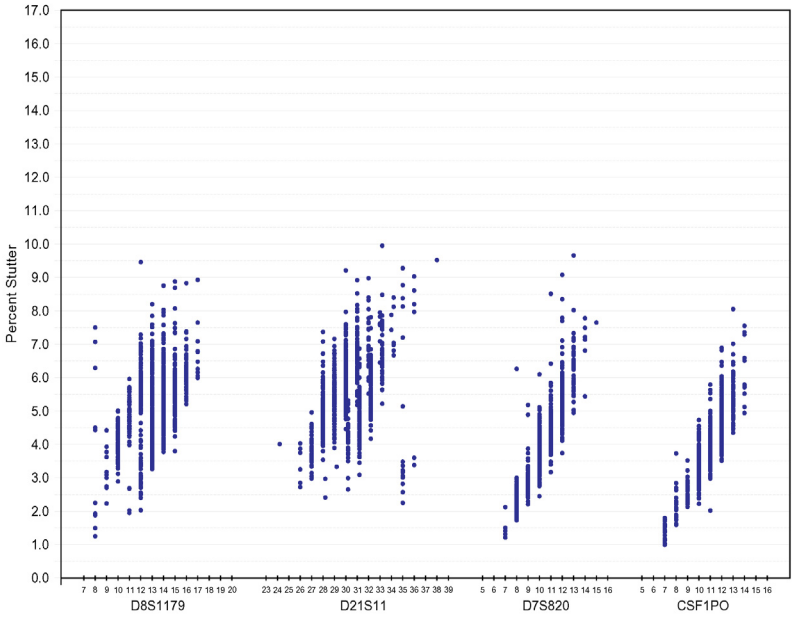

Figure 5-2 Stutter percentages for D8S1179, D21S11, D7S820, and CSF1PO loci

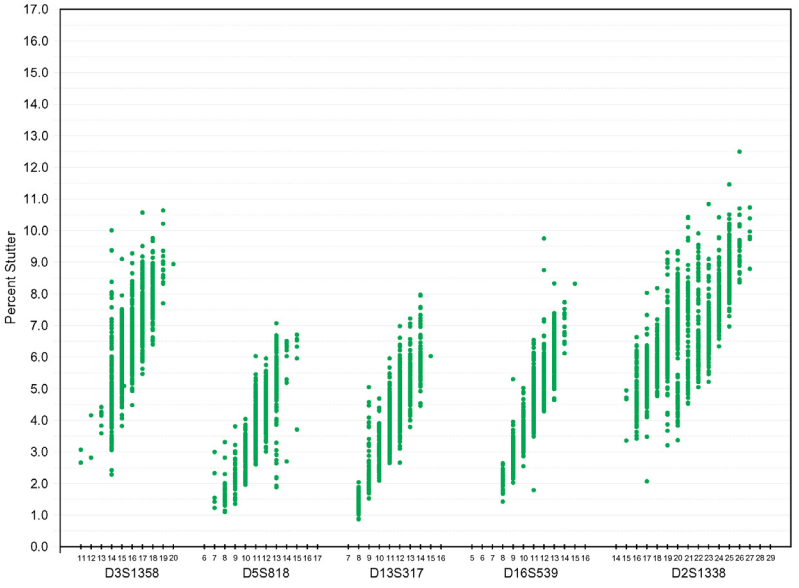

Figure 5-3 Stutter percentages for D3S1358, D5S818, D13S317, D16S539, and D2S1338 loci

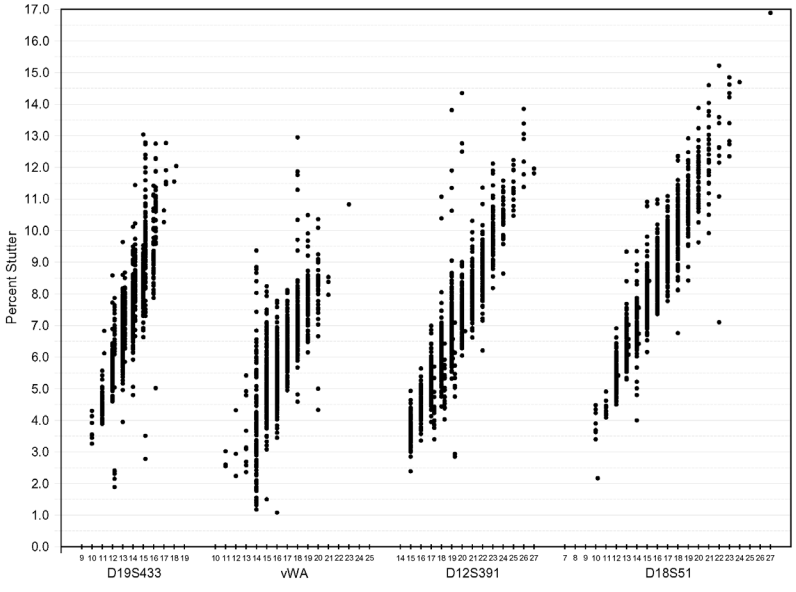

Figure 5-4 Stutter percentages for D19S433, vWA, D12S391, and D18S51 loci

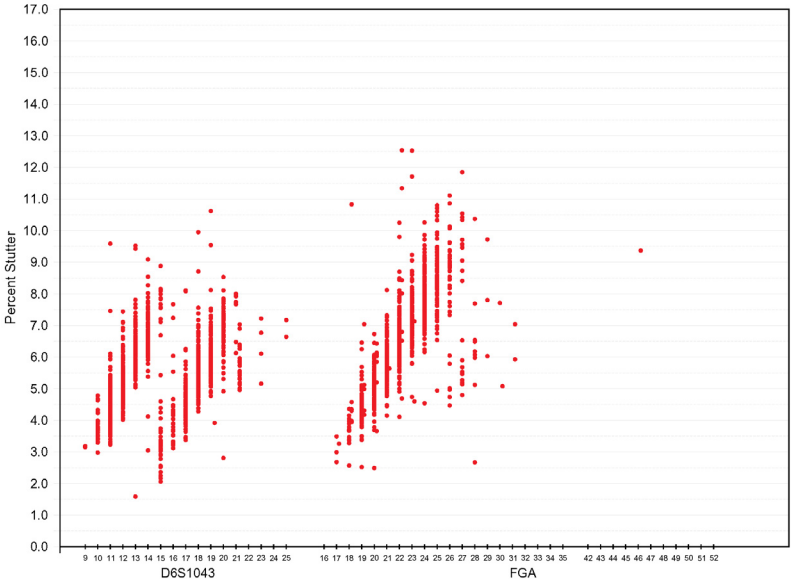

Figure 5-5 Stutter percentages for D6S1043 and FGA loci

Table 5-2 Marker-specific stutter percentages (ratios used in GeneMapper *ID* AmpFLSTR_Sinofiler_panels_v1) for Sinofiler Kit loci

Locus	% Stutter
CSF1PO	8.5
D12S391	14.5
D13S317	8
D16S539	10
D18S51	16
D19S433	13.5
D21S11	10
D2S1338	13
D3S1358	11
D5S818	7.5
D6S1043	11
D7S820	10
D8S1179	9.5
FGA	13
vWA	13.5

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 nontemplate addition results in a PCR product that is one nucleotide longer than the actual target sequence. The PCR product with the extra nucleotide is referred to as the "+A" form.

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

The final extension step gives the AmpliTaq Gold® DNA polymerase additional time to complete +A addition to all double-stranded PCR products. STR systems (where each allele is represented by two peaks that are one nucleotide apart) that have not been optimized for +A addition may have “split peaks.”

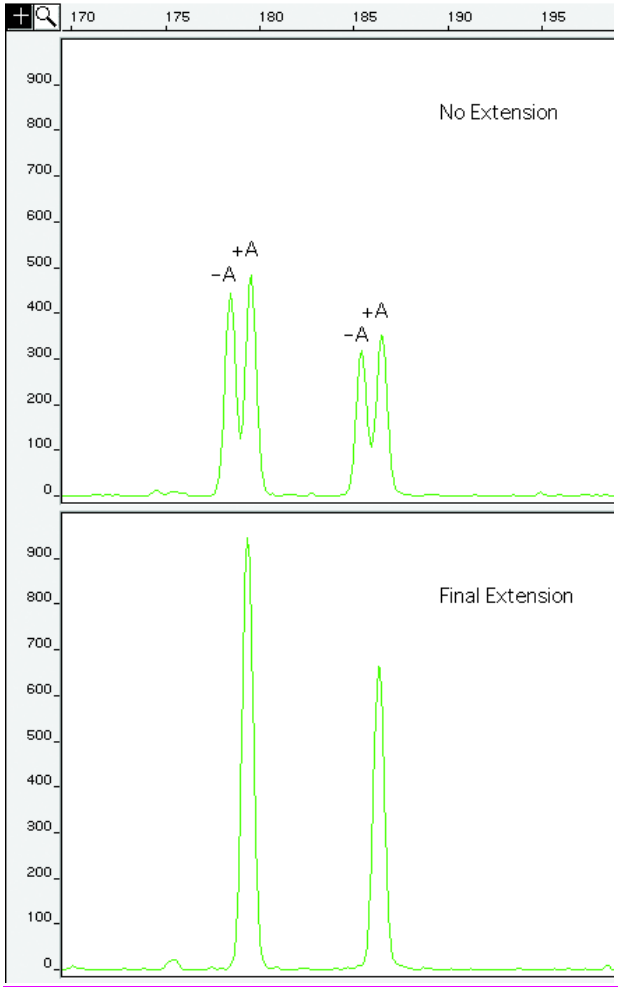

Figure 5-6 Omitting the final extension step results in split peaks due to incomplete A nucleotide addition. Data are from an ABI PRISM® 310 Genetic Analyzer using another AmpFℓSTR® kit.

Lack of complete +A nucleotide addition may be observed in Sinofiler kit results when the amount of input DNA is greater than the recommended protocols, 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 may also result in off-scale data.

Artifacts

Artifacts and anomalies are seen in all molecular biological systems. Artifacts are typically reproducible, and anomalies are nonreproducible, intermittent occurrences that are not consistently observed in a system, for example, spikes and baseline noise. Artifacts have been seen in data produced on genetic analyzers when using the Sinofiler kit. In amplified samples, artifacts in the non-calling region may appear in the blue (95–100 nt) dye. Low-level artifacts in the calling region may appear in the blue (118 nt), green (97, 120, and 189 nt), and black (95–100, 164 nt) dyes, depending on the sensitivity of the instrument.

Figure 5-7 on page 5-27 shows examples of baseline noise and artifacts in an electropherogram while using the Sinofiler kit. Genotyping may result in the detection of these artifacts as off-ladder alleles, or "OL Alleles." You should consider possible noise and artifacts when interpreting data.

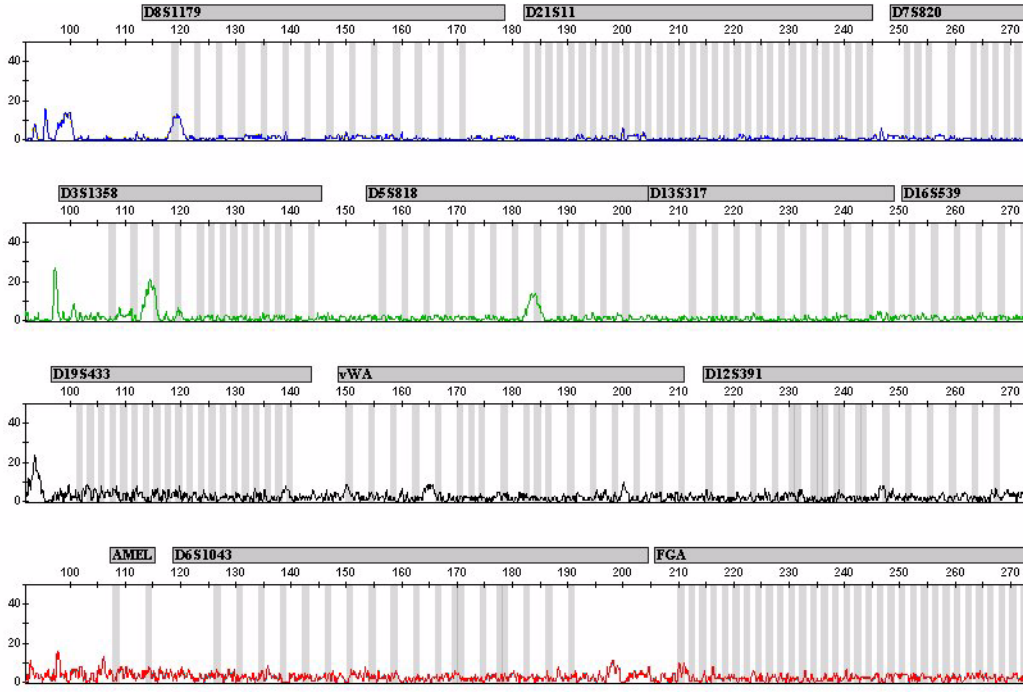

Figure 5-7 Examples of baseline noise and reproducible artifacts in data produced on the Applied Biosystems 3130*xl* Genetic Analyzer

Note that a high degree of magnification (y-axis) is used to illustrate these artifacts.

Characterization of Loci

SWGDAM Guideline 2.1

"The basic characteristics of a genetic marker must be determined and documented." (SWGDAM, July 2003)

This section describes basic characteristics of the 15 loci and the sex-determining marker, amelogenin that are amplified with the AmpFℓSTR® Sinofiler™ PCR Amplification Kit. These loci have been extensively characterized by other laboratories.

Nature of the Polymorphisms

The primers for the amelogenin locus flank a 6-nucleotide deletion within intron 1 of the X homologue. Amplification results in 107-nt and 113-nt products from the X and Y chromosomes, respectively. (Sizes are the actual nucleotide size according to sequencing results, including 3´ A nucleotide addition.) The remaining Sinofiler 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-nt repeat units.

The loci D6S1043 and D12S391 amplified by the Sinofiler kit are compound STR markers. The D6S1043 alleles contain repeat unit sequences (ATCT) and (ATGT) that can differ in number and relative position within the repeat region. The most common repeat motif (ATCT) is referred to as the core repeat sequence. The D12S391 locus consists of repetitive basic structures of $(AGAT)_x(AGAC)_y(AGAT)_z$. Sequence variations within the repeat region and allele distributions in these two loci have been reported in various populations (Chen *et al.,* 1999; Chen *et al.,* 2004; Glock *et al.,* 1997; Hu *et al.,* 2004; Junge and Madea, 1998; Klintschar *et al.,* 1998; Li *et al.,* 2004; Liu *et al.,* 2005; Lu *et al.,* 2003; Shin *et al.,* 2004; Su *et al.,* 2004; Waiyawuth *et al.,* 1998; Wu *et al.,* 2004; Yu *et al.,* 2003).

All the alleles in the AmpFℓSTR® Sinofiler™ Allelic Ladder have been been subjected to sequencing at Applied Biosystems. In addition, other groups in the scientific community have sequenced alleles at some of these loci. Among the various sources of sequence data on the Sinofiler kit loci, there is consensus on the repeat patterns and structure of the STRs.

Inheritance

The Centre d'Etude du Polymorphisme Humain (CEPH) has collected DNA from families of Utah Mormon, French Venezuelan, and Amish descent. These DNA sets have been extensively studied all over the world and are routinely used to characterize the mode of

inheritance of various DNA loci. Each family set contains three generations, generally including four grandparents, two parents, and several offspring. Consequently, the CEPH family DNA sets are ideal for studying inheritance patterns (Begovich *et al.,* 1992).

Three CEPH family DNA sets were examined. 1 ng of DNA from each sample was amplified using the AmpFℓSTR® Sinofiler™ kit, followed by analysis using an Applied Biosystems 3130*xl* Genetic Analyzer. The families examined included #1333 (9 offspring), #1340 (7 offspring), and #1345 (7 offspring), representing 23 meiotic divisions.

In family #1340, we observed two parent/offspring pairs with mutations at locus D8S1179. In family #1333, one mutation was identified at locus D12S391. The genotypes differed by one repeat unit between the two generations. These samples were reamplified using the AmpFℓSTR® Sinofiler™ and AmpFℓSTR® Identifiler® kits to confirm the allele calls. Calculation of a mutation rate based on these data would be inaccurate due to the small sample size. The other parent-offspring allele transfers were in accordance with Mendelian rules, as expected.

Mapping

The Sinofiler kit loci have been mapped, and the chromosomal locations have been published (Nakahori *et al.,* 1991; Edwards *et al.,* 1992; Kimpton *et al.,* 1992; Kong *et al.,* 2004; Lareu *et al.,* 1996; Mills *et al.,* 1992; Sharma and Litt, 1992; Li *et al.,* 1993; Straub *et al.*, 1993; Barber and Parkin, 1996).

Species Specificity

SWGDAM Guideline 2.2

"For techniques designed to type human DNA, the potential to detect DNA from forensically relevant nonhuman species should be evaluated." (SWGDAM, July 2003)

The AmpFℓSTR® Sinofiler™ PCR Amplification Kit provides the required specificity for detecting primate alleles. Other species do not amplify for the loci that are tested.

Nonhuman Studies

Nonhuman DNA may be present in forensic casework samples. The data from Sinofiler kit experiments on nonhuman DNA sources are shown in Figure 5-8.

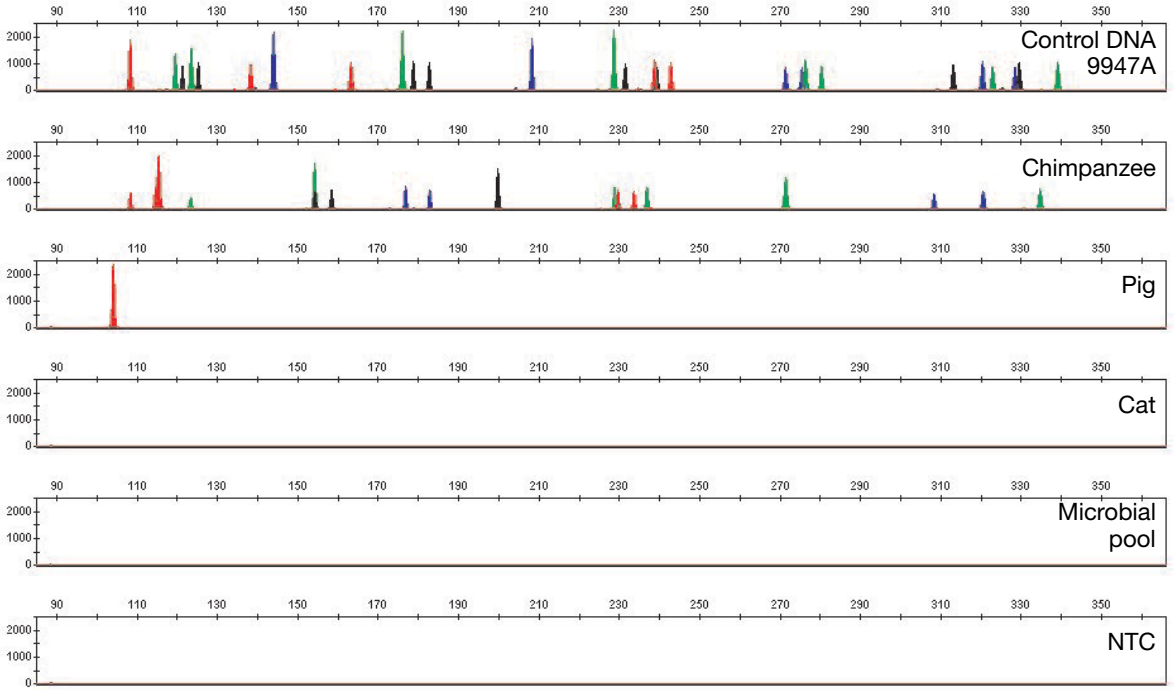

Figure 5-8 Representative electropherograms from a species-specificity study including positive and non-template controls (NTC)

Figure 5-8 on page 5-30 shows amplification for: control DNA 9947A (1 ng, panel 1), chimpanzee (1 ng, panel 2), pig (10 ng, panel 3), cat (10 ng, panel 4), microbial DNA pool (equivalent to 10^5 copies of *Candida albicans*, *Neisseria gonorrhoeae*, *E. coli 0157:H7*, *Bacillus subtilis,* and *Lactobacillus rhamnosus,* panel 5), and the negative control (panel 6). The extracted DNA samples were amplified with the Sinofiler kit and analyzed using the Applied Biosystems 3130*xl* Genetic Analyzer.

- Primates: gorilla, chimpanzee, orangutan, and macaque (1 ng each)
- Non-primates: mouse, dog, pig, cat, horse, hamster, rat, chicken, and cow (10 ng each)
- Microorganisms: *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Bacillus subtilis,* and *Lactobacillus rhamnosus* (equivalent to 10^5 copies)

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

The microorganisms, chicken, cat, hampster, rat, and mouse did not yield detectable product. Horse, cow, dog, and pig produced a 104-bp fragment near the amelogenin locus in PET® dye.

Sensitivity

SWGDAM Guideline 2.3

"When appropriate, the range of DNA quantities able to produce reliable typing results should be determined." (SWGDAM, July 2003)

Importance of Quantitation

The optimal amount of input DNA added to the AmpFℓSTR® Sinofiler™ PCR Amplification Kit should be between 0.50 and 1.25 ng. The DNA sample should be quantitated prior to amplification using a system such as the Quantifiler® Human DNA Quantification Kit (PN 4343895). The final DNA concentration should be 0.05 to 0.125 ng/µL so that 0.50 to 1.25 ng of DNA is added to the PCR reaction in a volume of 10 µL. If the sample contains degraded DNA, amplification of additional DNA may be beneficial. In Figure 5-9 on page 5-33 the control DNA 9947A was serially diluted from 1 ng to 0.062 ng. Full profiles (27 PCR products) were consistently obtained at 0.125 ng, but occasional partial profiles (missing from 1 to 3 alleles) were observed at 0.062 ng.

Effect of DNA Quantity on Results

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

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

  Off-scale data is a problem because:

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

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

- Incomplete +A nucleotide addition

  The sample can be reamplified using less DNA.

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

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.

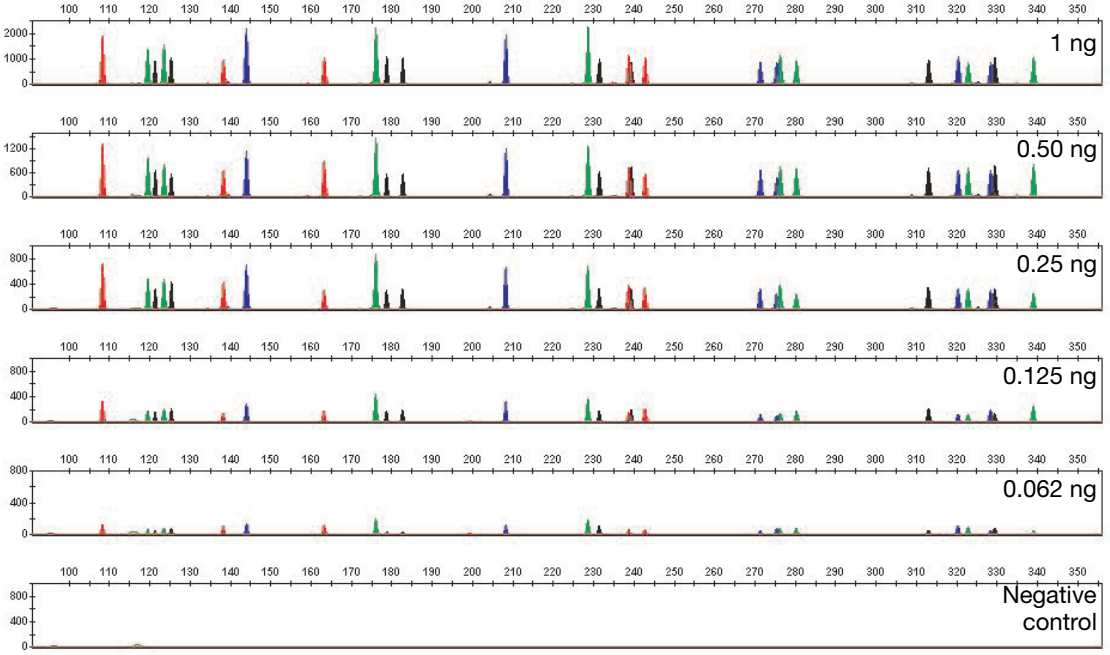

Figure 5-9 Effect of amplifying varying amounts of of control DNA 9947A and negative control

Note that the y-axis scale is magnified for the lower amounts of DNA, analyzed using the Applied Biosystems 3130*xl* Genetic Analyzer.

Stability

SWGDAM Guideline 2.4

"The ability to obtain results from DNA recovered from biological samples deposited on various substrates and subjected to various environmental and chemical insults has been extensively documented. In most instances, assessment of the effects of these factors on new forensic DNA procedures is not required. However, if substrates and/or environmental and/or chemical insults could potentially affect the analytical process, then the process should be evaluated using known samples to determine the effects of such factors." (SWGDAM, July 2003)

Degraded DNA

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

Degraded DNA was prepared to examine the potential for preferential amplification of loci. High-molecular-weight Raji DNA was sonicated and incubated with increasing doses of DNase I (0 to 6 Units) for 20 minutes (Bender *et al.* 2004). The DNA was examined by capillary electrophoresis analysis to determine the average size of the DNA fragments at each time point.

One ng of degraded DNA was amplified using the AmpFℓSTR® Sinofiler™ PCR Amplification Kit. As the DNA became increasingly degraded, the larger size loci became undetectable (Figure 5-10 on page 5-35).

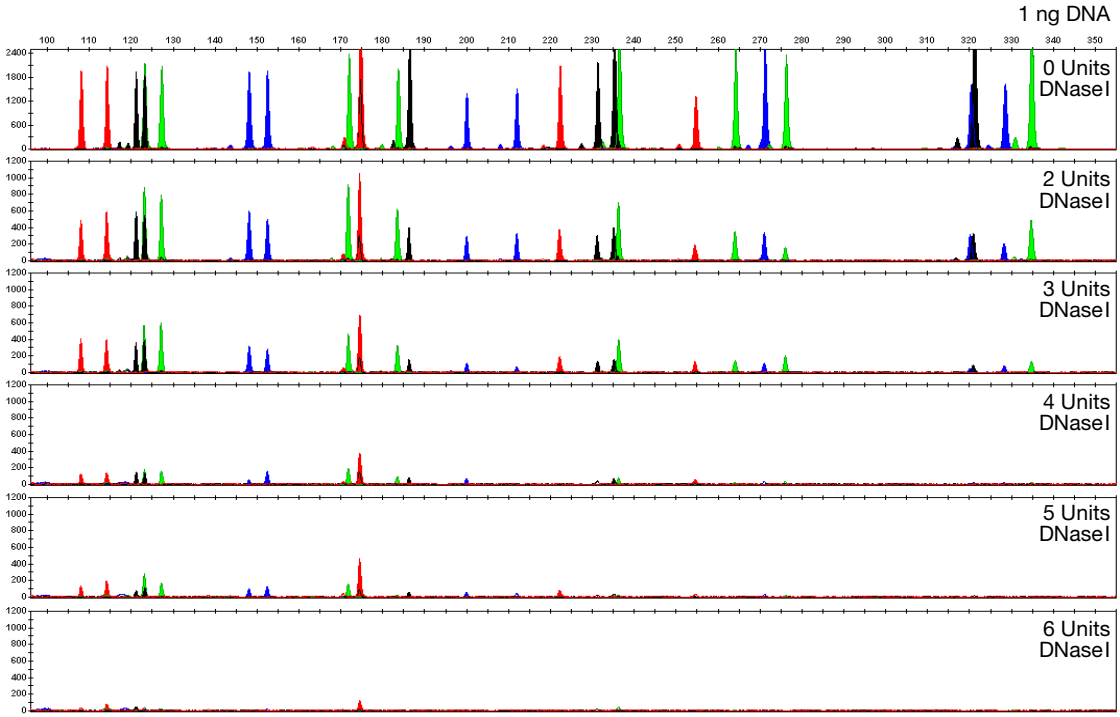

Figure 5-10 Amplification of 1-ng Raji DNA samples sonicated and then treated with 0, 2, 3, 4, 5, and 6 units of DNase I.

Effect of Inhibitors — Hematin

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.

To examine the effects of hematin on the amplification results obtained by the Sinofiler kit, male DNA 007 (1 ng input) was amplified with increasing concentrations of hematin: 0 µM, 40 µM, 50 µM, 60 µM, and 70 µM (Figure 5-11 on page 5-36). No preferential amplification was observed in the presence of increasing amounts of hematin.

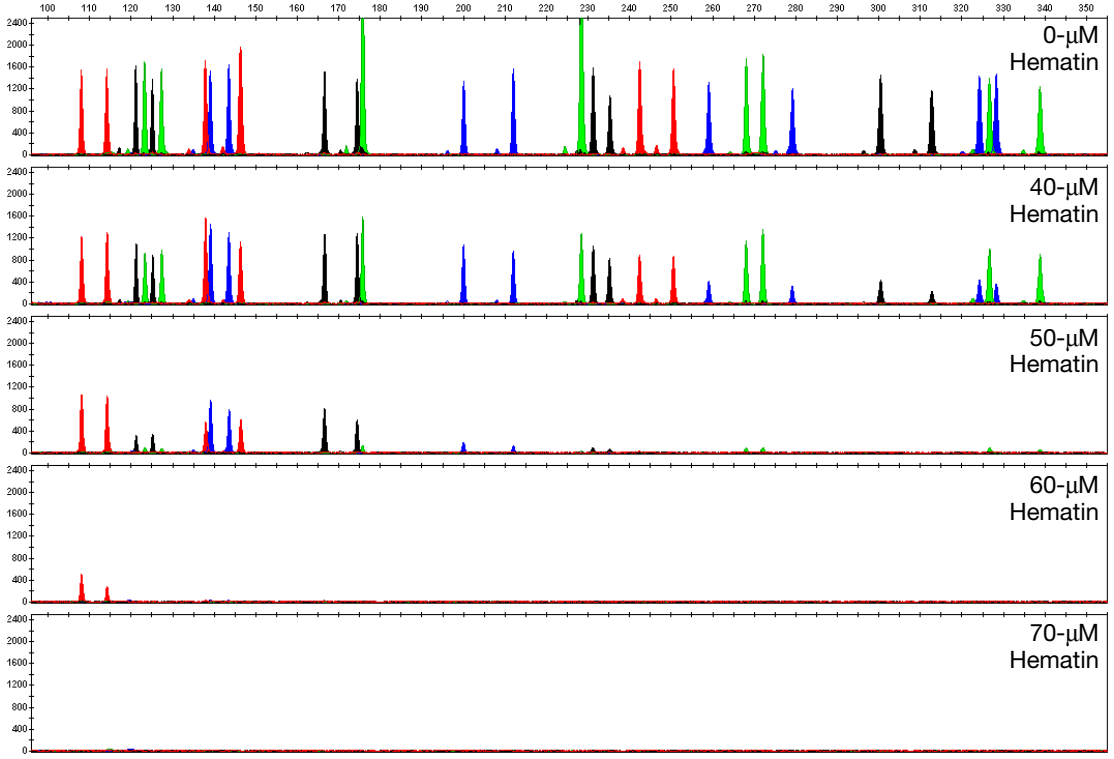

Figure 5-11 Amplification with the AmpFℓSTR® Sinofiler™ kit in the presence of varying concentrations of hematin, analyzed on the Applied Biosytstems 3130*xl* Genetic Analyzer.

Mixture Studies

SWGDAM Guideline 2.8

"The ability to obtain reliable results from mixed source samples should be determined." (SWGDAM, July 2003)

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

Mixture Studies

Evidence samples that contain body fluids and/or tissues originating from more than one individual are an integral component of forensic casework. Therefore, it is essential to ensure that the DNA typing system must be able to detect DNA mixtures. Mixed samples can be distinguished from single-source samples in a variety of ways:

- The presence of greater 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, minimum, and maximum peak height ratios observed for alleles in the AmpFℓSTR® Sinofiler™ PCR Amplification Kit loci in unmixed population database samples are shown inTable 5-3:

Table 5-3 Peak height ratios for 1 ng of input DNA

Allele	Number of Observations (n)	Mean	Median	Minimum	Maximum
CSF1PO	581	88.8	90.4	55.8	99.9
D12S391	688	87.9	89.2	57.4	100
D13S317	590	88.4	89.3	59.5	99.9
D16S539	621	88.6	89.8	52.4	100
D18S51	703	87.6	88.3	57.5	100
D19S433	637	89.8	90.6	62.6	100
D21S11	689	89.4	90.8	62.3	100
D2S1338	702	87.2	88.8	45.1	99.9
D3S1358	584	90.5	92.0	57.5	99.9
D5S818	575	89.9	91.3	62.0	100
D6S1043	674	90.0	91.5	61.9	100
D7S820	646	88.6	89.9	50.6	100
D8S1179	643	89.8	91.0	58.9	100
FGA	683	88.4	89.7	54.2	100
vWA	664	89.5	91.0	63.9	100

If an unusually low peak height ratio is observed for one locus, but there are no other indications that the sample is a mixture, you can reamplify and reanalyze the sample 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
- A SNP in one of the primer binding sites
- 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 (see 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 different allele peak heights on Applied Biosystems instruments provides an additional means to resolve mixed genotypes. The 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).

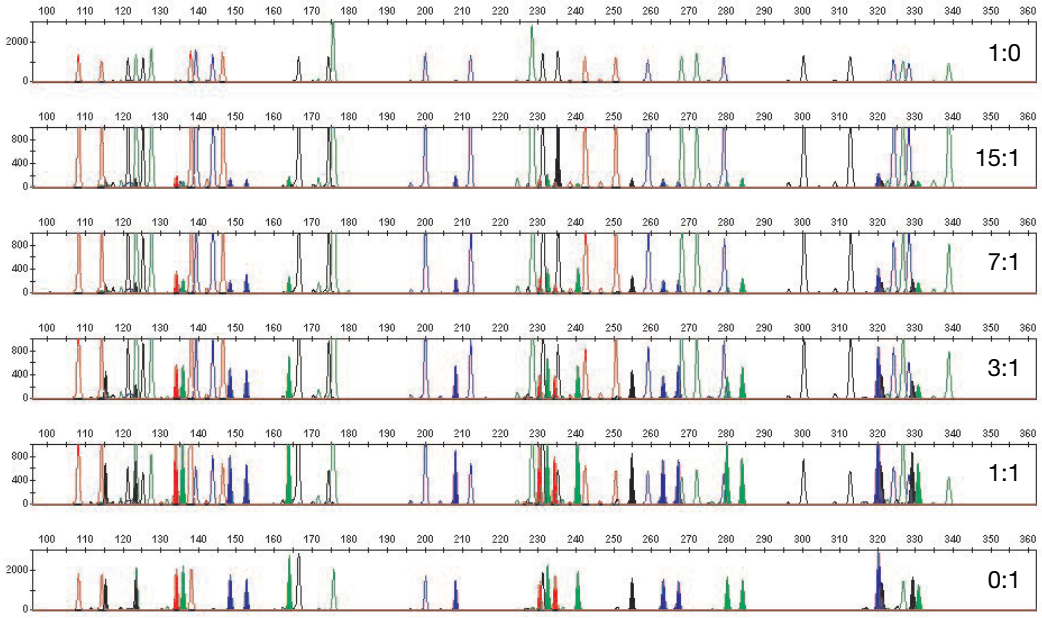

Figure 5-12 Amplification of DNA mixtures at various ratios

Limit of Detection of the Minor Component

Mixtures of two DNA samples were examined at various ratios (0:1, 1:1, 3:1, 7:1, 15:1, 1:0). The total amount of genomic input DNA mixed at each ratio was 1 ng. The samples were amplified in a GeneAmp® PCR System 9700, then electrophoresed and detected using an Applied Biosystems 3130*xl* Genetic analyzer.

The results of the mixed DNA samples are shown in Figure 5-12, where samples A and B were mixed according to the indicated ratios. The minor component allele calls at non-overlapping loci are highlighted. The amplification of the minor contributor at 3:1 and 7:1 (0.875:0.125 ng) mixture ratios was readily typeable. 15:1 ratios generally resulted in partial profiles for the minor component.

Table 5-4 shows the profiles of the samples in Figure 5-12 on page 5-40.

Table 5-4 Genotypes of mixed DNA samples

Allele	Profile Sample A (Control DNA 007)	Profile Sample B
D8S1179	12, 13	14, 15
D21S11	28, 31	28, 30
D7S820	7, 12	8, 9
CSF1PO	11, 12	10
D3S1358	15, 16	15, 18
D5S818	11	8, 11
D13S317	11	12, 14
D16S539	9, 10	12, 13
D2S1338	20, 23	20, 21
D19S433	14, 15	12.2, 14.2
vWA	14, 16	14
D12S391	18, 19	18, 24
D18S51	12, 15	17, 19
Amelogenin	X, Y	X, Y
D6S1043	12, 14	11, 12
FGA	24, 26	21, 22

The Sinofiler kit has been optimized to reliably amplify and type approximately 0.50 to 1.25 ng of single-source DNA.

Population Data

SWGDAM Guideline 2.7

"The distribution of genetic markers in populations should be determined in relevant population groups." (SWGDAM, July 2003)

Overview

To interpret the significance of a match between genetically typed samples, you must know the population distribution of alleles at each locus in question. If the genotype of the relevant evidence sample is different from the genotype of a suspects reference sample, then the suspect is excluded as the donor of the biological evidence that was tested. An exclusion is independent of the frequency of the two genotypes in the population.

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

Analysis across the three databases of 2,034 total chromosomes per locus revealed the following number of different alleles: 8 CSF1PO alleles, 14 D2S1338 alleles, 12 D3S1358 alleles, 9 D5S818 alleles, 25 D6S1043 alleles, 12 D7S820 alleles, 11 D8S1179 alleles, 21 D12S391 alleles, 10 D13S317 alleles, 8 D16S539 alleles, 23 D18S51 alleles, 19 D19S433 alleles, 24 D21S11 alleles, 28 FGA alleles, and 12 vWA alleles.

Conformity of the observed genotype frequencies with Hardy-Weinberg expectations (HWE) was examined in each sample population by the exact test using the HW-QuickCheck software (Bonferroni, 1936; Guo and Thompson, 1992, Kalinowski, 2006). A *p*-value >0.05 was obtained for all STRs, except D6S1043 in African-American, D3S1358 in Asian, D19S433 in Caucasian, and D12S391 in Hispanic samples. Considering the Bonferroni procedure and the fact that 15 tests for HWE were simultaneously performed on

the same population sample, the significance threshold is adjusted from 0.05 to 0.05/15 = 0.0033, which is clearly below the *p*-values observed in these loci. Hence, the departures from HWE were not significant.

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

AmpFℓSTR® Sinofiler™ Kit Allele Frequencies

Table 5-5 shows the AmpFℓSTR® Sinofiler™ kit allele frequencies in four populations, listed as percentages.

Table 5-5 AmpFℓSTR Sinofiler kit allele frequencies

Allele	African-American (n = 315)	Asian (n = 219)	Caucasian (n = 272)	Hispanic (n = 211)
CSF1PO				
6	‡	‡	‡	‡
7	5.56	0.68‡	‡	0.95‡
8	6.98	0.45‡	0.37‡	0.71‡
9	4.44	4.32	2.21	2.37
10	24.44	22.95	29.04	23.70
11	23.65	25.91	29.60	30.81
12	27.30	37.73	29.96	36.97
13	6.83	6.82	7.90	3.55
14	0.79	0.68‡	0.92	0.95‡
15	‡	‡	‡	‡
p-value	0.07	0.19	0.35	0.18
D12S391				
13	‡	‡	‡	0.24‡
14	‡	‡	‡	‡

Table 5-5 AmpFℓSTR Sinofiler kit allele frequencies *(continued)*

Allele	African-American (n = 315)	Asian (n = 219)	Caucasian (n = 272)	Hispanic (n = 211)
15	6.03	0.68‡	4.60	4.74
16	6.51	0.45‡	2.39	5.92
17	15.87	5.91	10.11	5.92
17.1	0.32‡	‡	‡	‡
17.3	0.48‡	‡	2.21	1.42
18	25.71	24.55	15.62	17.54
18.3	0.95	0.45‡	2.02	1.90
19	16.98	18.18	10.66	18.25
19.1	0.63‡	‡	‡	‡
19.3	0.16‡	‡	0.55‡	2.84
20	10.95	17.5	12.32	13.51
20.3	‡	0.23‡	0.37‡	0.24‡
21	6.83	10.68	13.05	10.66
22	3.33	9.32	13.42	9.24
23	2.06	5.91	8.46	3.55
24	1.90	3.18	2.76	1.18
25	1.11	1.59	0.92	1.90
26	0.16‡	0.23‡	0.55‡	0.71‡
27	‡	0.45‡	‡	0.24‡
28	‡	0.23‡	‡	‡
p-value	0.45	0.20	0.37	0.0287§

Table 5-5 AmpFℓSTR Sinofiler kit allele frequencies *(continued)*

Allele	African-American (n = 315)	Asian (n = 219)	Caucasian (n = 272)	Hispanic (n = 211)
D13S317				
6	‡	0.23‡	‡	‡
7	‡	0.23‡	‡	‡
8	3.81	29.32	9.74	8.77
9	1.59	11.82	5.70	14.93
10	3.02	15.91	7.35	9.24
11	30.48	22.73	29.78	24.17
12	44.92	14.55	30.70	27.49
13	11.90	4.09	11.76	10.43
14	3.97	0.45‡	4.96	4.98
15	0.32‡	0.23‡	‡	‡
p-value	0.38	0.20	0.23	0.19
D16S539				
5	‡	‡	‡	‡
8	3.65	0.45‡	1.10	2.37
9	20.48	27.27	12.32	11.37
10	10.79	17.27	4.96	14.22
11	30.79	24.77	32.35	30.09
12	17.14	19.77	28.49	26.54
13	15.56	9.32	19.12	14.45
14	1.59	0.68‡	1.47	0.95‡
15	‡	‡	0.18‡	‡

Table 5-5 AmpFℓSTR Sinofiler kit allele frequencies *(continued)*

Allele	African-American (n = 315)	Asian (n = 219)	Caucasian (n = 272)	Hispanic (n = 211)
p-value	0.06	0.45	0.13	0.40
D18S51				
7	‡	‡	‡	‡
9	‡	‡	‡	0.24‡
10	0.16‡	‡	1.10	0.24‡
10.2	0.16‡	‡	‡	‡
11	0.48‡	1.14	0.74‡	0.71‡
12	5.40	5.91	14.52	9.00
12.2	‡	‡	0.18‡	‡
13	4.60	15	13.05	15.17
13.2	0.79	‡	‡	‡
14	6.67	18.18	16.36	13.03
14.2	0.32‡	‡	‡	‡
15	18.73	22.95	11.40	10.90
15.2	‡	‡	‡	0.24‡
16	16.83	14.55	14.15	15.40
17	15.71	7.27	12.13	15.40
18	11.43	4.09	9.01	8.77
19	9.52	2.73	4.41	3.79
20	5.24	2.05	1.47	2.13
21	3.02	2.73	0.55‡	2.61
22	0.79	1.82	0.18‡	0.95‡

Table 5-5 AmpFℓSTR Sinofiler kit allele frequencies *(continued)*

Allele	African-American (n = 315)	Asian (n = 219)	Caucasian (n = 272)	Hispanic (n = 211)
23	0.16‡	0.68‡	0.55‡	0.95‡
24	‡	0.45‡	‡	0.24‡
25	‡	‡	0.18‡	‡
26	‡	‡	‡	‡
27	‡	‡	‡	0.24‡
p-value	0.37	0.55	0.39	0.41
D19S433				
9	‡	0.23‡	‡	‡
10	0.95	‡	‡	0.24‡
11	8.57	‡	0.18‡	1.18
11.2	0.16‡	‡	‡	0.24‡
12	11.43	3.86	8.64	7.11
12.1	‡	‡	0.18‡	‡
12.2	4.60	0.91‡	‡	1.18
13	26.67	32.05	26.10	23.22
13.2	4.92	3.64	1.10	5.69
14	20.79	21.14	33.64	25.83
14.2	4.92	12.05	2.76	5.69
15	6.51	5.45	16.91	14.45
15.2	4.76	16.14	2.21	9.24
16	1.75	1.36	6.62	3.08
16.2	3.33	2.73	1.10	2.13

Table 5-5 AmpFℓSTR Sinofiler kit allele frequencies *(continued)*

Allele	African-American (n = 315)	Asian (n = 219)	Caucasian (n = 272)	Hispanic (n = 211)
17	‡	‡	0.18‡	0.24‡
17.2	0.63‡	‡	‡	0.47‡
18	‡	‡	0.18‡	‡
18.2	‡	‡	0.18‡	‡
p-value	0.32	0.09	0.0091§	0.37
D21S11				
24	‡	‡	‡	‡
24.2	‡	‡	‡	0.24‡
25	‡	‡	‡	‡
26	0.32‡	‡	0.55‡	0.47‡
27	3.81	0.45‡	2.94	2.61
28	24.29	4.55	15.81	11.14
28.2	‡	0.68‡	‡	0.24‡
29	16.19	26.59	24.26	17.77
29.2	‡	0.23‡	‡	0.24‡
29.3	0.16‡	‡	0.18‡	‡
30	19.37	29.32	23.53	30.33
30.2	2.06	1.14	3.68	1.42
30.3	‡	0.68‡	‡	‡
31	9.84	9.77	5.51	5.92
31.2	6.19	7.73	9.19	10.43
32	2.06	3.41	1.65	0.71‡

Table 5-5 AmpFℓSTR Sinofiler kit allele frequencies *(continued)*

Allele	African-American (n = 315)	Asian (n = 219)	Caucasian (n = 272)	Hispanic (n = 211)
32.2	6.98	9.77	9.56	13.51
33	0.79	1.36	0.18‡	0.24‡
33.1	0.16‡	‡	‡	‡
33.2	3.81	2.73	2.39	3.55
34	0.32‡	‡	‡	‡
34.1	‡	0.23‡	‡	‡
34.2	0.16‡	0.91‡	0.37‡	0.71‡
35	2.54	‡	‡	0.47‡
35.2	‡	‡	‡	‡
36	0.79	‡	0.18‡	‡
37	‡	‡	‡	‡
38	0.16‡	‡	‡	‡
p-value	0.20	0.16	0.13	0.41
D2S1338				
<14	0.16‡	‡	0.18‡	‡
15	0.63‡	‡	0.18‡	‡
16	4.6	1.82	4.60	3.08
17	10.16	8.86	18.75	19.43
18	4.76	10.68	8.27	6.87
19	16.98	19.09	13.97	16.35
20	11.9	12.05	15.26	13.74
21	14.29	3.18	2.39	1.90

Table 5-5 AmpFℓSTR Sinofiler kit allele frequencies *(continued)*

Allele	African-American (n = 315)	Asian (n = 219)	Caucasian (n = 272)	Hispanic (n = 211)
22	11.75	5.68	2.76	6.64
23	7.46	16.14	9.01	14.93
24	8.1	15.23	12.13	8.53
25	6.19	5.91	10.66	6.64
26	2.54	0.91‡	1.65	1.66
27	0.48‡	‡	0.37‡	0.24‡
28	‡	‡	‡	‡
p-value	0.37	0.33	0.37	0.42
D3S1358				
9	0.48‡	‡	‡	0.24‡
11	0.16‡	‡	0.18‡	‡
12	0.16‡	0.23‡	‡	0.24‡
13	0.95	‡	‡	0.47‡
14	9.05	4.32	15.99	10.43
15	31.43	34.77	25.37	35.78
15.2	0.16‡	‡	‡	‡
16	27.62	31.82	25.92	26.07
17	22.54	20.91	17.46	16.35
18	6.98	7.05	13.42	9.95
19	0.48‡	0.23‡	1.47	0.47‡
20	‡	0.23‡	0.18‡	‡
p-value	0.08	0.0064§	0.27	0.42

Table 5-5 AmpFℓSTR Sinofiler kit allele frequencies *(continued)*

Allele	African-American (n = 315)	Asian (n = 219)	Caucasian (n = 272)	Hispanic (n = 211)
D5S818				
7	0.32‡	3.41	0.18‡	4.98
8	4.92	‡	0.74‡	0.47‡
9	1.27	7.05	4.78	3.08
10	7.46	20.23	4.96	4.27
11	25.08	30.91	36.40	38.15
12	34.76	22.95	33.82	31.75
13	23.97	14.09	17.83	16.35
14	1.43	0.68‡	0.92	0.71‡
15	0.79	0.23‡	0.37‡	0.24‡
16	‡	‡	‡	‡
p-value	0.16	0.39	0.37	0.15
D6S1043				
<8	‡	‡	0.18‡	‡
9	0.16‡	‡	‡	‡
10	1.59	2.73	1.65	1.90
11	10.48	11.36	26.47	16.35
12	22.54	13.86	27.94	20.38
13	9.68	11.14	6.80	12.32
14	5.71	16.36	4.96	12.56
15	5.56	1.82	1.10	2.61
16	3.65	0.23‡	0.92	0.24‡

Table 5-5 AmpFℓSTR Sinofiler kit allele frequencies *(continued)*

Allele	African-American (n = 315)	Asian (n = 219)	Caucasian (n = 272)	Hispanic (n = 211)
17	7.62	2.27	6.43	6.40
18	11.75	19.55	7.90	9.00
18.2	‡	0.23‡	‡	‡
19	13.65	13.18	9.74	7.58
19.3	0.16‡	‡	‡	‡
20	5.71	5.45	5.15	2.37
20.3	‡	‡	‡	1.42
21	0.32‡	1.36	0.55‡	0.47‡
21.3	0.32‡	‡	0.18‡	4.74
22	‡	‡	‡	‡
22.3	0.16‡	‡	‡	0.71‡
23	0.48‡	‡	‡	0.24‡
23.3	‡	‡	‡	0.47‡
24	0.16‡	‡	‡	‡
24.3	‡	‡	‡	0.24‡
25	0.32‡	‡	‡	‡
p-value	0.0416§	0.22	0.33	0.06
D7S820				
6	0.16‡	‡	‡	‡
7	0.79	0.23‡	1.29	1.42
8	19.05	13.64	18.01	13.27
9	10.63	5.45	14.34	8.77

Table 5-5 AmpFℓSTR Sinofiler kit allele frequencies *(continued)*

Allele	African-American (n = 315)	Asian (n = 219)	Caucasian (n = 272)	Hispanic (n = 211)
10	32.38	18.41	28.12	27.96
10.2	‡	0.23‡	‡	‡
11	21.90	36.14	21.51	23.46
11.3	‡	‡	‡	0.24‡
12	12.70	22.73	13.24	19.19
13	2.06	1.82	2.76	4.74
14	0.32‡	0.68‡	0.55‡	0.95‡
15	‡	0.23‡	0.18‡	‡
p-value	0.53	0.23	0.18	0.37
D8S1179				
8	0.16‡	‡	1.65	0.95‡
9	0.48‡	0.23‡	1.65	0.47‡
10	1.90	11.82	8.09	8.06
11	3.81	11.14	7.72	6.64
12	11.27	12.5	15.62	7.82
13	19.52	20.68	31.99	29.62
14	34.13	17.73	19.30	28.67
15	20.79	14.55	10.66	13.74
16	6.98	8.86	3.12	3.55
17	0.95	1.82	0.18‡	0.47‡
18	‡	0.23‡	‡	‡
19	‡	‡	‡	‡

Table 5-5 AmpFℓSTR Sinofiler kit allele frequencies *(continued)*

Allele	African-American (n = 315)	Asian (n = 219)	Caucasian (n = 272)	Hispanic (n = 211)
p-value	0.41	0.27	0.35	0.11
FGA				
17	0.32‡	0.45‡	‡	‡
17.2	0.16‡	‡	‡	‡
18	0.79	2.27	1.47	0.24‡
18.2	1.59	‡	‡	‡
19	6.67	6.36	5.88	6.64
19.2	0.79	‡	‡	‡
20	6.03	7.27	14.71	9.95
20.2	0.63‡	‡	0.37‡	0.24‡
21	10.63	11.82	19.85	15.17
21.2	‡	0.68‡	0.18‡	‡
22	19.52	17.05	17.83	12.09
22.2	0.16‡	1.14	0.55‡	0.47‡
23	17.30	23.41	15.07	14.22
23.2	‡	0.91‡	0.18‡	‡
24	16.98	17.05	15.26	17.06
24.2	‡	0.68‡	‡	‡
25	9.05	7.05	6.80	14.45
25.2	‡	0.23‡	‡	‡
26	4.29	1.82	1.10	5.69
26.2	‡	0.45‡	‡	‡

Table 5-5 AmpFℓSTR Sinofiler kit allele frequencies *(continued)*

Allele	African-American (n = 315)	Asian (n = 219)	Caucasian (n = 272)	Hispanic (n = 211)
27	2.86	0.68‡	0.55‡	2.84
28	0.95	0.23‡	0.18‡	0.71‡
29	0.32‡	‡	‡	0.24‡
30	0.16‡	‡	‡	‡
30.2	0.16‡	‡	‡	‡
31.2	0.32‡	‡	‡	‡
32.2	‡	‡	‡	‡
33.2	‡	‡	‡	‡
34.2	0.16‡	‡	‡	‡
42.2	‡	‡	‡	‡
43.2	‡	‡	‡	‡
44.2	‡	‡	‡	‡
45.2	‡	‡	‡	‡
46.2	0.16‡	‡	‡	‡
47.2	‡	‡	‡	‡
48.2	‡	‡	‡	‡
50.2	‡	‡	‡	‡
51.2	‡	‡	‡	‡
p-value	0.26	0.38	0.31	0.24
vWA				
11	0.48‡	‡	‡	0.24‡
12	0.32	‡	‡	0.24‡

Table 5-5 AmpFℓSTR Sinofiler kit allele frequencies *(continued)*

Allele	African-American (n = 315)	Asian (n = 219)	Caucasian (n = 272)	Hispanic (n = 211)
13	1.27	‡	0.37‡	‡
14	7.14	24.09	8.27	6.87
15	20.48	2.73	12.87	13.74
16	23.97	17.27	20.04	27.73
17	20.32	23.64	25.92	27.73
18	15.87	19.77	20.04	16.11
19	6.83	10.68	10.11	6.16
20	2.70	1.36	2.39	1.18
21	0.48‡	‡	‡	‡
22	‡	‡	‡	‡
23	0.16‡	‡	‡	‡
24	‡	‡	‡	‡
p-value	0.16§	0.44	0.54	0.11

‡ A minimum allele frequency (0.79% for the African-American database, 1.14% for the Asian database, 0.92% for the U.S. Caucasian database, and 1.18% for the U.S. Hispanic database) is suggested by the National Research Council in forensic calculations.

§ *p*-value <0.05 for exact test for HWE

Low-Frequency Alleles

Some alleles of the Sinofiler kit loci occur at a low frequency. For these alleles, a minimum frequency (five divided by 2n, where n equals the number of individuals in the database) was assigned for the Sinofiler kit African-American, Asian, U.S. Caucasian, and U.S. Hispanic databases, as suggested in the 1996 report of the Committee on DNA Forensic Science (National Research Council, 1996). These databases are summarized in Table 5-6 on page 5-59. The minimum reportable genotype frequency at each locus is: 1.42×10^{-4} for the

African-American database; 2.43×10^{-4} for the Asian database; 1.76×10^{-4} for the U.S. Caucasian database; and 2.57×10^{-4} for the U.S. Hispanic database [$p^2 + p(1–p)\,\overline{\theta}$, where $\overline{\theta} = 0.01$]. Hence, the minimum combined multilocus genotype frequency at 15 loci is: 1.87×10^{-58} for the African-American database; 6.15×10^{-55} for the Asian database; 4.63×10^{-57} for the U.S. Caucasian database; and 1.45×10^{-54} for the U.S. Hispanic database.

Concordance Studies

Applied Biosystems analyzed 300 samples by comparing allele calls between the AmpFℓSTR® Sinofiler™ and AmpFℓSTR® Identifiler® kits. The genotype data from all the analyzed samples were concordant between the Identifiler and Sinofiler kits.

Mutation Rate

Estimation of spontaneous or induced germline mutation at genetic loci can be achieved by comparing 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 that were 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; mutations were not detected at any of the other nine STR loci. The D18S11 mutation was represented by an increase of one 4-nt repeat unit, allele 17 was inherited as allele 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 AmpFℓSTR® Sinofiler™ PCR Amplification Kit loci.
- Methods for modifications of these mutation rates (to infer mutation rates indirectly for those loci where the rates are not large enough to be measured directly and/or to account for those events undetectable as Mendelian errors).

Probability of Identity

Table 5-6 shows the Probability of Identity (PI) values of the AmpFℓSTR® Sinofiler™ PCR Amplification Kit loci individually and combined.

Table 5-6 Probability of Identity values for the AmpFℓSTR® Sinofiler™ kit STR loci

Locus	African-American	Asian	Caucasian	Hispanic
CSF1PO	0.074	0.119	0.127	0.142
D12S391	0.041	0.046	0.025	0.026
D13S317	0.145	0.069	0.077	0.059
D16S539	0.070	0.082	0.101	0.084
D18S51	0.030	0.039	0.031	0.029
D19S433	0.037	0.067	0.081	0.049
D21S11	0.040	0.07	0.052	0.049
D2S1338	0.024	0.034	0.030	0.038
D3S1358	0.099	0.145	0.073	0.093
D5S818	0.101	0.083	0.140	0.122
D6S1043	0.028	0.034	0.057	0.029
D7S820	0.084	0.092	0.071	0.075
D8S1179	0.081	0.043	0.063	0.071
FGA	0.031	0.038	0.042	0.033
vWA	0.057	0.071	0.059	0.081
Combined	1.12×10^{-19}	8.37×10^{-19}	6.28×10^{-19}	2.31×10^{-19}

The P_I value is the probability that two individuals selected at random will have an identical Sinofiler kit genotype (Sensabaugh, 1982). The P_I values for the populations described in this section are then approximately $1/8.93 \times 10^{18}$ (African-American), $1/1.19 \times 10^{18}$ (Asian), $1/1.59 \times 10^{18}$ (U.S. Caucasian), and $1/4.33 \times 10^{18}$ (U.S. Hispanic).

Probability of Paternity Exclusion

Table 5-7 shows the Probability of Paternity Exclusion (P_E) values of the AmpFℓSTR® Sinofiler™ PCR Amplification Kit STR loci individually and combined.

Table 5-7 Probability of Paternity Exclusion values for the AmpFℓSTR® Sinofiler™ kit

Locus	African-American	Asian	Caucasian	Hispanic
CSF1PO	0.530	0.433	0.461	0.402
D12S391	0.696	0.649	0.767	0.682
D13S317	0.397	0.556	0.535	0.583
D16S539	0.530	0.564	0.581	0.558
D18S51	0.766	0.712	0.737	0.748
D19S433	0.678	0.676	0.461	0.655
D21S11	0.741	0.676	0.723	0.673
D2S1338	0.766	0.767	0.737	0.758
D3S1358	0.471	0.597	0.561	0.381
D5S818	0.471	0.589	0.473	0.416
D6S1043	0.690	0.694	0.665	0.682
D7S820	0.570	0.492	0.650	0.627
D8S1179	0.581	0.739	0.602	0.655
FGA	0.715	0.694	0.679	0.710
vWA	0.672	0.597	0.636	0.655
Combined	0.99999973	0.999999774	0.99999968	0.99999964

The P_E value is the probability, averaged over all possible mother-child pairs, that a random alleged father will be excluded from paternity after DNA typing of the Sinofiler kit STR loci (Chakraborty *et al.,* 1996).

Troubleshooting

A

In This Appendix

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

Troubleshooting

Table A-1 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 AmpFℓSTR® PCR Reaction Mix, AmpFℓSTR® Sinofiler™ Primer Set, or AmpliTaq Gold® DNA Polymerase.	Repeat amplification.
	No activation of AmpliTaq Gold DNA Polymerase	Repeat amplification, making sure to hold reactions initially at 95 °C for 11 min.
	Master Mix not vortexed thoroughly before aliquoting	Vortex the Master Mix thoroughly.
	AmpFℓSTR® Sinofiler™ 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.
	Incorrect thermal cycler parameters	Check the protocol for correct thermal cycler parameters.
	Tubes not seated tightly in the thermal cycler during amplification	Push reaction tubes firmly into contact with block after first cycle. Repeat test.
	GeneAmp PCR System 9600 heated cover misaligned	Align the GeneAmp 9600 heated cover properly so that white stripes align after twisting the top portion clockwise.
	Wrong PCR reaction tube	Use Applied Biosystems MicroAmp Reaction Tubes with Caps for the GeneAmp 9600 and 9700.
	MicroAmp™ Base used with tray/retainer set and tubes in GeneAmp® 9600 and 9700	Remove MicroAmp Base from tray/retainer set and repeat test.

Table A-1 Troubleshooting *(continued)*

Observation	Possible Causes	Recommended Actions
Faint or no signal from both the AmpFℓSTR® Control DNA 9947A and the DNA test samples at all loci. *(continued)*	Insufficient PCR product electrokinetically injected	For ABI PRISM® 3100/3100-*Avant* or 3130/3130*xl* instrument runs: Mix 1.0 µL of PCR product and 9 µL of Hi-Di™ Formamide/GeneScan™ 500 LIZ® solution. **WARNING CHEMICAL HAZARD**. **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
	Degraded formamide	Check the storage of formamide; do not thaw and refreeze multiple times. Try Hi-Di™ Formamide. **WARNING CHEMICAL HAZARD**. **Formamide** causes eye, skin, and respiratory tract irritation. It is a possible reproductive and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Table A-1 Troubleshooting *(continued)*

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 0.5 to 1.25 ng of DNA. Repeat test.
	Test sample contains PCR inhibitor (for example, heme compounds, certain dyes)	Quantitate DNA and add minimum necessary volume. Repeat test.
		Wash the sample in a Centricon®-100. 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.
	Dilution of test sample DNA in H_2O or wrong buffer (*e.g.*, wrong EDTA concentration)	Redilute DNA using TE Buffer (with 0.1 mM EDTA).
More than one allele present at a locus	Presence of exogenous DNA	Use appropriate techniques to avoid introducing foreign DNA during laboratory handling.
	Too much DNA in reaction	Use recommended amount of template DNA (0.5 to 1.25 ng).
	Mixed sample	See "Stutter Products" on page 5-18
	Amplification of stutter product (n-4 nt position)	
	Incomplete 3´ A base addition (n-1 nt position)	See "Addition of 3´ A Nucleotide" on page 5-23. Be sure to include the final extension step of 60 °C for 60 min in the PCR.
	Signal exceeds dynamic range of instrument (off-scale data)	Quantitate DNA and reamplify sample, adding 0.5 to 1.25 ng of DNA.
	Poor spectral separation (bad matrix)	Follow the steps for creating a matrix file.
		Confirm that Filter Set G5 modules are installed and used for analysis.

Table A-1 Troubleshooting *(continued)*

Observation	Possible Causes	Recommended Actions
Some but not all loci visible on electropherogram	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.
	Test sample contains high concentrations of a PCR inhibitor (for example, heme compounds, certain dyes)	Quantitate DNA, then add minimum necessary volume. Repeat test.
		Wash the sample in a Centricon-100.

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