AmpFℓSTR® Yfiler™

PCR Amplification Kit

User's Manual

Applied Biosystems

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Preface

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Purpose of This Guide

The *AmpFℓSTR® Yfiler™ PCR Amplification Kit User's Manual* provides information about the Applied Biosystems instruments, chemistries, and software associated with the AmpFℓSTR Yfiler PCR Amplification Kit (Yfiler kit).

Text Conventions

This guide uses the following conventions:

- **Bold** 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 bracket (>) 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**.
- Double brackets are used to indicate a field on a software screen, for example, "Click the arrow beside <Collection Setting>."

Pull-Out Chapters

This guide is designed to allow users to pull out Chapters 3, 4, and 5. The pull-out chapters have title and back pages, which indicate the chapter number and title.

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 size of the column affects the run time.

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

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

IMPORTANT! You must create a separate Sample Entry Spreadsheet for each 96-well plate.

Safety Alert Words

Safety alert words also appear in user documentation. For more information, see "Safety Alert Words" on page ix.

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:

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

– 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.

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

– 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

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 (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs.")
- 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 on 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

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document

- **Print Target** – To print the document
- **Save Target As** – To download a PDF version of the document to a destination that you choose

4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5. After you enter the required information, click **View/Deliver Selected Documents Now**.

Chemical Waste Hazard

WARNING **CHEMICAL WASTE HAZARD.** Some wastes produced by the operation of the instrument or system are potentially hazardous and can cause injury, illness, or death.

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, 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 eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **http://bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).

Additional information about biohazard guidelines is available at: **http://www.cdc.gov**

How to Obtain More Information

Related Documentation

The following documents are available on the HID Validation and Verification Atlas CD (PN 402800) and on the Applied Biosystems Web site (**http://docs.appliedbiosystems.com/search.taf**).

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 (DC v1.1)	4315834
ABI PRISM® 3100-Avant Genetic Analyzer User Guide (DC 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's Manual (Macintosh)	903565
ABI PRISM® 310 Genetic Analyzer User Guide (Windows NT)	4317588
Protocols for Processing AmpFℓSTR® PCR Amplification Kit Products with the ABI PRISM® 377 DNA Sequencer and Windows NT OS User Bulletin	4340648
New Features and Installation Procedures for GeneMapper® ID Software Version 3.2 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
ABI PRISM® GeneScan® Analysis Software version 3.1 User's Manual (Macintosh)	4306157
ABI PRISM® GeneScan® Analysis Software v3.7 for the Windows NT Platform	4308923

GeneScan® Software Reference Guide ABI PRISM® 310 Analyzer	4303189
ABI PRISM® GeneScan® Analysis Software for the Windows NT Platform Overview of the Analysis Parameters and Size Caller User Bulletin	4335617
ABI PRISM® Genotyper® 2.5 Software: User's Manual (Macintosh)	904648
ABI PRISM® Genotyper® 3.7 NT Software User's Manual	4309947
ABI PRISM® Genotyper® 3.7 NT Software Applications Tutorials	4309961
Quantifiler™ Human DNA Quantification Kits User's Manual	4344790
AmpFℓSTR® Profiler Plus® PCR Amplification Kit User's Manual	4303501

For additional documentation, see "How to Obtain Support" below.

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

How to Obtain Support

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

At the Support page, you can:

- 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

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Overview 1

This chapter covers:

Product Overview

Purpose

The AmpFℓSTR® Yfiler™ PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that amplifies 17 Y-STR loci in a single PCR reaction. The kit amplifies the loci in the

- "European minimal haplotype" (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393)
- Scientific Working Group-DNA Analysis Methods (SWGDAM)-recommended Y-STR panel (European minimal haplotype plus DYS438 and DYS439)
- Additional highly polymorphic loci: DYS437, DYS448, DYS456, DYS458, DYS635 (Y GATA C4), and Y GATA H4

Product Description

The Yfiler kit contains all the necessary reagents for the amplification of human male-specific DNA.

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

- 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

Loci Amplified by the Kit

The following table shows the loci amplified by the Yfiler kit and the corresponding dyes used. The AmpFℓSTR Yfiler Kit Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the allelic ladder and the genotype of the Control DNA 007 are listed in the table.

Table 1-1 AmpFℓSTR Yfiler Kit loci and alleles

Locus Designation	Alleles Included in Yfiler Kit Allelic Ladder[a]	Dye Label	DNA 007 Genotype
DYS456	13—18	6-FAM™	15
DYS389I	10—15		13
DYS390	18—27		24

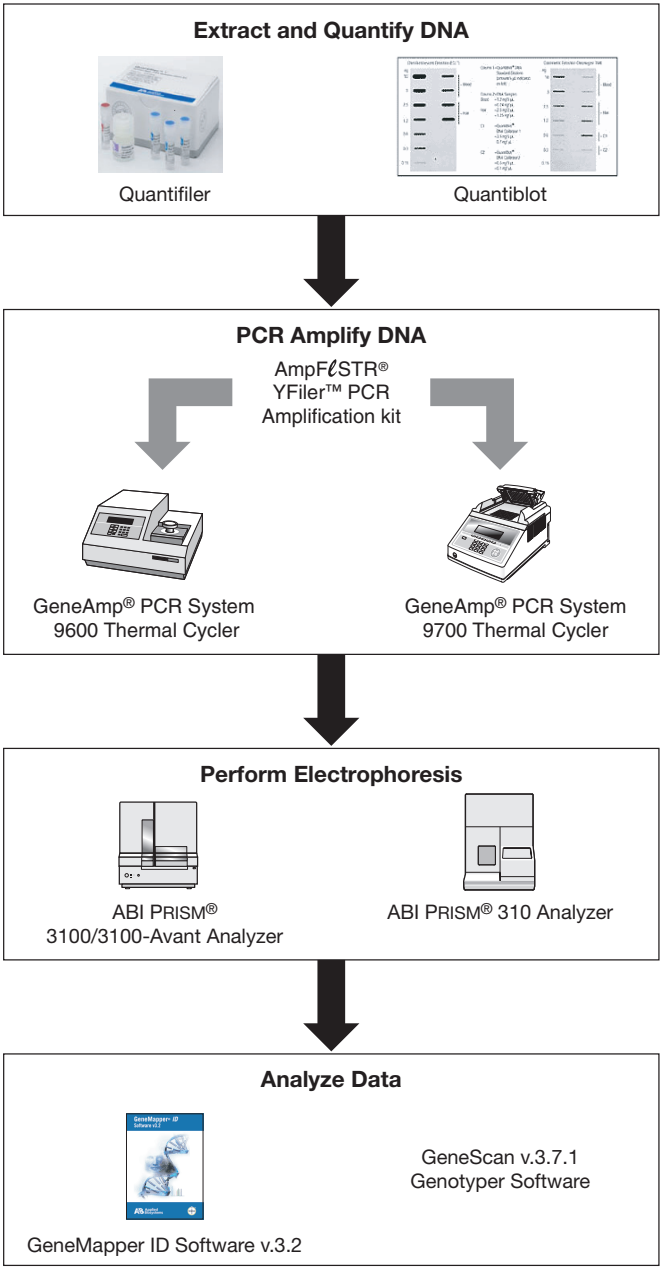
Table 1-1 AmpFℓSTR Yfiler Kit loci and alleles *(continued)*

Locus Designation	Alleles Included in Yfiler Kit Allelic Ladder[a]	Dye Label	DNA 007 Genotype
DYS389II	24—34		29
DYS458	14—20	VIC®	17
DYS19	10—19		15
DYS385 a/b	7—25		11,14
DYS393	8—16	NED™	13
DYS391	7—13		11
DYS439	8—15		12
DYS635	20—26		24
DYS392	7—18		13
Y GATA H4	8—13	PET®	13
DYS437	13—17		15
DYS438	8—13		12
DYS448	17—24		19

a. See "About the AmpFℓSTR Yfiler Kit Allelic Ladder" on page 5-53 for more information about the Yfiler Kit allelic ladder.

Procedural Overview

Y-STR Workflow



Instrument and Software Overview

This section provides information about the data collection and analysis software versions required to run the Yfiler 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 records sample fluorescence on the detection system hardware, 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
3100/3100-*Avant*	Windows NT[a]	1.1 (3100) 1.0 (3100-*Avant*)	• GeneMapper® *ID* 3.2 • GeneScan 3.7.1 + GenoTyper 3.7
	Windows 2000[a]	2.0	• GeneMapper® *ID* 3.2
310	Windows 2000	3.0	• GeneMapper *ID* 3.2 • GeneScan 3.7.1 + GenoTyper 3.7
	Macintosh OS 9.0	2.1	• GeneMapper *ID* 3.2 • GeneScan 3.1.2 + GenoTyper 2.5.2

a. Applied Biosystems conducted validation studies for YFiler 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® Yfiler™ 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 Size Standard.

How Multicomponent Analysis Works

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

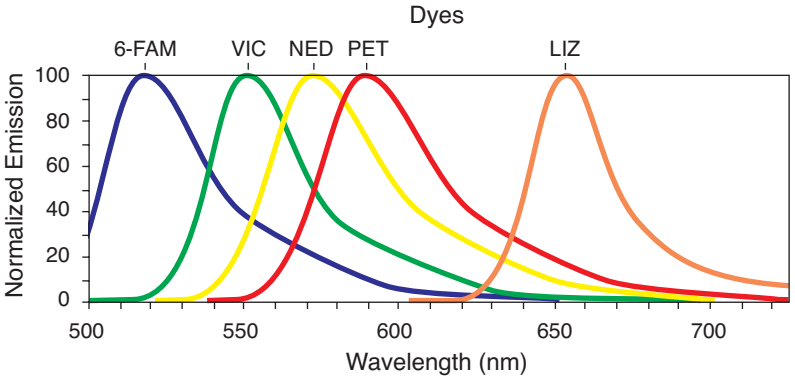


Figure 1-1 Emission spectra of the five dyes used in the AmpFℓSTR Yfiler PCR Amplification Kit

Materials and Equipment

Kit Contents and Storage

Each Yfiler™ kit contains materials sufficient to perform 100 reactions at a 25-µL reaction volume

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

Table 1-2 Yfiler kit contents

Reagent	Contents	Quantity	Storage
AmpFℓSTR Yfiler Primer Set	Forward and reverse primers to amplify human male DNA target	1 tube, 0.55 mL	2 to 8 °C
AmpFℓSTR Yfiler PCR Reaction Mix	$MgCl_2$, dNTPs, and bovine serum albumin in buffer with 0.05% sodium azide	1 tube, 1.1 mL/tube	2 to 8 °C
AmpFℓSTR Yfiler Allelic Ladder	Allelic ladder containing amplified alleles (refer to "Loci Amplified by the Kit" on page 1-2 for a list of alleles included in the ladder)	1 tube, 50 µL	2 to 8 °C
AmpFℓSTR Control DNA 007	0.10 ng/µL human male genomic DNA in 0.05% sodium azide and buffer (refer to "Loci Amplified by the Kit" on page 1-2 for profile)	1 tube, 0.3 mL	2 to 8 °C
AmpliTaq Gold® DNA Polymerase	DNA polymerase, 5 U/µL	2 tubes, 50 µL/tube	–15 to –25 °C
AmpFℓSTR Control DNA 9947A	10 ng/µL human female cell line DNA in 0.05 % sodium azide and buffer	1 tube, 25 µL	2 to 8 °C

Standards for Samples

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

- **Control DNA 007 –** A positive control for evaluating the efficiency of the amplification step and STR genotyping using the AmpFℓSTR Yfiler Kit Allelic Ladder.
- **GeneScan-500 LIZ Size Standard** – Used for obtaining base pair sizing results. The GeneScan-500 LIZ Size Standard is designed for sizing DNA fragments in the 35–500 bp range, and it contains 16 single-stranded fragments of 35, 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490, and 500 bases. This standard has been evaluated as an internal lane size standard and it yields precise sizing results for AmpFℓSTR Yfiler kit PCR products. Order the GeneScan-500 LIZ Size Standard (PN 4322682) separately.
- **AmpFℓSTR Yfiler Kit Allelic Ladder** – Developed by Applied Biosystems for accurate characterization of the alleles amplified by the AmpFℓSTR Yfiler kit. The AmpFℓSTR Yfiler Allelic Ladder contains the majority of alleles reported for the 17 loci. Refer to "Loci Amplified by the Kit" on page 1-2 for a list of the alleles included in the AmpFℓSTR Yfiler kit.

Equipment and Materials Not Included

Tables 1-3 through 1-4 list required and optional equipment and materials not supplied with the Yfiler kit. Unless otherwise noted, many of the items are available from major laboratory suppliers (MLS).

Table 1-3 Equipment

Equipment	Source
ABI PRISM® 3100/3100-*Avant* Genetic Analyzer	Contact your local Applied Biosystems sales representative
ABI PRISM® 310 Genetic Analyzer	
GeneAmp® PCR System 9600	
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

Table 1-3 Equipment

Equipment	Source
Tabletop centrifuge with 96-well plate adapters (optional)	Major Laboratory Supplier (MLS)

Table 1-4 User-supplied materials

Material	Source
AmpFℓSTR® Yfiler™ PCR Amplification Kit	4359513
3100/3100-Avant Analyzer materials	
96-Well Plate Septa	4315933
3100 Capillary Array, 36-cm	4315931
36-cm 3100-Avant Capillary Array	4333464
3100 Performance Optimized Polymer 4 (POP-4™)	4316355
Autosampler 96-well Plate Kit	4316471
GeneScan™ 500 LIZ® Size Standard	4322682
10× Genetic Analyzer Buffer with EDTA	402824
DS-33 (Dye Set G5) Matrix Standard, Kit for 3100/3100-*Avant* analyzers	4345833
MicroAmp® Optical 96-Well Reaction Plate	N801-0560
3100 Instrument Consumable Reservoir Septa	4315932
Array-fill syringe, 250-µL glass syringe	4304470
Polymer-reserve syringe, 5.0-mL glass 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	

Table 1-4 User-supplied materials *(continued)*

Material	Source
310 Capillaries, 47 cm × 50 µm i.d. (internally uncoated) (green)	402839
0.5 mL Sample Tray	5572
96-Well Tray Adaptor (for 9700 thermal cycler trays)	4305051
GeneScan™ 500 LIZ® Size Standard	4322682
10× Genetic Analyzer Buffer with EDTA	402824
Genetic Analyzer Retainer Clips (96-Tube Tray Septa Clips)	402866
Genetic Analysis Sample Tubes (0.5 mL)	401957
Genetic Analysis Septa for 0.5 mL Sample Tubes	401956
Matrix Standard Set DS-33 (6FAM™, VIC®, NED™, PET®, and LIZ® dyes) for 310/377 systems	4318159
MicroAmp® 8-strip Reaction Tubes	N801-0580
MicroAmp® 96-Well Support Base (holds 0.2-mL reaction tubes)	N801-0531
MicroAmp® 96-Well Full Plate Cover	N801-0550
MicroAmp® 96-Well Tray/Retainer Sets	403081
POP-4™ Performance Optimized Polymer	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 Trays for Tubes with Caps	N801-0541
MicroAmp® Reaction Tubes with Caps, 0.2-mL	N801-0540
MicroAmp® 8-Strip Reaction Tubes	N801-0580

Table 1-4 User-supplied materials *(continued)*

Material	Source
MicroAmp® Caps, 8 Caps/Strip	N801-0535
MicroAmp® 96-Well Tray/Retainer Sets	403081
MicroAmp® 96-Well Support Base	N801-0531
MicroAmp® Optical 96-Well Reaction Plate	N801-0560
Other user-supplied materials	
Hi-Di™ Formamide, 25mL	4311320
Aerosol resistant pipette tips	MLS
Microcentrifuge tubes	MLS
Pipettors	MLS
Tape, labeling	MLS
Tube, 50-mL Falcon	MLS
Tube decapper, autoclavable	MLS
Deionized water, PCR grade	MLS
Tris-HCL, pH 8.0	MLS
0.5-M EDTA	MLS
Vortex	MLS

Extracting and Quantifying DNA

2

This chapter covers:

Extracting DNA

Overview

Extraction procedures can be classified as organic or nonorganic. Depending on the material received, scientists should determine which procedure is appropriate for each piece of evidence.

DNA extracted from fresh or frozen whole blood, peripheral blood lymphocytes, blood stains, sperm cells, paraffin blocks, teeth, hair, tissue, bone, and other biological samples can be PCR amplified and analyzed using the AmpFℓSTR® YFiler™ PCR Amplification Kit.

The quality of the DNA (degree of degradation), its purity, and its quantity in a sample influence the efficiency of PCR amplification. Decreased amplification is usually caused by highly degraded DNA, the presence of PCR inhibitors, insufficient DNA (quantity), or any combination of these factors.

DNA Extraction Methods

Many DNA extraction procedures—including phenol-chloroform, Chelex®, and FTA™ paper—are currently in use. Regardless of which method you use, handle all samples carefully to prevent sample-to-sample contamination or contamination by extraneous DNA. When possible, process evidence samples separately from reference samples.

Phenol-Chloroform Method

This method removes proteins and other cellular components from nucleic acids, resulting in relatively pure DNA preparations. Double-stranded DNA extracted by this method is suitable for use with AmpFℓSTR YFiler kit amplifications, provided it is not significantly degraded.

The phenol-chloroform method is often used for extracting DNA from large samples, when the amount of DNA is expected to exceed 100 ng.

Chelex Method

The Chelex method of DNA extraction involves fewer steps than the phenol-chloroform method and consequently results in fewer opportunities for cross-sample contamination. The single-stranded DNA extracted by this method is suitable for AmpFℓSTR YFiler kit amplification.

FTA Paper Extraction

The FTA paper extraction process begins as soon as blood is spotted on FTA paper. Upon spotting, cells are lysed and DNA is immobilized within the paper matrix. DNA is then purified by a series of washes, after which the DNA is ready for PCR amplification. Refer to Figure 3-1 on page 3-10 for AmpFℓSTR Yfiler kit results from a 1.2-mm FTA bloodstain punch.

Sample Storage and Chain of Custody

The proper storage of samples and DNA specimens is essential to ensure that the DNA profiles obtained are accurate and meaningful.

Additionally, the proper chain of custody is vital to maintaining the integrity of each particular specimen.

Quantifying DNA

Importance of Quantitation

By quantifying the DNA in a sample, you ensure that there is enough DNA for adequate amplification. You can determine the smallest volume necessary to obtain 0.5 to 1.0 ng of DNA.

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

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

  Off-scale data is a problem for two reasons:

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

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

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. Detailed information about how the kits work, kit specificity and sensitivity, and other frequently asked questions are provided in the cited references.

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. • Able to detect 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)
QuantiBlot® Human DNA Quantitation Kit (PN N808-0114)	**Properties:** • High specificity for human DNA • Detects single-stranded or degraded DNA **How it works:** A biotinylated probe specific for the human D17Z1 sequence is hybridized to sample DNA that has been immobilized via slot blot onto a nylon membrane. The subsequent binding of horseradish peroxidase/streptavidin enzyme conjugate (HRP-SA) to the bound probe allows for either colorimetric or chemiluminescent detection.	*QuantiBlot Human DNA Quantitation Kit Product Insert*[a] *QuantiBlot® Human DNA Quantitation Kit: Human Identification: Product Bulletin* (PN 112PB04-02)

a. Contact your Applied Biosystems sales representative to obtain a copy of the product insert.

Chapter 3

PCR Amplification

PCR Amplification 3

This chapter covers:

PCR Work Areas

Work Area Setup and Lab Design

Many resources are available for the appropriate design of a PCR laboratory.

- If you are using the Yfiler kit for forensic DNA testing, refer to "Forensic Laboratories: Handbook for Facility Planning, Design, Construction and Moving," National Institute of Justice, 1998 (http://www.ojp.usdoj.gov/nij/scidocs.htm).
- If you are using the Yfiler kit for parentage DNA testing, refer to the "Guidance for Standards for Parentage Testing Laboratories," American Association of Blood Banks, 6th edition, 2004.

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

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

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

PCR Setup Work Area

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

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

Amplified DNA Work Area

IMPORTANT! The GeneAmp® PCR Systems should be placed in the Amplified DNA Work Area.

You can use the following systems:

- 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 Yfiler™ kit contains materials sufficient to perform 100 reactions at a 25-µL reaction volume

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

Table 3-1 Yfiler kit contents

Reagent	Contents	Quantity	Storage
AmpFℓSTR Yfiler Primer Set	Forward and reverse primers to amplify human male DNA target	1 tube, 0.55 mL	2 to 8 °C
AmpFℓSTR Yfiler PCR Reaction Mix	$MgCl_2$, dNTPs, and bovine serum albumin in buffer with 0.05% sodium azide	1 tube, 1.1 mL/tube	2 to 8 °C
AmpFℓSTR Yfiler Allelic Ladder	Allelic ladder containing amplified alleles (refer to "Loci Amplified by the Kit" on page 1-2 for a list of alleles included in the ladder)	1 tube, 50 µL	2 to 8 °C
AmpFℓSTR Control DNA 007	0.10 ng/µL human male genomic DNA in 0.05% sodium azide and buffer (refer to "Loci Amplified by the Kit" on page 1-2 for profile)	1 tube, 0.3 mL	2 to 8 °C
AmpliTaq Gold® DNA Polymerase	DNA polymerase, 5 U/µL	2 tubes, 50 µL/tube	–15 to –25 °C
AmpFℓSTR Control DNA 9947A	10 ng/µL human female cell line DNA in 0.05 % sodium azide and buffer	1 tube, 25 µL	2 to 8 °C

User-supplied Reagents

In addition to the reagents supplied with the Yfiler kit, it is recommended that you use low TE buffer. 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 • 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.

Preparing the Reactions

To prepare the reactions:

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

Component	Volume Per Reaction (µL)
AmpFℓSTR Yfiler Kit PCR Reaction Mix	9.2
AmpFℓSTR Yfiler Kit Primer Set	5.0
AmpliTaq Gold® DNA Polymerase	0.8

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

2. Prepare the reagents:
   a. Thaw the PCR reaction mix and primer set, then vortex 3 to 5 seconds and centrifuge briefly before opening the tubes.
   b. Vortex the AmpliTaq Gold DNA Polymerase for 3 to 5 seconds and centrifuge briefly.

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

3. Pipette the required volumes of components into an appropriately sized polypropylene tube.
4. Vortex the PCR master mix for 3 to 5 seconds, then centrifuge briefly.
5. Dispense 15 µL of the PCR master mix into each reaction well.

To prepare the reactions: ***(continued)***

6.	Add 10 µL of sample or control (0.1ng/µL) to the appropriate wells using MicroAmp® Reaction Tubes or a MicroAmp® Optical 96-Well Reaction Plate. The final reaction volume should be 25 µL.
7.	Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders to remove any bubbles. **Note:** If a tabletop centrifuge with 96-well plate adapters is not available, you can omit this step.
8.	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 (30 cycles)			Final Extension	Final Hold
	De-nature	Anneal	Extend		
HOLD	CYCLE			HOLD	HOLD
95 °C 11 min	94 °C 1 min	61 °C 1 min	72 °C 1 min	60 °C 80 min	4 °C ∞

2. Load the plate into the thermal cycler.

   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. 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. Before performing the procedure, keep hands away until the heated cover and sample block reach room temperature.

4. Start the run.

To run PCR: ***(continued)***

5. Store the amplified DNA.

If you are storing the DNA...	Then place 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. Our 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 27 cycles. It is recommended that each laboratory determine the cycle number based upon individual validation studies.

In the example shown in Figure 3-1, a 1.2-mm punch of a bloodstained FTA card was purified using three washes with FTA Purification Reagent and two washes with 1X TE buffer. After drying at room temperature overnight, the punch was then amplified directly in the MicroAmp® tube for 27 cycles.

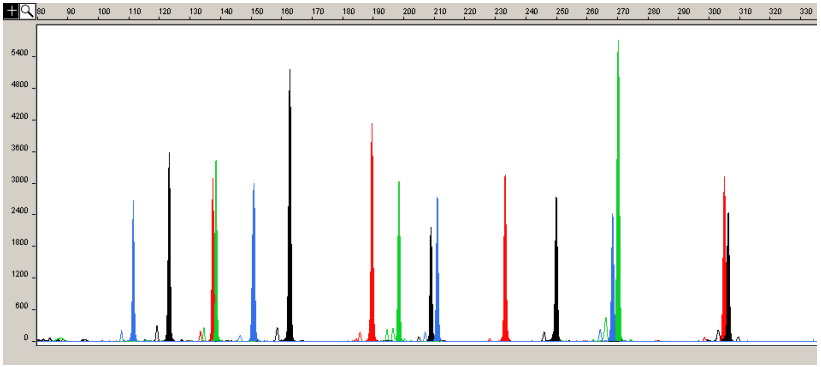


Figure 3-1 AmpFlSTR Yfiler kit results from a 1.2-mm FTA bloodstain punch (27 cycle amplification), analyzed on the ABI PRISM 3100 Genetic Analyzer.

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Chapter 4

Electrophoresis

Performing Electrophoresis 4

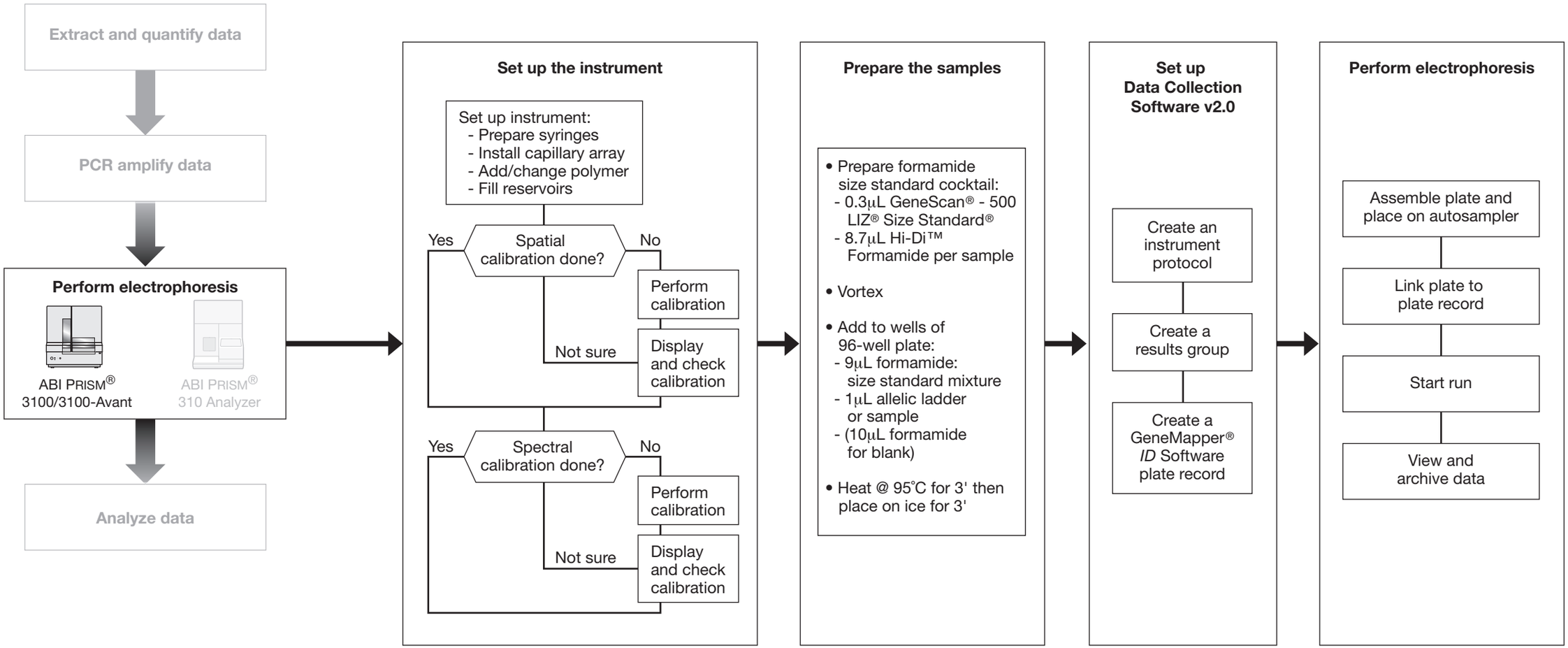
This chapter covers:

Section 4.1 ABI PRISM 3100/3100-*Avant* Genetic Analyzer Setup

This section covers:

Overview

Flowchart



3100/3100-*Avant* Analyzer Quick Reference Table

The following table provides information users familiar with the 3100/3100-*Avant* instruments can utilize to get started analyzing samples from Yfiler™ kits.

Condition	Setting
Dye Set	Matrix Standard Set, DS-33 (PN 4345833)
Filter Set	G5vb
Size Standard	GeneScan™ 500 LIZ® Size Standard (PN 4322682)
Run Module	• For Data Collection Software v2.0: HIDFragmentAnalysis36_POP4_1 • For Data Collection Software v1.1 (3100 instrument): GeneScan36vb_POP4DyeSetG5Module • For Data Collection Software v1.0 (3100-*Avant* instrument): GeneScan36Avb_POP4DyeSetG5Module **Note:** Before the first run on 3100/3100-Avant instruments running Data Collection Software v1.1 or v1.0, you must edit the default module parameters. Refer to page 34 of *ABI PRISM® 3100 and 3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products* (PN 4332345).
Analysis Module	G500Analysis.gsp
Polymer	3100 Performance Optimized Polymer 4 (POP4) (7 mL) (PN 4316355)
Capillary Array	3100 Capillary Array (PN 4315931) 36-cm 3100-*Avant* Capillary Array (PN 4333464)
Running Buffer	10× Genetic Analyzer Buffer with EDTA (25 mL) (PN 402824)

Before You Begin

Before using the instrument, use the following checklist to determine if regular maintenance tasks have been performed for the 3100/3100-*Avant* instrument.

	Condition	Task
❒	Have the syringes been replaced within the last three months?	Replace the syringes, as described in Chapter 1 of the *ABI PRISM 3100/3100-Avant Genetic Analyzers User Guide*.
❒	Have the capillary arrays been replaced within the last 100 runs?	Replace the capillary arrays, as described in Chapter 7 of the *ABI PRISM 3100/3100-Avant Genetic Analyzers User Guide*. New capillary arrays may be required if you noticed the following conditions in a previous run: • Poor sizing precision or allele calling • Poor resolution and/or decreased signal intensity
❒	Have you replenished the electrophoresis reagents?	Replenish the reagents, as described in Chapter 1 of the *ABI PRISM 3100/3100-Avant Genetic Analyzers User Guide* (PN 4347102).
❒	Have you performed a spatial calibration?	Perform a spatial calibration each time you: • Install or replace a capillary • Temporarily remove the capillary array from the detection block Refer to Chapter 2 of the *ABI PRISM 3100/3100-Avant Genetic Analyzers User Guide* for information about performing spatial calibration.
❒	Have you performed a spectral calibration?	Perform a spectral calibration. A spectral calibration creates a matrix to correct for the overlapping of fluorescence emission spectra of the dyes. See "Performing a Spectral Calibration" on page 4-9 for information.

Setting-up the 3100/3100-*Avant* Instrument

Kit Contents and Storage

Each Yfiler™ kit contains materials sufficient to perform 100 reactions at a 25-µL reaction volume

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

Table 4-1 User-supplied Materials

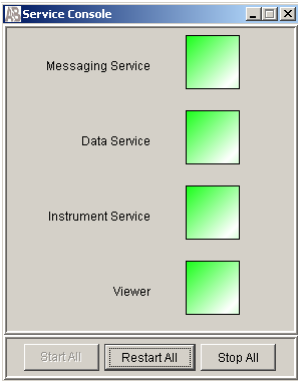
Material	Source
AmpFℓSTR® Yfiler™ PCR Amplification Kit	4359513
3100/3100-Avant Analyzer materials	
96-Well Plate Septa	4315933
3100 Capillary Array, 36-cm	4315931
36-cm 3100-Avant Capillary Array	4333464
3100 Performance Optimized Polymer 4 (POP-4™)	4316355
Autosampler 96-well Plate Kit	4316471
GeneScan™ 500 LIZ® Size Standard	4322682
10× Genetic Analyzer Buffer with EDTA	402824
DS-33 (Dye Set G5) Matrix Standard, Kit for 3100/3100-*Avant* analyzers	4345833
MicroAmp® Optical 96-Well Reaction Plate	N801-0560
3100 Instrument Consumable Reservoir Septa	4315932
Array-fill syringe, 250-µL glass syringe	4304470
Polymer-reserve syringe, 5.0-mL glass 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).	

Setting-up the 3100/3100-*Avant* Instrument

Following is a summary of the tasks involved in setting up the 3100/3100-*Avant* instrument for use with Data Collection Software v2.0. For detailed information about these tasks, refer to *ABI PRISM® 3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0 User Bulletin* (PN 4350218).

For information about setting-up the 3100/3100-Avant instrument for use with other Data Collection Software versions, refer to the appropriate user manuals. "Related Documentation" on page xiii provides a list of related documentation.

To set up the 3100/3100-*Avant* instrument:

1.	Power on the computer. The OrbixWeb™ Daemon software automatically launches.
2.	Power on the 3100/3100-*Avant* instrument; wait for solid green light.
3.	Select **Start > Programs > Applied Biosystems > Data Collection > Run Data Collection 3100 v2.0** or **Run Data Collection 3100-*Avant* v2.0**. The Service Console opens; when all the applications are running, the Data Collection Viewer window opens. 
4.	Fill the reservoirs and place them on the autosampler.
5.	Install or replace the capillary array, if necessary.
6.	Install the polymer blocks, if necessary.
7.	Add or change the polymer, if necessary.

To set up the 3100/3100-*Avant* instrument: *(continued)*

8.	Prepare the syringes.
9.	Perform spatial and spectral calibration, if necessary.

Performing a Spectral Calibration

A spectral calibration creates a matrix (or spectral) to correct for the overlapping of fluorescence emission spectra of the dyes. Data collection software applies this spectral to raw data during the multicomponenting process.

Perform a spectral calibration

- When you use a new dye set on the instrument
- When you change the capillary array length or polymer type
- After the laser or CCD camera has been realigned by a service engineer
- If you begin to see a decrease in spectral separation (“pull-up” and/or “pull-down” peaks)

For more information about performing spectral calibrations, refer to *ABI PRISM® 3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0 User Bulletin* (PN 4350218).

For information about performing spectral calibrations when using other Data Collection Software versions, refer to the appropriate user manual. “Related Documentation” on page xiii provides a list of related documentation.

To perform a spectral calibration for 3100/3100-*Avant* Data Collection Software v2.0:

1. Prepare the spectral calibration (matrix) standards for Dye Set G5.
   a. Thaw and thoroughly mix the contents of the Matrix Standard Set DS-33 tube, then spin briefly in a microcentrifuge.
   b. Combine the following in a labeled 1.5-mL microcentrifuge tube:

Reagent	Volume (µL) 3100 Analyzer	Volume (µL) 3100-*Avant* Analyzer
Matrix Standard Set DS-33	5	2
Hi-Di™ Formamide	195	78
Final Volume	**200**	**80**

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.

   c. Vortex thoroughly to mix, then spin briefly in a microcentrifuge.
   d. Heat the tube at 95 °C for 3 min to denature the DNA, then
   e. Immediately place the tube on ice for 3 min.

To perform a spectral calibration for 3100/3100-*Avant* Data Collection Software v2.0: *(continued)*

2. Dispense the appropriate amount of denatured standard into the wells of a reaction plate.

Instrument	Plate Type	Volume	Wells
3100	96-Well	10 µL	A1 to H2
3100-*Avant*	96-Well	10 µL	A1, B1, C1, D1

3. Prepare the plate assembly, and place the plate assembly onto the autosampler.

4. Create a spectral instrument protocol.

   a. In the Tree pane of the Data Collection Software v2.0, click **GA Instruments > ga3100** or **ga3100-*Avant* > Protocol Manager**.

   b. In the Instrument Protocols pane, click **New** to open the Protocol Editor dialog box.

   c. Complete the Protocol Editor dialog box:
      - Type: **Spectral**
      - Dye Set: **G5**
      - Polymer: **POP4**
      - Array Length: **36-cm**
      - Chemistry: **Matrix Standard**
      - Run Module: **Spect36_POP4_**1

      Note: If using DC v1.1 or 1.0, select **Spect36vb_POP4_DefaultModule** as the run module. For more information about performing spectral calibrations for Data Collection Software v1.1 or 1.0, refer to *ABI PRISM® 3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products User Bulletin*(PN 4332345).

   d. Click **OK** to save the protocol.

To perform a spectral calibration for 3100/3100-*Avant* Data Collection Software v2.0: *(continued)*

5.	Create a plate record. a. In the Tree pane of the Data Collection Software v2.0, click **GA Instruments > ga3100** or **ga3100-*Avant* > *instrument name* > Run Scheduler**. b. In the Run Scheduler view, enter a new plate name in the Scan or Type Plate ID field, then click **Search**. c. In the Create new plate dialog box, click **Yes**. d. Complete the New Plate Dialog box: – Name: <Enter a name for the plate.> – Application: **Spectral Calibration** – Plate Type: **96-Well** – Owner Name: <Enter a name for the owner.> – Operator Name: <Enter a name for the operator.> e. Click **OK**.
6.	In the Spectral Calibration Plate Editor dialog box, enter the following information: a. In the Sample Name column of row A, enter a sample name, then click the next cell. The value 100 is automatically displayed in the Priority column. b. In the Comments column of row A, enter any additional comments or notations for the sample at the corresponding position of the plate. c. In the Instrument Protocol 1 column of row A, select the protocol you created in step 4 on page 4-11. d. Highlight the entire row (row A). e. Select **Edit > Fill Down Special**. The software automatically fills in the appropriate well numbers for a single run. f. Click **OK**.
7.	Link your reaction plate and start the run.
8.	View the status after the run.

To perform a spectral calibration for 3100/3100-*Avant* Data Collection Software v2.0: *(continued)*

9.	Review and evaluate the spectral calibration profile for each capillary.

For more information about performing spectral calibrations, refer to *ABI PRISM® 3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0 User Bulletin* (PN 4350218) or Chapter 3 of the *ABI PRISM 3100/3100-Avant Genetic Analyzer User Guide* (PN 4347102).

Preparing Samples for Electrophoresis

Required Materials

Refer to "Kit Contents and Storage" on page 4-7 for a list of materials.

Preparing the Samples

Prepare the samples for electrophoresis immediately prior to 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 (µL) per reaction
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 amount 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 min at 95 °C.
7.	Immediately place the plate on ice for 3 min.

Setting Up the Electrophoresis Run

Compatible Data Collection and Analysis Software

The following table lists data collection and analysis software that you can use to analyze YFiler data.

Operating System	Data Collection Software	Analysis Software	References
Windows NT[a]	1.1 (3100 Analyzer) 1.0 (3100-*Avant* Analyzer)	GeneMapper® *ID* 3.2 GeneScan 3.7.1 + GenoTyper 3.7	*ABI PRISM 3100 Genetic Analyzer User Manual* (Data Collection Software v1.1) (PN 4315834) *ABI PRISM® 3100-Avant Genetic Analyzer User Guide* (Data Collection Software v1.0) (PN 4333549) *ABI PRISM® 3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFlSTR® PCR Amplification Kit PCR Products User Bulletin*(PN 4332345)
Windows 2000[a]	2.0	GeneMapper *ID* 3.2	This section

a. Applied Biosystems conducted validation studies for Yfiler using these configurations.

Software Setup Summary

Setting up the Data Collection Software v2.0 involves the following three tasks:

- "Creating an Instrument Protocol" on page 4-17
- "Creating a Results Group" on page 4-17
- "Creating a GeneMapper ID Software Plate Record for Autoanalysis" on page 4-21

Note: Before the first run on 3100/3100-Avant instruments running Data Collection Software v1.1 or v1.0, you must edit the default module parameters. Refer to page 34 of *ABI PRISM® 3100 and 3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products* (PN 4332345).

Creating an Instrument Protocol

You must create an instrument protocol before the first run for any AmpFℓSTR PCR Amplification Kit. You can use the same instrument protocol for all subsequent runs.

To create an instrument protocol:

1.	In the Tree pane of Data Collection Software v2.0, click **GA Instruments > ga3100** or **ga3100-*Avant* > Protocol Manager**.
2.	In the Instrument Protocol pane, click **New** to open the Protocol Editor dialog box.
3.	Complete the Protocol Editor dialog box: a. Enter a name for the protocol. b. Optional: Enter a description for the protocol. c. In the Type drop-down list, select **REGULAR**. d. In the Run Module drop-down list, select **HIDFragmentAnalysis36_POP4_1**. e. In the Dye Set drop-down list, select **G5**.
4.	Click **OK**.

Creating a Results Group

Results groups are used to analyze, name, sort, and deliver samples from a run. Typically, you modify the results group for each fragment analysis run.

To create a results group:

1.	In the Tree pane of Data Collection Software v2.0, click **GA Instruments > Results Group**.
2.	Click **New**. The Results Group Editor displays.
3.	Complete the General tab: a. Type a Results Group Name. The name can be used in naming and sorting sample files. It must be unique. b. Optional: Type a Results Group Owner. c. Optional: Type a Results Group Comment.

To create a results group: ***(continued)***

4. Select the **Analysis** tab, then:

   a. Select an Analysis Type.

If you select...	Then...
<None>	Only unanalyzed sample files are generated.
GeneMapper-Generic	Autoanalysis is not enabled and only.fsa files are generated.
GeneMapper-<*Instrument Name*>	Autoanalysis of completed runs is enabled.

Note: Step b below applies only to GeneMapper-<*Instrument Name*>.

   b. Select an Analysis Action.

If you select...	Then...	Use with Setting from Automated Processing tab (page 4-21)
Do Autoanalysis	Samples are analyzed after each run of 16 or 4 samples.	**When every run completes**
Do Autoanalysis and Results Entry Group Complete	Samples are analyzed after all samples using the same results group have been run.	**Only when the result group is complete**

   c. Type the GeneMapper *ID* software Login ID.

   d. Type the GeneMapper *ID* software login password.

The login ID and password are created in the GeneMapper *ID* software Options Users tab.

To create a results group: ***(continued)***

5. Select the **Destination** tab, then use the default destination or define a new location for data storage.

To use a...	Then...
default location	skip to step 6
custom location	complete steps a to c.

   a. Click **Use Custom Location**, then click **Browse** to navigate to a different location for saving.

   b. Click **Test** to test the Location path name connection:

      If it passes, a message box displays "Path Name test successful."

      If it fails, a message box displays "Could not make the connection. Please check that the Path Name is correct." click and try to establish a connection.

   c. Click **OK**.

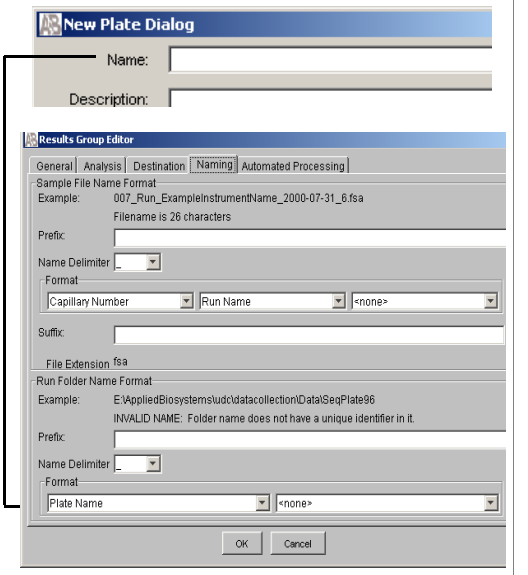
To create a results group: ***(continued)***

6. Select the **Naming** tab.

   Use the Naming table to customize the sample file and run folder names. For more information about sample-naming conventions, refer to Chapter 5 of the *ABI PRISM 3100/3100-Avant Genetic Analyzers User Guide* (PN 4347102).

   Typically, plates are grouped by plate name (by selecting **Plate Name** under Run Folder Name Format.) If you select "Plate Name", the software stores all sample files in one folder based on the plate name. The software uses the value for Name (in the New Plate Dialog box) when creating run folders.

   If you select Plate Name as a run folder naming parameter, the software uses the value of the Name field of the New Plate Dialog when creating run folders.



   IMPORTANT! Sample name, run folder name, and path name, combined, cannot exceed 250 characters.

   IMPORTANT! You must select at least one Format element for the Sample File and the Run Folder Name Formats in order to proceed within the Results Group.

   Note: The run folder is stored in the following path: Applied Biosystems\UDC \Data Collection\Data.

To create a results group: ***(continued)***

6. *(continued)*

   Note: If you choose elements from the Format lists that do not create unique Sample file or Run folder names, the following warning message appears below the Example line: INVALID NAME: Filename does not have a unique identifier in it.

   You can proceed to start a run without removing the warning message.

   If you want to remove the warning message, select an additional Format element that distinguishes one file from another (for example, the capillary number is unique while the instrument name is not).

7. Select the Automated Processing tab.

If you select...	Then...	Use with Setting from Analysis tab (page 4-18)
Only when the result group is complete	Samples are analyzed after all samples using the same results group have been run.	**Do Autoanalysis and Results Entry Group Complete**
When every run completes	Samples are analyzed after each run of 16 or 4 samples.	**Do Autoanalysis**

8. Click **OK** to save the Results Group.

Creating a GeneMapper *ID* Software Plate Record for Autoanalysis

Refer to *ABI PRISM® 3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0 User Bulletin* (PN 4350218) and Chapter 5 of the *ABI PRISM 3100/3100-Avant Genetic Analyzers User Guide* (PN 4347102) for more information about creating these files.

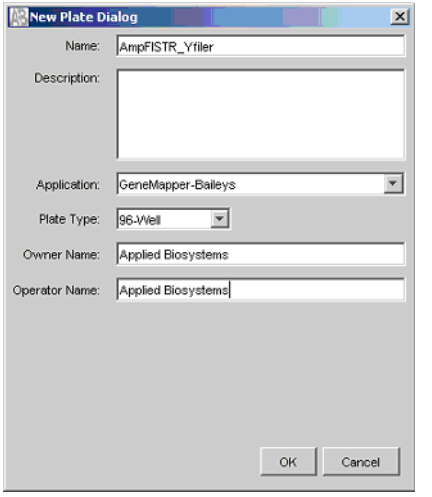
To set up data collection software for electrophoresis:

1. In the Tree pane of the Data Collection software, click **GA Instruments > ga 3100 or ga 3100-Avant > Plate Manager**.

To set up data collection software for electrophoresis: ***(continued)***

2. Complete the New Plate dialog box:
   a. Enter a name for the plate.
   b. (Optional) Enter a description for the plate record.
   c. In the Application drop-down list, select:
      – **GeneMapper-Generic** (for manual analysis) or
      – **GeneMapper-<*Instrument Name*>** (for autoanalysis)
   d. In the Plate Type drop-down list, select 96-Well.
   e. Enter a name for the owner.
   f. Enter a name for the operator.

   The following figure shows an example of a completed New Plate dialog box.



   g. Click **OK**.

   The GeneMapper Software Plate Editor opens.

To set up data collection software for electrophoresis: ***(continued)***

3. Complete the plate record.

Column	Value
Sample Name[a]	Enter a name for the sample.
Comment	Enter any additional comments or notations for the sample.
Priority	The value 100 is automatically displayed. Change the priority value, if desired.
Sample Type[b]	From the drop-down list, select a sample type that corresponds to the sample in that well.
Size Standard[b]	Select GS500LIZ.
Panel[b]	Select the AmpFℓSTR Yfiler panel from the drop-down list.
Analysis Method[b]	Select the appropriate analysis method from the drop-down list. Refer to "Creating HID Analysis Methods" on page 5-12.
SNP Set	Leave blank.
User-Defined columns 1 to 3	Enter any additional text, as necessary.
Results Group 1[a]	Select the Results Group that you created for the AmpFℓSTR Yfiler kit (see "Creating a Results Group" on page 4-17).
Instrument Protocol[a]	Select the Instrument Protocol that you created for the AmpFℓSTR Yfiler kit (see "Creating an Instrument Protocol" on page 4-17).

a. Required fields for both manual and autoanalysis.
b. Additional required fields for autoanalysis.

4. If you want to perform more than one run:
   a. Select **Edit > Add Sample Run**.
      Additional Results Group, Instrument Protocol, and Analysis Method columns are added to the right end of the plate record.
   b. Complete the columns for the additional runs.

To set up data collection software for electrophoresis: ***(continued)***

5.	Click **OK** to save, then close the plate record. **IMPORTANT!** After you click **OK** in the Plate Editor, the Data Collection Software stores the completed plate record in the Plate Manager database. Once the plate is in the Plate Manager database, you can search for, edit, delete, or export a plate record.

For additional information about setting up Data Collection software for electrophoresis, refer to the *ABI PRISM 3100/3100-Avant Genetic Analyzers User Guide* (PN 4347102).

Performing Electrophoresis

Preparing the Plate Assembly

To prepare the plate assembly:

1.	Place the reaction plate into the plate base provided with the instrument.
2.	Align the septa strip on the reaction plate.
3.	Snap the plate retainer onto the reaction plate and plate base.
4.	Verify that the holes of the plate retainer and septa strip are aligned.
5.	Place the plate assembly on the autosampler.

Running the Plate on the 3100/3100-*Avant* Instrument

To run the plate on the 3100/3100-*Avant* instrument:

1.	Search for your plate record. For more information about searching for plate records, refer to Chapter 6 of the *ABI PRISM 3100/3100-Avant Genetic Analyzer User Guide* (PN 4347102).

To run the plate on the 3100/3100-*Avant* instrument: ***(continued)***

2.	Select the plate record you want to run, then click the plate position indicator that corresponds to the plate you are linking. **Note:** The 3100-*Avant* instrument has only one plate position to link to a plate record. The plate position indicator changes from yellow to green when linked and the green run button is active.
3.	Verify that the active spectral calibration matches your dye set and capillary array length.
4.	Verify that the Autoanalysis Manager is running.
5.	Click the green run button, then click **OK** in the Processing Plates dialog box.

Viewing Run Data

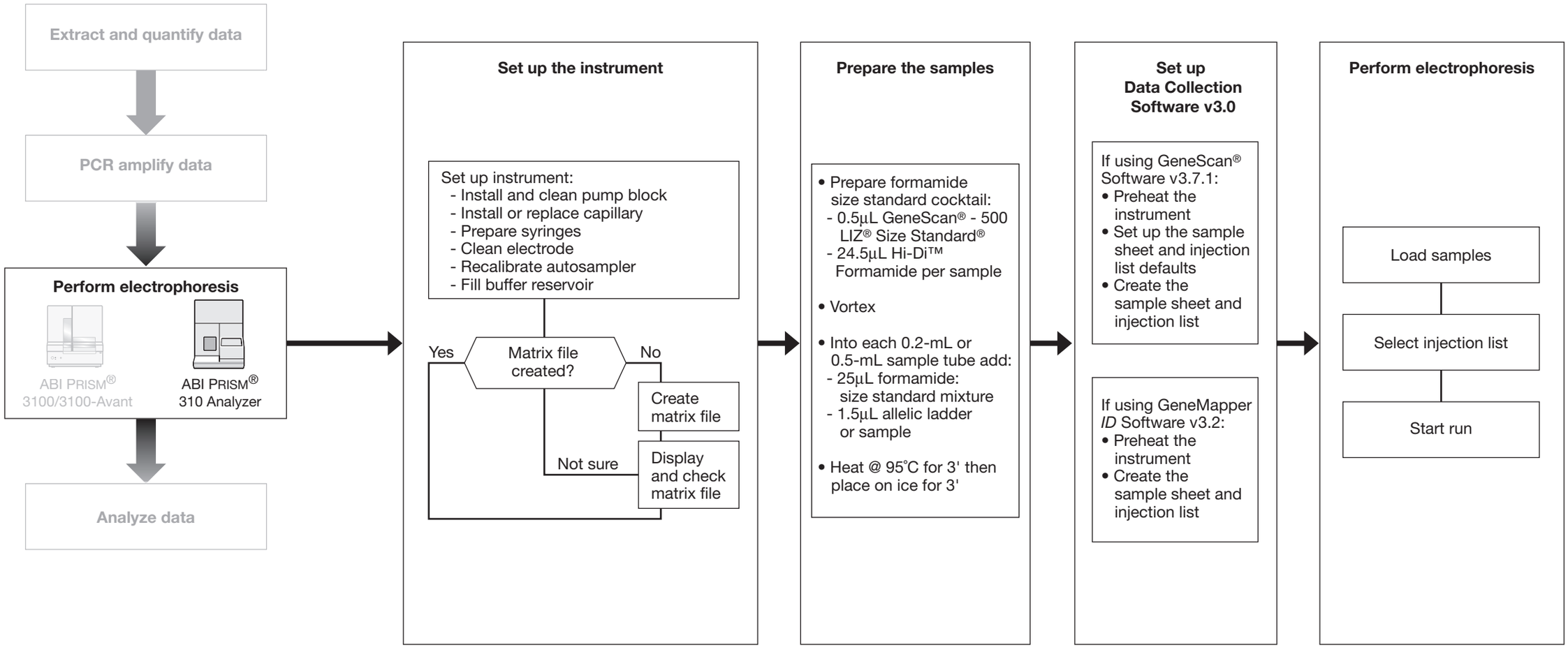
You can view data both during a run and after a run. Refer to *ABI PRISM® 3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0 User Bulletin* (PN 4350218) or Chapter 6 of the *ABI PRISM 3100/3100-Avant Genetic Analyzer User Guide* (PN 4347102) for more information about viewing run data.

Section 4.2 ABI PRISM 310 Genetic Analyzer Setup

This section covers:

Overview

Flowchart



310 Analyzer Quick Reference Tables

The following tables provide information that users familiar with the 310 instrument can use to get started analyzing samples from Yfiler™ Kit experiments.

Condition	Setting
Dye Set	DS-33 Matrix Standards (PN 4312131)
Filter Set	G5v2
Size Standard	GeneScan™ 500 LIZ® Size Standard (PN 4322682)
Run Module	GS STR POP4 (1mL)G5v2
Analysis Method	• If using GeneMapper *ID* software, create an analysis method as explained in "Creating HID Analysis Methods" on page 5-12 • If using GeneScan software: AnalyzeGSsample.bat
Polymer	3100 Performance Optimized Polymer 4 (POP4) (7 mL) (PN 4316355)
Capillary	310 Capillaries, 47cm x 50µm i.d. (internally uncoated) (PN 402839)
Running Buffer	10X Genetic Analyzer Buffer with EDTA (25 mL) (PN 402824)

Before You Begin

Before using the instrument, use the following checklist to determine if the 310 instrument is ready to use for electrophoresis.

	Condition	Task
❒	Have you checked the electrode?	The electrode must not be bent and must be clean. For more information refer to: • Chapter 3 of the *ABI PRISM 310 Genetic Analyzer User Guide* (PN 4317588) (for Windows) • Chapter 4 of the *ABI PRISM 310 Genetic Analyzer User Manual* (PN 903565) (for Macintosh)
❒	Have you cleaned the pump block?	The pump block must be clean. Refer to the appropriate user manual (listed above) for more information about cleaning the pump block.
❒	Has the capillary been replaced within the last 100 runs?	A new capillary may be required if you noticed the following conditions in a previous run: • Poor sizing precision or allele calling • Poor resolution and/or decreased signal intensity Refer to the appropriate user manual (listed above) for more information about replacing the capillary.
❒	Have you checked the syringe?	Two o-rings should be present, the ferrule firmly seated, and the syringe should be clean. For more information refer to the appropriate user manual (listed above).
❒	Have the syringes been replaced within the last three months?	Replace the syringes, as described in the appropriate user manual (listed above).
❒	Have you replenished the electrophoresis reagents?	Replenish the reagents, as described in the appropriate user manual (listed above).
❒	Do you have a valid matrix?	The 310 instrument uses a matrix file to correct for the overlapping of fluorescence emission spectra of the dyes. For information about creating a matrix file, refer to "Creating a Matrix File for the 310 Genetic Analyzer" on page 4-34 and Chapter 6 of *ABI PRISM GeneScan Analysis Software v3.7 for the Windows NT Platform* (PN 4308923).

Setting Up the 310 Genetic Analyzer

Kit Contents and Storage

Each Yfiler™ kit contains materials sufficient to perform 100 reactions at a 25-µL reaction volume

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

Table 4-2 User-supplied materials

Material	Source
310 Analyzer materials	
310 Capillaries, 47 cm × 50 µm i.d. (internally uncoated) (green)	402839
0.5 mL Sample Tray	5572
96-Well Tray Adaptor (for 9700 thermal cycler trays)	4305051
GeneScan™ 500 LIZ® Size Standard	4322682
10× Genetic Analyzer Buffer with EDTA	402824
Genetic Analyzer Retainer Clips (96-Tube Tray Septa Clips)	402866
Genetic Analysis Sample Tubes (0.5 mL)	401957
Genetic Analysis Septa for 0.5 mL Sample Tubes	401956
Matrix Standard Set DS-33 (6FAM™, VIC®, NED™, PET®, and LIZ® dyes) for 310/377 systems	4318159
MicroAmp® 8-strip Reaction Tubes	N801-0580
MicroAmp® 96-Well Support Base (holds 0.2-mL reaction tubes)	N801-0531
MicroAmp® 96-Well Full Plate Cover	N801-0550
MicroAmp® 96-Well Tray/Retainer Sets	403081

Material	Source
POP-4™ Performance Optimized Polymer	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).	

Setting Up the 310 Instrument

Following is a summary of the tasks involved in setting up the 310 instrument. For detailed information about these tasks, refer to

- Chapter 3 of the *ABI PRISM 310 Genetic Analyzer User Guide* (PN 4317588) (for Windows)
- Chapter 4 of the *ABI PRISM 310 Genetic Analyzer User Manual* (PN 903565) (for Macintosh)

To set up the 310 instrument:

1.	Power on the computer and log on, power on the 310 instrument, then start the data collection software.
2.	If necessary: • install and clean the pump block • install or replace the capillary • prepare the syringes • clean the electrode • recalibrate the autosampler
3.	Prime the pump block.
4.	Fill the buffer reservoirs.
5.	If necessary, create a matrix file.

Installing the G5V2 Run Module

The G5v2 run module is required for the 310 instrument to analyze information from Yfiler kits.

To install the G5v2 run module:

1.	Close all windows and applications.
2.	Insert the G5v2 Module Software CD (PN 4339037) into the computer CD-ROM drive. Alternatively, you can download the module file from **https://www.appliedbiosystems.com/support/software/310/modules.cfm**
3.	Navigate to the 310 Data Collection Modules folder. The default path is D:\AppliedBio\310\Modules.

To install the G5v2 run module: ***(continued)***

4.	Copy **GS STR POP4 (1 mL) G5v2.md5** into the Modules folder.
5.	Close all files, then eject the CD.

Creating a Matrix File for the 310 Genetic Analyzer

Overview

The precise spectral overlap between the five dyes is measured by analyzing DNA fragments labeled with each of the dyes (6-FAM™, VIC®, NED™, PET®, and LIZ®) in separate injections on a capillary. These dye-labeled DNA fragments are called matrix standard samples.

Instruments that do not perform multicomponenting, such as the 310 and 377 instruments, the analysis software (GeneMapper *ID* or GeneScan) analyzes the data from each of the five dye samples and creates a matrix file.

The matrix file contains information about normalized fluorescence intensities that represent a mathematical description of multicomponent overlap that is observed between the five dyes.

Because matrix file values vary between different instruments and between different virtual filter sets and run conditions on a single instrument, you must create a matrix file for each instrument and for a particular set of run conditions.

You can apply the appropriate matrix file to data on subsequent runs on the same instrument, as long as the electrophoresis conditions are constant from run to run.

Creating a Matrix File (DC v3.0)

To create a matrix file for the 310 instrument (Data Collection Software v3.0):

1. Prepare the matrix standards for Dye Set G5.
   a. Thaw and thoroughly mix the contents of the five-color matrix tubes, then spin briefly in a microcentrifuge.
   b. For each matrix standard, combine:
      – 1 µL of standard
      – 12 µL Hi-Di Formamide in labeled 0.5 mL Genetic Analyzer Sample tubes

   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.

   IMPORTANT! Do not include the GeneScan™ 500 LIZ® Size Standard when preparing matrix standards.

   c. Vortex thoroughly to mix, then spin briefly in a microcentrifuge.
   d. Heat the tube at 95 °C for 5 min to denature the DNA.
   e. Immediately place the tube on ice for 3 min.
   f. Place the tubes in the appropriate sample tray.

2. Set up the run.
   a. In the Data Collection Software, select **File >New**, then click **GeneScan Smpl Sheet 48 Tube** or **GeneScan Smpl Sheet 96 Tube**, as appropriate.
   b. Complete the sample sheet, then save and close it.
   c. Select **File > New**, then click **GeneScan Injection List**.
   d. Complete the injection list, making sure to select
      – the sample sheet you set up in step b.
      – Module: **GS STR POP4 (1mL) G5v2**
      – Matrix File: **none**

3. Click **Run** to run the matrix samples.

To create a matrix file for the 310 instrument (Data Collection Software v3.0): *(continued)*

4.	Create the matrix file using GeneMapper *ID* or GeneScan analysis software. *If you are using GeneMapper ID analysis software:* a. Navigate to the GeneMapper Manager and click the **Matrices** tab. b. Click the **New** tab and the Matrix editor is displayed. c. In the Matrix editor, enter the appropriate values. d. Click **Create** and the values are displayed in the Matrix Result table. e. Click **OK**. *If you are using GeneScan analysis software*: a. Select **File > New**, then click the **Matrix** icon. b. In the Make New Matrix dialog box, – Indicate the number of dyes by selecting **5** in the Number of Dyes dropdown. – Select the sample file that corresponds to each dye by clicking a button (B, G, Y, R, or O), then selecting the appropriate sample file. – Enter the starting point for each file. The Start At point should be after the primer peak. c. Click **OK**. A successful matrix opens an untitled Matrix Values window with a 5x5 matrix of numerical values.
5.	Use the **Save As** command to name and save the matrix file. • Choose a name that reflects the chemistry and run conditions. • Save the matrix file in the ABI folder: `D:\AppliedBio\Shared\Analysis\SizeCaller\Matrix`

Verifying the Accuracy of the Matrix File

To verify the accuracy of the matrix file:

1.	Apply the new matrix file to the Matrix Standard Sample Files as follows: a. In the **Analysis Control** window, highlight the **Sample File** column by clicking in the **Sample File** title row. b. Select **Sample** > **Install New Matrix**. c. Select the new matrix file (located in the ABI folder in the System folder), and click **Open**.
2.	Analyze the matrix standard samples as follows: a. Select **Settings** > **Analysis Parameters**, and verify that the settings are correct. b. In the **Analysis Control** window, select all five colors in each sample row for all of the matrix standard samples. c. Click the **Analyze** button.
3.	In the **Results Control** window, examine the results for all five colors for each of the matrix standard samples. For example, the 6-FAM dye matrix standard results should have peaks for blue. Evaluate the baseline. A pattern of pronounced peaks or dips in any of the other four colors indicates that the color separation may not be optimal.

If this verification test does not pass, then the capillary may not have been aligned properly in the instrument during the run. To correct this problem:

a. Tape the capillary to the heat plate so that the capillary is immobilized during the run.

b. Repeat the experiment, making sure that the capillary is placed carefully in the laser detection window.

Once a satisfactory matrix file has been made, this matrix file can be applied to subsequent runs. It is not necessary to run matrix standard samples for each new capillary.

Setting Up the Electrophoresis Run

Compatible Data Collection and Analysis Software

The following table lists data collection and analysis software that you can use to analyze YFiler Kit data.

Operating System	Data Collection Software	Analysis Software	References
Windows 2000	3.0	• GeneMapper® *ID* 3.2 • GeneScan 3.7.1 + GenoTyper 3.7	"Setup for Data Collection Software 3.0 with GeneMapper ID Software" on page 4-43 "Setup for Data Collection Software 3.0 with GeneScan Software" on page 4-38
Macintosh OS 9.0	2.1	• GeneMapper *ID* 3.2 • GeneScan 3.1.2 + GenoTyper 2.5.2	The *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (PN 4338775) Chapter 3 of the *AmpFℓSTR® Identifiler™ PCR Amplification Kit User's Manual* (PN 4323291)

Setup for Data Collection Software 3.0 with GeneScan Software

Overview

Setting up the electrophoresis run involves three tasks:

1. Preheating the instrument (optional)
2. Setting Up GeneScan Sample Sheet and Injection List Defaults
3. Creating a Sample Sheet and Injection List for the Run

Preheating the Instrument

Setting the run temperature prior to starting a run is optional; this heating step occurs automatically at the beginning of the GS STR POP4 (1 mL) G5v2 run module. However, preheating the instrument prior to a run saves time.

To preheat the instrument to the run temperature:

1.	Make sure the instrument doors are closed.
2.	Launch the Data Collection software.
3.	Set the temperature: a. Select **Window > Manual Control**. b. Select **Temperature Set** from the popup menu. c. Set the temperature to 60 °C. d. Click **Execute**. **Note:** It takes up to 30 min for the instrument to reach the 60 °C run temperature.

Setting GeneScan Software Sample Sheet and Injection List Defaults

When you create a new sample sheet, the data collection software automatically fills in portions of the sample sheet based on settings specified in the Preferences dialog. This section provides information on changing the default preferences.

To set sample sheet and injection list defaults:

1.	If necessary, launch the Data Collection Software.
2.	Select **Windows > Preferences**.
3.	In the Preferences dialog box, select **GeneScan Injection Sample Sheet Defaults**.
4.	Set the size standard color for 5-Dye to orange (O).
5.	In the Preferences dialog box, select **GeneScan Injection List Defaults**. The software displays the default settings, as shown in the following figure.

To set sample sheet and injection list defaults: *(continued)*

6. Select the following settings:

Setting	Value
5-Dye Module	**GS STR POP4 (1 mL) G5v2**
Matrix File	Select the appropriate matrix file. **Note:** A valid matrix file, created using DS-33 and Filter Set G5v2 module, must be available in the GeneScan/GSMatrix folder.
Autoanalyze With	• Select **AnalyzeGSSample.bat** if you want to send data automatically to GeneScan software. **Note:** You can select any saved Analysis Parameters file.AnalyzeGSSample.bat contains typical analysis parameter settings. For more information about setting analysis parameters, see "Defining Analysis Parameters" on page 5-27. • Select **none** if you do not want to use the autoanalysis feature.
Size Standard	Assign the appropriate size standard.

7. Click **OK** to save your changes.

Creating a Sample Sheet and Injection List for the Run

You can prepare the sample sheet at any time before the preparation of samples and save it in the Sample Sheet folder for later use.

To create a sample sheet and injection list:

1. If necessary, launch the Data Collection Software.
2. Select **File > New**, then click  **(GeneScan Sample Sheet)**.

To create a sample sheet and injection list: ***(continued)***

3. Complete the sample sheet.

Column	Value
Sample Name	Enter a name for the sample. This column indicates which sample is in which tube of the sample tray.
Standard	Click in the column beside O. The software displays a diamond to indicate that orange is the size standard.
Sample Info	Copy the information from the Sample Name column. TIP: Complete the Sample Name column, then copy and paste the information into the Sample Info column.

4. Click **File > Save As** to save the sample sheet in the Sample Sheets folder.

5. Select **File > New**, then click  **(GeneScan Injection List)**.

6. Select the appropriate sample sheet from the Sample Sheet popup menu (at the top left of the Injection List window).

To create a sample sheet and injection list: *(continued)*

7. For autoanalysis, complete the following information:

Setting	Value
Analysis Parameters	Select **Module > Module GS STR POP4 (1 mL) G5** for every injection.
Matrix	Select the matrix file for the injections from the Matrix file popup menu. **IMPORTANT!** Use only matrix files created using DS-33 (6-FAM, VIC, NED, PET, and LIZ dyes) and the Filter Set G5v2 module. To perform autoanalysis, this matrix file must be located in the GeneScan\GSMatrix folder.
Size Standard	Select the size standard from the Size Standard popup menu.

Note: If you edited the default sample sheet and injection list defaults (as described in "Setting GeneScan Software Sample Sheet and Injection List Defaults" on page 4-39), you do not need to perform this step.

Note: If you are performing a manual analysis, you only need to complete the run module field.

8. Save the injection list. By default, the injection list is saved in the Run folder.

Setup for Data Collection Software 3.0 with GeneMapper *ID* Software

Overview

Setting up the electrophoresis run involves three tasks:

1. Preheating the instrument (optional)
2. Creating a Sample Sheet and Injection List for the Run

Preheating the Instrument

Preheat the instrument as described in "Preheating the Instrument" on page 4-38.

Creating a Sample Sheet and Injection List for the Run

You can prepare the sample sheet at any time before the preparation of samples and save it in the Sample Sheet folder for later use.

To create a sample sheet and injection list:

1. If necessary, launch the Data Collection Software.
2. Select **File > New**, then click  **(GeneScan Sample Sheet)**.
3. Complete the sample sheet.

Column	Value
Sample Name	Enter a name for the sample. This column indicates which sample is in which tube of the sample tray.
Standard	Click in the column beside O. The software displays a diamond to indicate that orange is the size standard.
Sample Info	Copy the information from the Sample Name column. TIP: Complete the Sample Name column, then copy and paste the information into the Sample Info column.

To create a sample sheet and injection list: ***(continued)***

4.	Click **File > Save As** to save the sample sheet in the Sample Sheets folder.
5.	Select **File > New**, then click  **(GeneScan Injection List)**.
6.	Select the appropriate sample sheet from the Sample Sheet popup menu (at the top left of the Injection List window).
7.	Save the injection list. By default, the injection list is saved in the Run folder.

Preparing Samples for Electrophoresis

Required Materials

IMPORTANT! Refer to "Kit Contents and Storage" on page 4-31 for a list of materials.

Preparing the Samples

To prepare the 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 Internal 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 amount 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 the samples for electrophoresis: ***(continued)***

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
5.	Seal the tubes with the 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 tubes in a thermal cycler for 3 min at 95 °C.
7.	Immediately place the tubes on ice for 3 min.

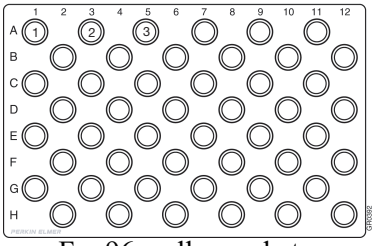
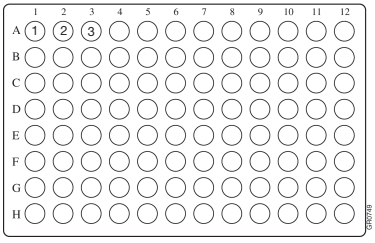
Performing Electrophoresis

Preparing the Plate Assembly and Loading the Samples

To load the samples:

1.	Open the instrument door and press the **Tray** button to present the autosampler.

To load the samples: ***(continued)***

2.	Load the sample tubes on a 48-well or 96-well sample tray, as shown in the following illustration: • For 48-well sample tray:  • For 96-well sample tray: 
3.	Place the sample tray on the autosampler.
4.	Press the **Tray** button to retract the autosampler.
5.	Close the instrument door.

Running the Samples on the 310 Instrument

To run the samples on the 310 instrument:

1.	If necessary, launch the data collection software.
2.	Open the GeneScan Software Injection list that you saved earlier.
3.	Click **Run**. If you did not preheat the instrument, the run module brings the instrument up to the 60 °C run temperature. This process can take up to 30 minutes. The run begins once the run temperature is reached.

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Chapter 5

Analyzing Data

Analyzing Data 5

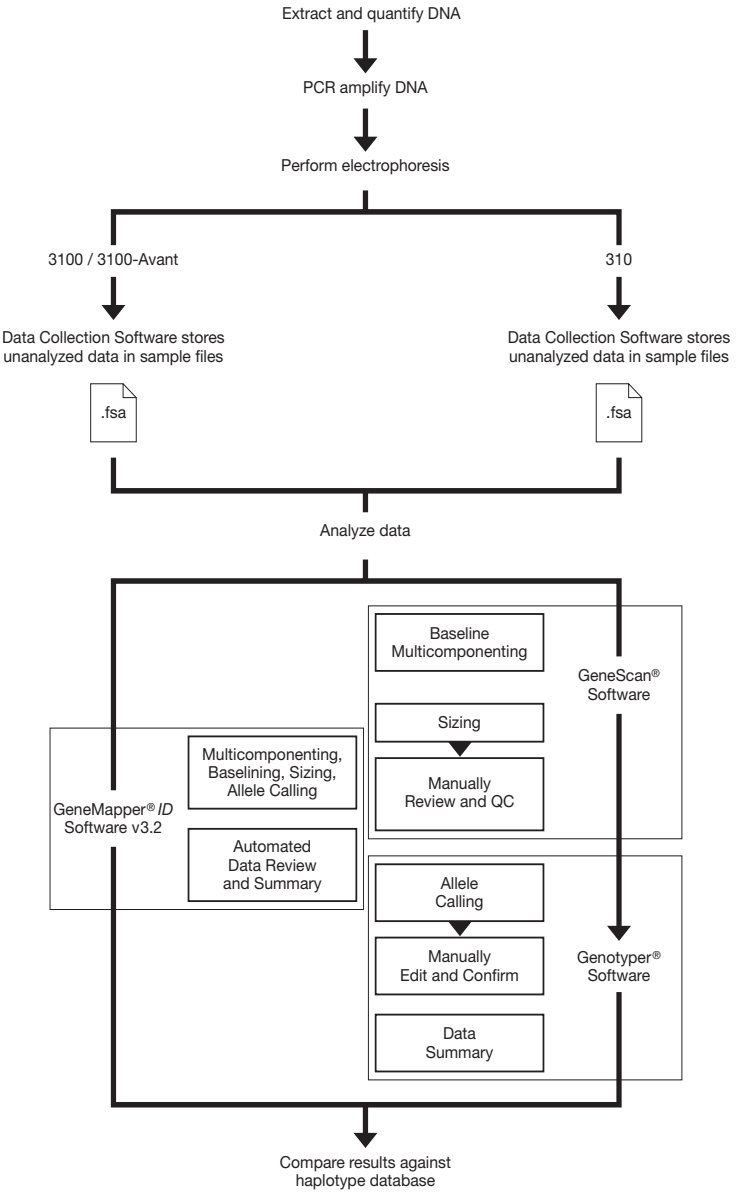
This chapter covers:

Section 5.1 Data Analysis Overview

This section covers:

Overview

After electrophoresis, the Data Collection software stores information for each sample in an.fsa file. Using analysis software, you can then analyze and interpret the data.



Section 5.2 Using GeneMapper *ID* Software v3.2 to Analyze AmpFℓSTR Yfiler Kit Data

This section covers:

Overview

What Does GeneMapper *ID* Software Do?

GeneMapper *ID* Software is an automated genotyping software solution for forensic, paternity, and database data analysis and other genotyping needs. The GeneMapper *ID* software combines GeneScan® Analysis Software and the Genotyper® Software functionality in a single, rules-based analysis package.

Instruments

Refer to "Instrument and Software Compatibility" on page 1-5 for a list of compatible instruments.

Before You Start

When using GeneMapper® *ID* Software version 3.2 to perform Human Identification (HID) analysis with AmpFℓSTR® kits, consider the following:

- HID analysis requires the presence of 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 a single run folder are considered to be from a single run.

  When the software imports multiple run folders into a project, only ladders within a single run folder are used for calculating allelic bin offsets and subsequent genotyping.
- Allelic ladder samples need to be identified 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_Yfiler panels in the Panel Manager.
- Lanes or injections containing the allelic ladder should be analyzed with the same analysis method and parameters used for samples.
- Alleles not found in the AmpFℓSTR® Allelic Ladders do exist. These off-ladder alleles may contain full and/or partial repeat units. An off-ladder allele is defined as an allele falling outside of the ±0.5-bp 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 should be rerun to verify the result.

Setting Up GeneMapper *ID* Software v3.2 for Analyzing AmpFℓSTR Yfiler Kit Data

Perform the following tasks before you analyze sample (.fsa) files for the first time:

1. Import panels and bins into the Panel Manager, as explained in "Importing Panels and Bins" on page 5-8.
2. Create an analysis method with the appropriate bin set option, as explained in "Creating HID Analysis Methods" on page 5-12.
3. Define custom views of analysis tables.

   Refer to Chapter 1 of the *GeneMapper® ID Software versions 3.1 and 3.2 Human Identification Analysis Tutorial* (PN 4335523) for more information.
4. Define custom views of plots.

   Refer to Chapter 1 of the *GeneMapper® ID Software versions 3.1 and 3.2 Human Identification Analysis Tutorial* (PN 4335523) for more information.
5. Optionally, view and set HID analysis options (in Options tab).

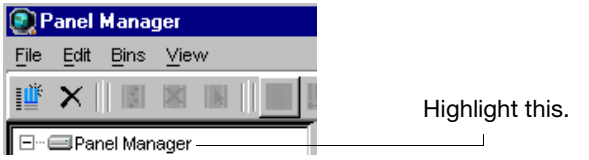
   Analysis options allow you to automatically set values for plate record fields. Refer to Chapter 1 of the *GeneMapper® ID Software versions 3.1 and 3.2 Human Identification Analysis Tutorial* (PN 4335523) for more information.
6. If necessary, convert any GeneScan software sample files generated on the 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).

Note: For more detailed information about GeneMapper 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). Refer to the *GeneMapper® ID Software version 3.2 Human Identification Analysis User* Bulletin (PN 4352543)

Importing Panels and Bins

Use this procedure to import panels and bin sets into the GeneMapper software database for subsequent analysis and to view imported panels, markers, and bins. Import the panels and bin sets the first time you use the software and when updated versions of panels and bin sets are provided.

To import panels and bin sets:

1.	Start the GeneMapper *ID* software: a. Select **Start > Programs > Applied Biosystems >GeneMapper > GeneMapper ID**. b. In the login box that appears, make the following selections and click **OK**. – User Name: (type in a unique user name) – Password: (type in a password of 6-10 characters)
2.	Create a new password: The first time you start the software, you are prompted to change the password. When the password dialog box opens: • Leave the Old Password box blank. • Type a the New Password. • Type the new password again to verify it. The GeneMapper Project window opens with a blank, untitled project.
3.	Select **Tools > Panel Manager** to open the Panel Manager.
4.	Locate and 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.

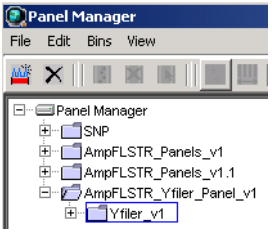
To import panels and bin sets: ***(continued)***

5.	Select **AmpFLSTR_Yfiler_Panel_v1**, then click **Import**. **Note:** Importing this file creates a new folder in the navigation pane of the Panel Manager, AmpFlSTR_Yfiler_Panel_v1. This folder contains the panels and associated markers.

To import panels and bin sets: ***(continued)***

6. Import **AmpFLSTR_Yfiler_Binset_v1**:

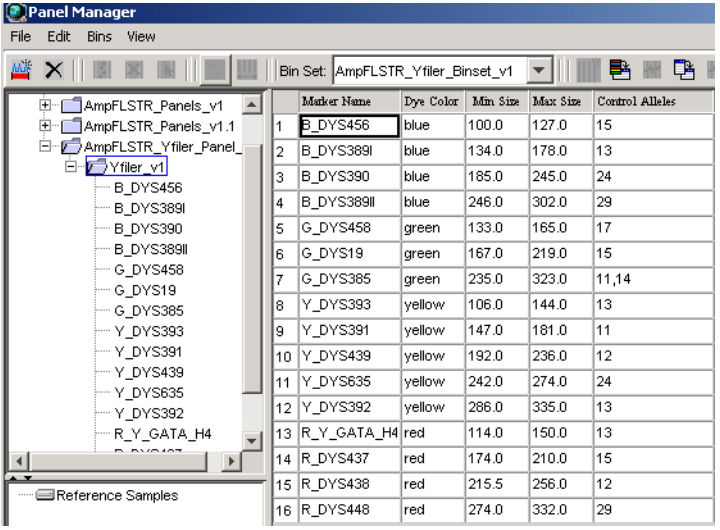
   a. Select the **AmpFLSTR_Yfiler_Panel_v1** folder in the navigation pane.



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

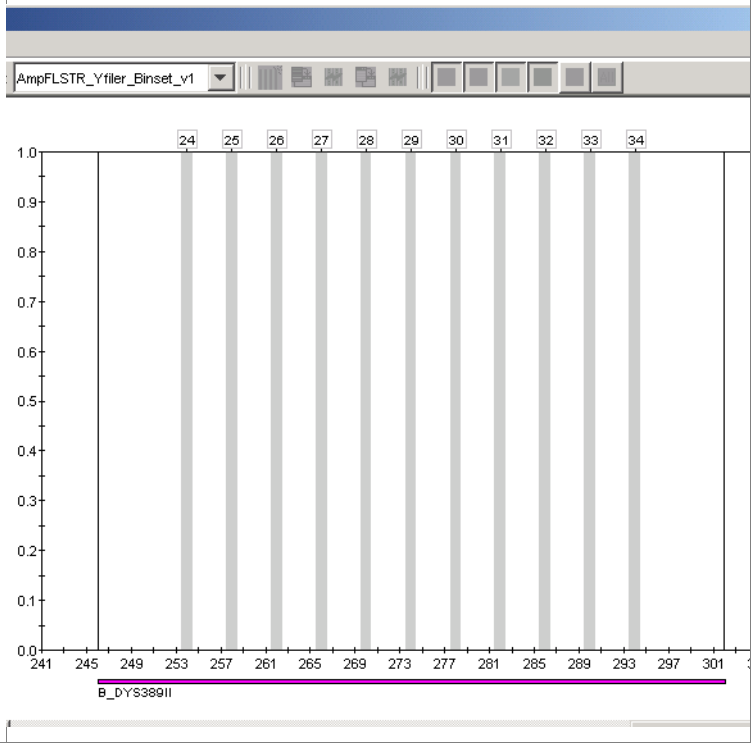
   c. Select **AmpFLSTR_Yfiler_Binset_v1**, then click **Import**.

Note: Importing this file associates the bin set with the panels in the AmpFlSTR_Yfiler_Panel_v1 folder.



	Marker Name	Dye Color	Min Size	Max Size	Control Alleles
1	B_DYS456	blue	100.0	127.0	15
2	B_DYS389I	blue	134.0	178.0	13
3	B_DYS390	blue	185.0	245.0	24
4	B_DYS389II	blue	246.0	302.0	29
5	G_DYS458	green	133.0	165.0	17
6	G_DYS19	green	167.0	219.0	15
7	G_DYS385	green	235.0	323.0	11,14
8	Y_DYS393	yellow	106.0	144.0	13
9	Y_DYS391	yellow	147.0	181.0	11
10	Y_DYS439	yellow	192.0	236.0	12
11	Y_DYS635	yellow	242.0	274.0	24
12	Y_DYS392	yellow	286.0	335.0	13
13	R_Y_GATA_H4	red	114.0	150.0	13
14	R_DYS437	red	174.0	210.0	15
15	R_DYS438	red	215.5	256.0	12
16	R_DYS448	red	274.0	332.0	29

To import panels and bin sets: ***(continued)***

7.	View the imported panels: a. Select the **AmpFLSTR_Yfiler_Panel_v1** folder in the navigation pane to view the panel contained in this folder in the right pane. b. Double-click the **AmpFLSTR_Yfiler_Panel_v1** folder in the navigation pane to display the list of panels below it.
8.	View the markers and display the Bin view: a. Select the **Yfiler_v1** folder in the navigation pane to display the list of markers it contains in the right pane. b. Double-click the **Yfiler_v1** folder in the navigation pane to display the list of markers below it. c. Select **DYS389II** in the navigation pane to display the Bin view for the marker in the right pane. 

To import panels and bin sets: *(continued)*

9.	Add the Yfiler_vl panel to the project window by clicking **Apply**, then **OK**. **Note:** If you close the Panel Manager without clicking OK, the panels and bins will not be available for analysis.
10.	In the first sample row, under the column labeled Panel, double-click **None**, open the folder to display a list of panels, and double-click on the panel you want, to assign a panel set.

Creating HID Analysis Methods

Two analysis methods are suitable for HID analysis using Yfiler kits:

- HID_Classic – Provides users with the same analysis parameters and produces results similar to those obtained for data analyzed with GeneScan Software v3.1.2 for the Macintosh OS. This algorithm allows laboratories that have optimized analysis parameter settings on the Macintosh OS to use GeneMapper *ID* software to analyze their data.
- HID_Advanced – Provides users with the same analysis parameters available in GeneScan Software v3.7.1 for the Windows OS.

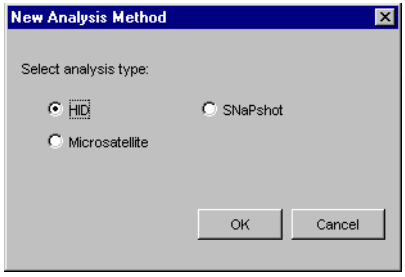
To create an analysis methods for the HID Classic Mode:

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

To create an analysis methods for the HID Classic Mode:

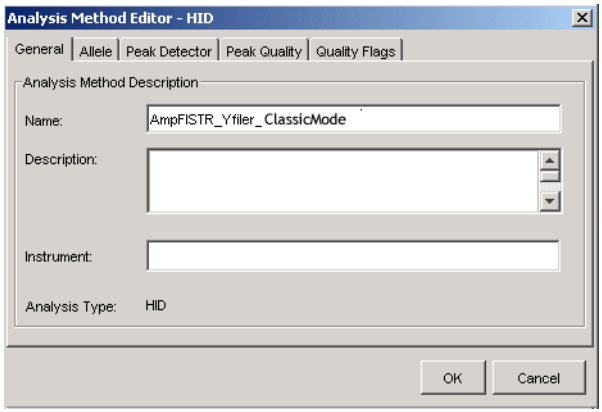
2. Create an analysis method for HID Classic:

   a. Select the **Analysis Methods** tab and click **New** to open the New Analysis Method dialog box.



   b. Select **HID** and click **OK** to open the Analysis Method Editor with the General tab selected.

   c. In the General tab, enter an analysis name for the method, such as AmpFlSTR_Yfiler_ClassicMode.



3. Select the settings shown in Table 5-1, "HID Classic analysis method settings."

   IMPORTANT! You must select your settings on all the tabs before you Click **OK** to save the analysis method and return to GeneMapper Manager!

HID Classic Settings

Table 5-1 HID Classic analysis method settings

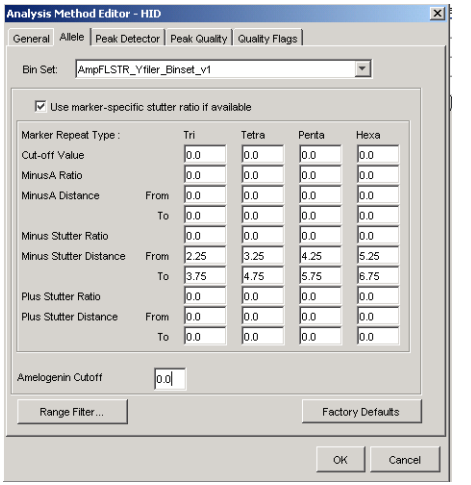
Tab	Settings
General	Name: AmpFℓSTR_Yfiler_ClassicMode
Allele	 **Note:** GeneMapper® *ID* Software v3.2 allows you to specify four types of marker repeat motifs: tri, tetra, penta, and hexa. You can enter parameters for each type of repeat in the appropriate column. **Note:** Select "Use marker-specific stutter ratio if available". If the box is selected, the software applies the stutter ratio filters specific to the Yfiler panel (defined by Applied Biosystems). **Note:** For more information about allele filters, refer to Chapter 3 of the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (PN 4338775) and User Bulletin New Features and Installation Procedures for *GeneMapper® ID Software Version 3.2* (PN 4352543).

Table 5-1 HID Classic analysis method settings *(continued)*

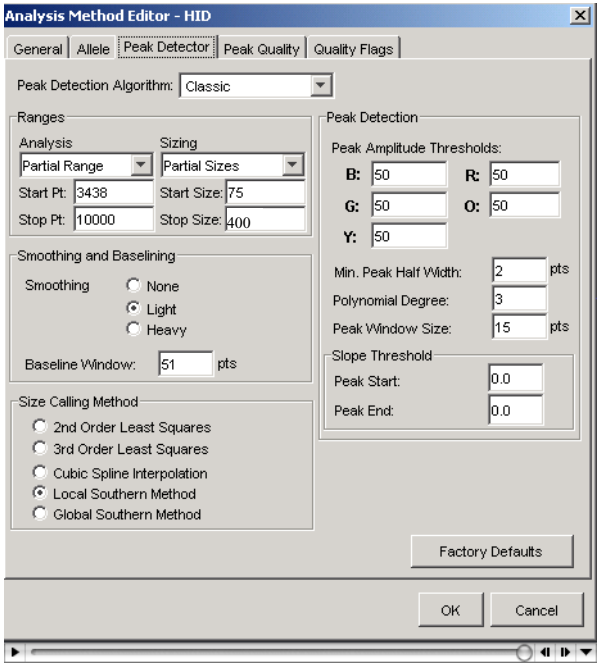
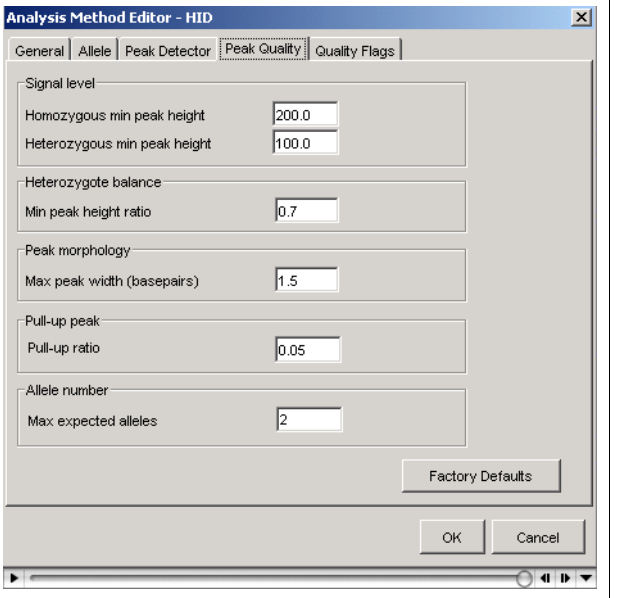
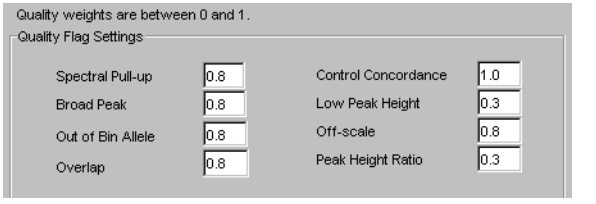
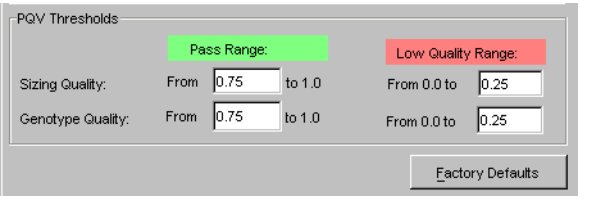
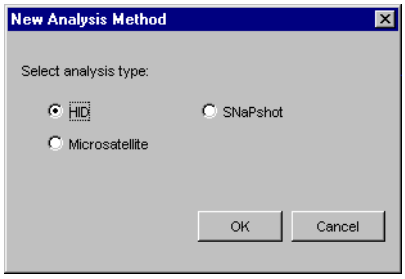
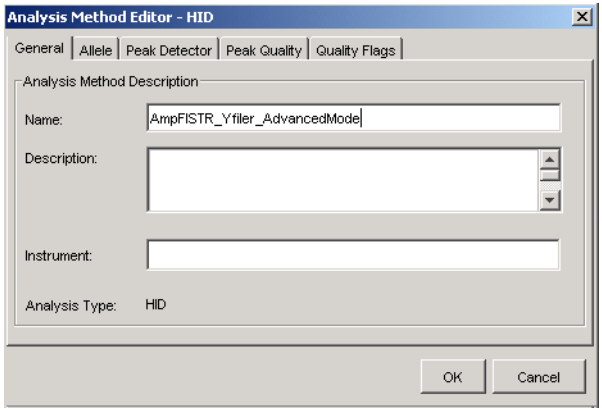
Tab	Settings
Peak Detector	 The software uses the peak detection parameters to specify the minimum peak height to limit the number of peaks detected. Although GeneMapper *ID* software displays peaks that fall below the specified height in electropherograms, the software does not label or determine the genotype of these peaks. The analysis range is set by the user based on location of the primer peak and size standard peaks. **Note:** For more information, on peak detection algorithms, refer to Appendix A of the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (PN 4338775) and User Bulletin New Features and Installation Procedures for *GeneMapper® ID Software Version 3.2* (PN 4352543).

Table 5-1 HID Classic analysis method settings *(continued)*

Tab	Settings
Peak Quality	
Quality Flags	Quality flag settings:  PQV thresholds: 

To create an analysis method for the HID Advanced Mode:

1.	Select **Tools > GeneMapper Manager** to open the GeneMapper Manager.
2.	Create an analysis method for HID_Advanced: a. Select the **Analysis Methods** tab and click **New** to open the New Analysis Method dialog box. b. Select **HID** and click **OK** to open the Analysis Method Editor with the General tab selected.  c. In the General tab, enter the name for the analysis method, such as AmpFlSTR_Yfiler_AdvancedMode. 
3.	Select the settings shown in Table 5-2, "HID_Advanced analysis method settings." **IMPORTANT!** You must select your settings on all the tabs before you Click **OK** to save the analysis method and return to GeneMapper Manager!

HID_Advanced Settings

Table 5-2 HID_Advanced analysis method settings

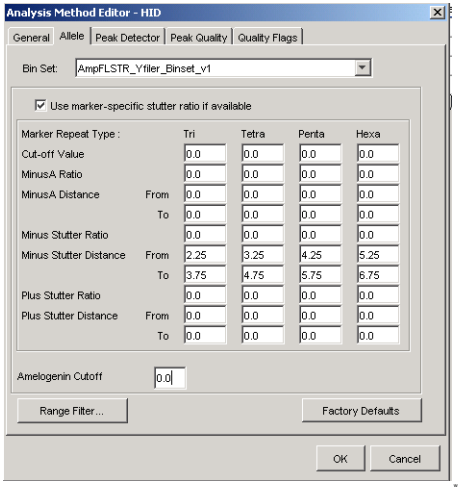
Tab	Settings
General	Name: AmpFℓSTR_Yfiler_AdvancedMode
Allele	 **Note:** GeneMapper® *ID* Software v3.2 allows you to specify four types of marker repeat motifs: tri, tetra, penta, and hexa. You can enter parameters for each type of repeat in the appropriate column. **Note:** Select "Use marker-specific stutter ratio if available". If the box is selected, the software applies the stutter ratio filters specific to the Yfiler panel (defined by Applied Biosystems). **Note:** For more information about allele filters, refer to Chapter 3 of the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (PN 4338775) and User Bulletin New Features and Installation Procedures for *GeneMapper® ID Software Version 3.2* (PN 4352543).

Table 5-2 HID_Advanced analysis method settings *(continued)*

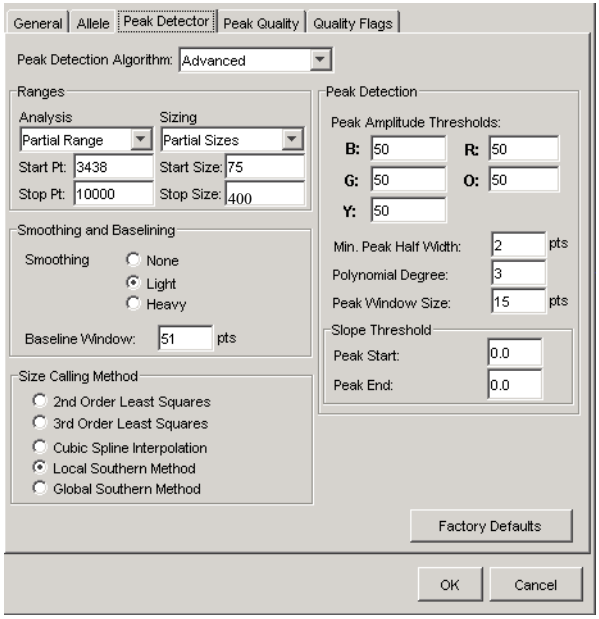
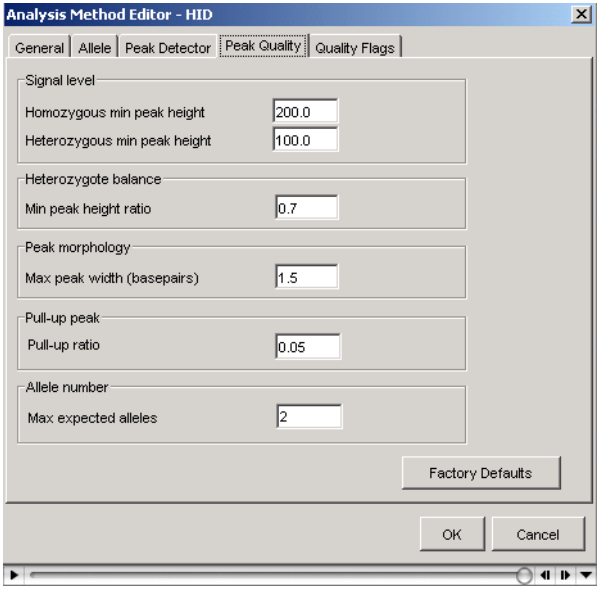
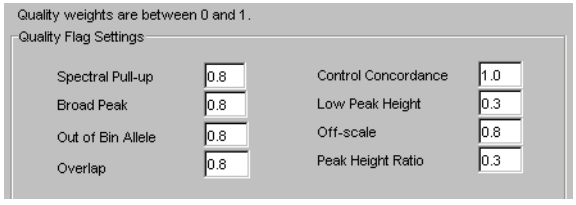
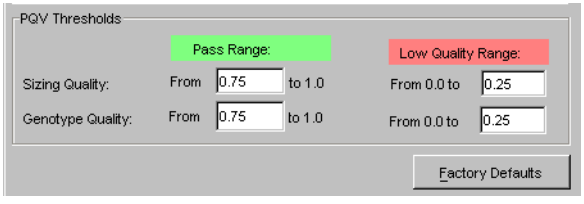
Tab	Settings
Peak Detector	 The software uses the peak detection parameters to specify the minimum peak height to limit the number of peaks detected. Although GeneMapper *ID* software displays peaks that fall below the specified height in electropherograms, the software does not label or determine the genotype of these peaks. The analysis range is set by the user based on location of the primer peak and size standard peaks. **Note:** For more information, on peak detection algorithms, refer to Appendix A of the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (PN 4338775) and User Bulletin New Features and Installation Procedures for *GeneMapper® ID Software Version 3.2* (PN 4352543).

Table 5-2 HID_Advanced analysis method settings *(continued)*

Tab	Settings
Peak Quality	
Quality Flags	Quality flag settings:  PQV thresholds: 

Analyzing Sample Files With GeneMapper *ID* Software

To analyze a project:

1. From the Project window, select **File > Add Samples** to Project to navigate to the disk or directory containing the sample files.

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

Parameter	Analysis Method	
	Advanced Method	**Classic Method**
Table Setting	Select **AmpFℓSTR Table** from the drop-down list.	Select **AmpFℓSTR Table** from the drop-down list.
Sample Type	Select the sample type.	Select the sample type.
Analysis Method	**AmpFℓSTR_Yfiler_ AdvancedMode**	**AmpFℓSTR_Yfiler_ ClassicMode**
Panel	**AmpFℓSTR_Yfiler_ Panel_v1**	**AmpFℓSTR_Yfiler_ Panel_v1**
Size Standard[a]	**CE_G5_HID_ GS500**[b,c]	Define a new size standard.[d]
Matrix	Select a matrix for 310 instruments only.	Select a matrix for 310 instruments only.

a. 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).

b. The following fragments are defined for the CE_G5_HID_GS500 size standard provided with the AmpFlSTR kits: 75, 100, 139, 150, 160, 200, 300, 340, 350, 400, 450. For additional information about size standards, refer to Appendix A of the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (PN 4338775).

c. The 250-bp peak is not included in the size standard definition because this peak can be used as an indicator of precision within a run.

d. Refer to Chapter 2 of the *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (PN 4335523).

To analyze a project: *(continued)*

3.	Click (Analyze), type a name for the project (in the Save Project dialog), then click **OK** to initiate 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). See Figure 5-1. • The genotypes tab becomes available after analysis.

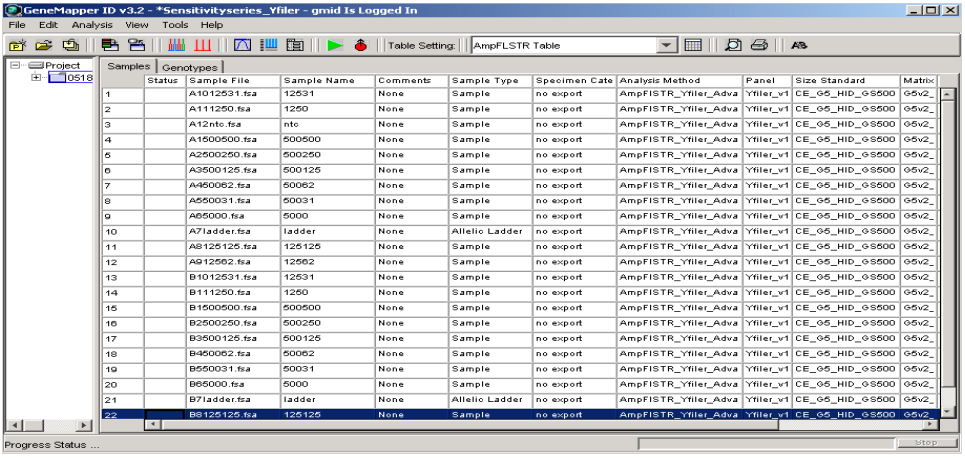


Figure 5-1 Project Window

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

Examining and Editing GeneMapper *ID* Software Results

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).

To examine and edit GeneMapper *ID* Software results:

1.	Examine the size standard. a. Assess whether samples pass the sizing criteria. Examine the flags in the SQ column to assess sizing quality. A green square indicates that a sample has passed the sizing criteria. b. Check the size standards for any samples that do not pass the sizing criteria. **Note:** Beginning with v3.2, GeneMapper *ID* software automatically labels size standards. Refer to the *New GeneMapper® ID Software Version 3.2 User Bulletin* (PN 4352543).
2.	Examine the allelic ladder calls. a. In the Samples View tab, find the plots for all allelic ladders. b. Display AmpFℓSTR Genotyping Plot Setting. c. Verify that the allelic ladder is called correctly for each marker. **Note:** Deselecting Controls to Top will display each color within the allelic ladder. d. Close the Samples View window.
3.	Examine data. a. Select low quality samples (indicated by red octagons or yellow triangles). b. It is recommended that a user carefully review all PQVs that display a yellow triangle or red octagon.

To examine and edit GeneMapper *ID* Software results: ***(continued)***

4.	Close the Samples Plot window and save the project.

For more information about any of these tasks, refer to the following documents:

- *New Features and Installation Procedures for GeneMapper® ID Software Version 3.2 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)

Section 5.3 Using GeneScan® Analysis Software to Analyze Yfiler Kit Data

This section covers:

Analyzing Sample Files Using GeneScan Software

Summary

Analyzing sample files using GeneScan software involves the following steps:

1. Creating a project, and if necessary, installing and applying a matrix.
2. Defining analysis parameters.
3. Defining a size standard.
4. Performing data analysis.

Creating a Project

A project is a file containing references to a set of sample files that you want to analyze and display together.

You can create a new project and add any combination of sample files, allowing you to analyze and display samples from different runs. Adding a sample file to the project sets up a link between the project and the sample file. The file itself is not imported into the project.

If you enabled autoanalysis, the Data Collection Software automatically launches the GeneScan application, creates a project, and adds sample files to the project.

If you did not enable autoanalysis, you must create the project manually and add the sample files to the project, as explained in the following procedure.

To create a new project:

1.	Select **File > New**. The Create New dialog box opens.
2.	Click the **Project** icon. An untitled Analysis Control window opens.
3.	Add sample files to the project by • Selecting **Project > Add Sample Files** to add one or more sample files from the hard drive. • Selecting **Project > Add "file name"** to add open sample files.

To create a new project: ***(continued)***

4.	Click **Finish** when you have added all relevant sample files. The sample files appear in the Analysis Control window.
5.	For sample files generated by ABI PRISM® 310 and 377 Analyzers, install and apply a matrix to the sample files. a. Click the **Sample** column to select all the sample files in the project. b. Click **Sample > Install New Matrix**. c. Navigate to the location that contains the matrix file, then select it. **Note:** Data Collection Software on the ABI PRISM 3100/3100-*Avant* Analyzers performs multicomponenting; you do not need to perform this step when analyzing sample files from those instruments.

Note: The GeneScan Analysis Software version 3.7 CD-ROM contains two applications, Mac to Win and Win to Mac, which you can use to convert sample files created on one platform to files that can be read by the other platform. Refer to Chapter 4 of the *ABI PRISM GeneScan Analysis Software v3.7 for the Windows NT Platform* (PN 4308923) for detailed information about this process.

Defining Analysis Parameters

Analysis parameters are defined in the Analysis Settings dialog box.

Figure 5-2 shows the default analysis parameter settings for GeneScan analysis software v3.7.1 on the Windows NT operating system.

This range is set by the user.

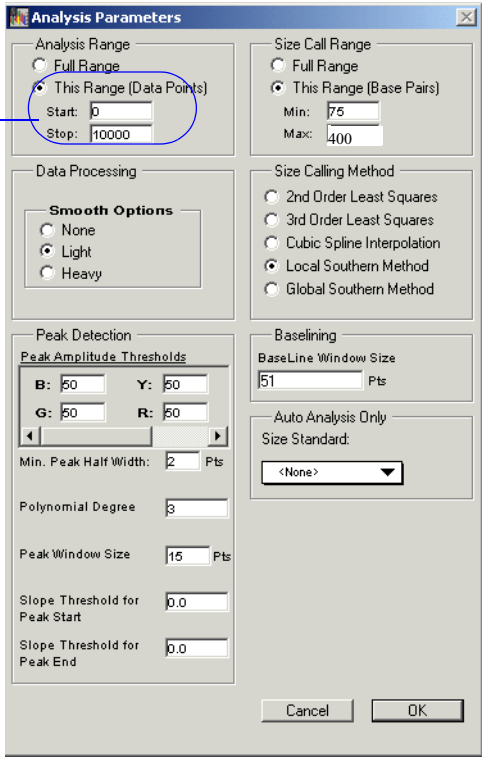


Figure 5-2 Analysis Parameters dialog box with default settings

Table 5-3 lists the individual parameters and explains how to set them.

Table 5-3 GeneScan analysis parameters

Parameter	Procedure
Analysis Range[a]	1. Click the **This Range (Data Points)** radio button. 2. Look at the raw data and enter the values that are appropriate for all sample files in the project. These data points affect data in the results display. 3. Enter Start and Stop data point numbers in the entry fields. Select the **Start** data point just before the first peak of interest, the 75 bp size standard peak. At a minimum, select the **Stop** data point just after the last peak of interest, the 400 bp size standard peak. (See Figure 5-2 on page 5-28.)
Smooth Options	The default parameter for Smooth Options is light.
Peak Amplitude Thresholds[a]	1. Select a Peak Amplitude Threshold (PAT) for each dye color. 2. Use the active scroll bar to enter the PATs for each of the five colors. 3. After analysis, the GeneScan table contains data for all peaks with a height above that specified by the PAT. **Note:** Applied Biosystems suggests that you determine the PATs appropriate for your analysis. Conduct sensitivity experiments in your laboratory with each instrument to evaluate the PATs used for analysis.
Min. Peak Half Width	The Min Peak Half Width for use with the AmpFℓSTR products is 2 Pts.
Peak Window Size	The default parameter for peak window size is 15.
Polynomial Degree	The default parameter for polynomial degree is 3.
Slope Threshold for Peak Start	The default parameter for slope threshold for peak start is 0.0.

Table 5-3 GeneScan analysis parameters *(continued)*

Parameter	Procedure
Slope Threshold for Peak End	The default parameter for slope threshold for peak end is 0.0.
Size Call Range[a]	Click the **This Range (Base Pairs)** radio button and enter the values of **75** for Min and **400** for Max.
Size Calling Method[a]	Click the **Local Southern Method** radio button for sizing of the AmpFℓSTR products. This method determines the sizes of fragments by using the reciprocal relationship between fragment length and mobility.
Baseline Window Size	Refer to the user bulletin (P/N 4335617) for more information.
Auto Analysis Only	Refer to the user bulletin (P/N 4335617) for more information.

a. Same for Windows v3.7.1 and Macintosh v3.1.2.

To define analysis parameters:

1.	In the Parameters column of the Analysis Control window, click on the arrow beside <Analysis Parameters> (for any of the sample files).
2.	Select **Define New**. The Analysis Settings dialog box opens.
3.	Modify the default settings as necessary.
4.	Click **Save**. Make sure to save the file in the following location: `C:\AppliedBio\Shared\Analysis\SizeCaller\Params`

For additional information about analysis parameters, refer to

- *ABI PRISM® GeneScan Analysis Software v3.7 for the Windows NT Platform* (PN 4308923)

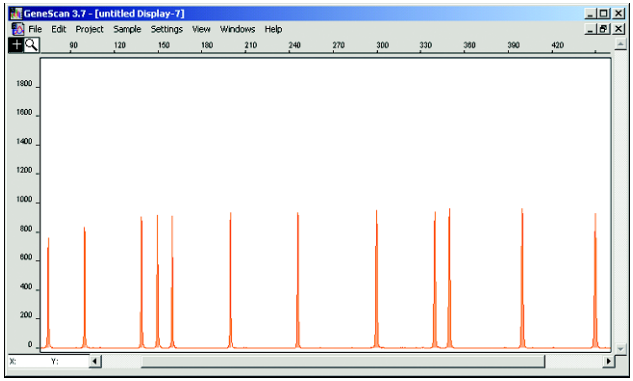
- *ABI PRISM® GeneScan Analysis version 3.1 User's Manual* (Macintosh) (PN 4306157)

Defining a Size Standard

While GeneScan software is shipped with several built-in size standard definitions, it is sometimes useful or necessary to create your own size standard definition.

For Yfiler kits, you create a size standard definition based on the GeneScan-500 LIZ® size standard, without the 250-bp peak.

To define the size standard for Yfiler kits:

1.	In the Size Standard column of the Analysis Control window, click the arrow beside <Collection Setting> (for any of the sample files).
2.	Click **Define New**. The software launches the size standard definition utility.
3.	Define the known size standard peaks for the GeneScan 500 LIZ Size Standard (75, 100, 139, 150, 160, 200, [250], 300, 340, 350, 400). **IMPORTANT!** Do not assign the 250-bp peak—skip a row or assign a size of 0. This peak can be used as an indicator of precision within a run. 
4.	Save the size standard definition in C:\AppliedBio\Shared\Analysis\SizeCaller\SizeStandards

To define the size standard for Yfiler kits: *(continued)*

5.	In the Analysis Control window, apply the size standard definition to all sample files in the project.

Performing Data Analysis

To analyze the sample files:

1.	In the Analysis Control window, select the blue, green, yellow, red, and orange columns. The Analyze button is activated.
2.	Confirm that the orange dye (LIZ) is assigned to the size standard, as indicated by a diamond symbol.
3.	If the diamond symbol does not appear in the orange boxes, **Ctrl + Click** (or ⌘-click) to place a diamond in the box.
4.	Click **Analyze**.
5.	After the analysis is complete, confirm that the sizes for the peaks in the GeneScan-500 LIZ Size Standard have been correctly assigned. a. Select **Window > Results Control** and examine the orange GeneScan-500 LIZ Size Standard peaks in overlapping groups of 16 samples (Quick Tile Off). Be sure to select **View > Align By Size**. b. While the samples are tiled, check the 250-bp peaks (sized as approximately 246 bp) in the enlarged view window. Remember that this peak was not defined in the size standard. The tiled 250-bp peaks should size consistently—they should all overlap. In a typical run, the 250-bp peaks all fall within a size window of approximately 1 bp. Temperature fluctuations in the laboratory may cause variations >1 bp. c. Scroll through the tables to verify correct GeneScan-500 LIZ peak assignments. d. Check the GeneScan-500 LIZ Size Standard peaks in the remaining samples, taking note of which samples (if any) have incorrect peak assignments.

To analyze the sample files: ***(continued)***

6.	View AmpFℓSTR Yfiler kit results (using the Results Control window). Refer to the *ABI PRISM® GeneScan Analysis Software v3.7 for the Windows NT Platform* (PN 4308923) and the *ABI PRISM® GeneScan Analysis version 3.1 User's Manual* (Macintosh) (PN 4306157) for printing options.

Viewing GeneScan® Software Results

After sample files have been analyzed, use the Results Control window to display the results from each injection into a capillary. The Results Control window displays the newly analyzed sample files and allows the user to specify the format of the results. Selecting both the Electropherogram and Tabular Data icons is recommended for reviewing results.

Section 5.4 Using Genotyper® Software to Analyze Yfiler Kit Data

This section covers:

Overview

Information in this Section

This chapter describes the use of ABI PRISM® Genotyper® Software v3.7 in conjunction with the AmpFℓSTR® Yfiler™ Kit Template and the Microsoft® Windows® operating system to automatically genotype samples.

- If you have not worked with the Yfiler Template before, "Understanding the AmpFℓSTR Yfiler Kit Template" on page 5-37 describes the macros in the template.
- If you are familiar with the Yfiler Template, "Using the AmpFℓSTR Yfiler Kit Template for Automatic Genotyping" on page 5-44 provides instructions for using the template.
- If you prefer to genotype samples manually, "Manual Genotyping Against the AmpFℓSTR Yfiler Kit Allelic Ladder" on page 5-53 explains how.

Instruments

Refer to "Instrument and Software Compatibility" on page 1-5 for a list of compatible instruments.

About the Software

Genotyper® software is used to automatically convert allele sizes obtained from ABI PRISM® GeneScan® Analysis Software into allele designations and to build tables containing the genotype information. The software assigns genotypes by comparing the sizes obtained for the unknown sample alleles with the sizes obtained for the alleles in the allelic ladder.

Refer to the *ABI PRISM® Genotyper® 3.7 NT Software User's Manual* (PN 4309947) and *ABI PRISM® Genotyper® 3.7 NT Software Applications Tutorials* (PN 4309961) for more detailed information about the Genotyper software.

Before Running Genotyper Software

GeneScan Analysis Software sample data (particularly the allelic ladder) must meet the requirements described in this section before you can use the macros in the AmpFℓSTR Yfiler Kit Template.

Sample Info Column Requirements

- All samples must have a unique sample description in the Sample Info column of the GeneScan software sample sheet for the macros in the AmpFℓSTR Yfiler Kit Template to build a table. Samples with an empty Sample Info column are not incorporated into the table of genotypes.

- Lanes or injections that contain the AmpFℓSTR Yfiler Allelic Ladder must have the word “ladder” in the Sample Info column. The Kazam macro uses the first lane or injection of ladder to determine the sizes in the allele categories that will be used for genotyping.

  You can skip the first lane or injection of allelic ladder and use the second lane or injection of allelic ladder for genotyping instead. To do so, remove the word “ladder” from the Sample Info column in all four sample dye colors for the first lane or injection of allelic ladder in the Dye/lanes window after importing the sample files, but before running the Kazam macro. Make sure that the word “ladder” is entered for Sample Info in the second lane or injection of allelic ladder.

  See step 4 on page 5-45 for a description of how to access the Sample Info column in the Dye/lanes window.

GeneScan Analysis Software Peak Recognition Requirements

- All allele peaks in the allelic ladder for each locus must be “recognized” (labeled) in the GeneScan Analysis Software (that is, each allele peak must have an entry in the GeneScan table).
- All allele peaks in each allelic ladder must have a peak height value in relative fluorescence units (RFU) greater than the Peak Amplitude Threshold (PAT) specified in the GeneScan software Analysis Parameters.
- All allele peaks in each allelic ladder must be resolved. For example, the DYS439, 12, 13, and 14 alleles must be resolved so that each peak has an entry in the GeneScan software table.
- Sample allele peak heights must be greater than the GeneScan Software PAT in order to be recognized (labeled) by Genotyper software.

Understanding the AmpFℓSTR Yfiler Kit Template

About the Yfiler Template

Table 5-4 lists the macros contained in the Yfiler Kit Template and summarizes their functions. Detailed descriptions of each macro are provided in subsequent sections.

Table 5-4 Macros contained in the Yfiler Template

Macro Name	Function	Reference
Check GS500	Automatically labels the size standard peaks and examines the 250-bp peak.	—
Kazam	Automatically determines genotypes based on the allelic ladder, applying different stutter filters for each locus.	"Kazam Macro" on page 5-39
Kazam (20% Filter)	Automatically determines genotypes based on the allelic ladder, applying a 20% stutter filter to all loci.	"Kazam (20% Filter) Macro" on page 5-43
Make Allele Table	Stores genotyping information in a table. Can be used for data generated by the ABI PRISM® 310, 3100,and 3100-*Avant* instruments.	For more information about the Make Table macros, refer to Chapter 10 of the *AmpFℓSTR® Profiler Plus™ PCR Amplification Kit User's Manual* (PN 4303501).
310: Make Table	Stores genotyping information in a table. Can be used for data generated by the 310 instrument.	

Kazam Macro

The Kazam macro automatically determines genotypes relative to the allelic ladder.

For each locus, the Kazam macro calls the Calculate [locus] Offsets macros, which label peaks and filter (remove labels from) the stutter peaks. Consequently, the Kazam Macro allows a different stutter threshold to be calculated for each locus and thus provides maximum flexibility for customizing the filter that is used for each locus.

Removing Labels from Stutter Peaks by Applying Percentage Filters

The Kazam macro includes a step that removes labels from stutter peaks by applying a percentage filter. Labels are removed from peaks that are followed by a specified percent difference higher labeled peak within 2.25 to 3.75 bp for a trinucleotide repeat marker, 3.25 to 4.75 bp for a tetranucleotide repeat marker, 4.25 to 5.75 bp for a pentanucleotide repeat marker, and 5.25 to 6.75 bp for a hexanucleotide repeat marker. An additional filter for -2 bp stutter has been added for the DYS19 locus (1.50 to 2.50 bp) and a +3 bp stutter filter for the DYS392 locus (2.25 to 3.75 bp). The specified filter percentages for the loci are listed in Table 5-5.

Table 5-5 Kazam macro stutter filter percentages for Yfiler loci

Locus	% Stutter	% bp stutter (plus or minus)
DYS456	13.21	-
DYS389I	11.79	-
DYS390	10.4	-
DYS389II	13.85	-
DYS458	12.2	-
DYS19	11.4	10.21 (-2 bp)
DYS385	13.9	-
DYS393	12.58	-
DYS391	11.62	-

Table 5-5 Kazam macro stutter filter percentages for Yfiler loci *(continued)*

Locus	% Stutter	% bp stutter (plus or minus)
DYS439	11.18	-
DYS635 (YGATAC4)	10.75	-
DYS392	16.22	7.9 (+3 bp)
Y GATA H4	11.08	-
DYS438	4.28	-
DYS437	8.59	-
DYS448	4.96	-

The peak filtering included in the Kazam macro is intended only as a tool and guideline. Final conclusions should be based on careful examination of the STR profiles. For instructions on filtering stutter peaks, refer to "Examining and Editing Data" on page 5-47.

Viewing the Kazam Macro Steps

To view the Kazam macro steps, click **Kazam** in the Macro list, then select **View** > **Show Step Window**.

Calculating Offsets

The Calculate [locus] Offsets macros, which are automatically called by the Kazam macros, calculate offsets by

- Identifying the first allele peak in each allelic ladder
- Comparing reference sizes to allelic ladder sizes and determining the offset value
- Applying the offset value to each allele
- Applying the offset value to off-ladder and virtual alleles

These functions are described in greater detail in the following sections. The Calculate [locus] Offsets macros use offset categories, also described below, to perform these functions.

Categories

In the Genotyper software, each allele is defined by a category. Each category contains information about the allele size, size range, and dye color.

To view the list of categories in the AmpFℓSTR Yfiler Template, select **View** > **Show Categories**. Categories for each locus are listed under the locus name. (Note that the software calls a locus a group.)

In the Categories window, each locus has two sets of categories:

- **Allele categories** – Designated by <*locus name*>, for example, DYS392. The Genotyper software uses the categories in this group for allele assignment.
- **Offset categories** – Designated by <*locus name*.os>, for example, DYS392.os. The Calculate [locus] Offsets macros use the categories in this group to find the allele peaks in the allelic ladder and to determine the correct offset values for each allele category.

Identifying the First Allele Peak in Each Allelic Ladder

The macros use values specified in the first offset category (each allelic ladder has its own group of offset categories) to identify the first (leftmost) peak in each allelic ladder.

For example, the first offset category for the DYS392 allele, 7.os, instructs the Genotyper software to find all peaks in a range of ±7 bp around the reference size for the indicated allele. Each Calculate [locus] Offsets macro applies a percentage filter to all peaks in the ±7 bp range in the allelic ladder, avoiding the first stutter peak in each allelic ladder. Consequently, the first allele peak is identified as the leftmost peak.

Comparing Reference Sizes to Allelic Ladder Sizes

The base pair size indicated in each offset category is a reference size. The Calculate [locus] Offsets macros offset the reference sizes relative to the sizes obtained for the alleles in the allelic ladder. After the macros run, the calculated offset values are indicated in parentheses near the end of each category line in the Categories window.

For example, the sequenced size for allele 10 of locus DYS392 is 303 bp. The size obtained on a 3100 Genetic Analyzer for the allele for a particular injection was 300.39 bp.

- The offset value is calculated as 300.39 – 303 = –2.61
- The category size used for allele assignment is 300.39 (or 303 – 2.61). That is, the category sizes used for genotyping are equivalent to the allele sizes obtained in the lane or injection of allelic ladder

Applying the Appropriate Offset Value to Each Allele

Once the leftmost allele peak in each allelic ladder is identified, the offset value determined for this allele is applied to the relevant allele(s) in the allele categories.

For example, assume that the offset value determined by the 7.os category in the DYS392.os group is –2.67 for a particular lane or injection of allelic ladder. This offset value is then applied to the allele 7 category in the DYS392 group, thus setting the correct offset value for allele 7.

In order for the software to find the next allele peak in the DYS392 allelic ladder (allele 8), the offset value for the 7.os allele is also applied to the 8.os category. The result of this operation is that the 8.os category size will be 3 bp longer than the 7.os category. In other words, allele 8 is expected to be found at a size that is 3 bp longer than allele 7.

To maximize the ease of peak recognition, the size width for most offset categories is ±1 bp, as compared to the allele categories, which have a width of ±0.5 bp. Once allele 8 is recognized in the DYS392 allelic ladder, the correct offset value is calculated and assigned to the appropriate categories.

This process of peak recognition, offset calculation, and offset assignment is carried out for each of the alleles in each of the allelic ladders.

Off-Ladder Alleles and Virtual Alleles

In the previous example, the 7.os offset value (-2.67) is also applied to two other categories in the DYS392 group: "OL Allele?" and allele 6.

The OL Allele? category is specified to span the range of known DYS392 alleles to catch off-ladder alleles that do not size within one of the allele categories.

Allele 6 in this case is a "virtual" allele category, meaning that this allele is not present in the allelic ladder. The virtual category exists to assign an allele designation to allele 6, which is a known allele not included in the allelic ladder.

Because allele 6 is specified to have the same offset value as allele 7, the allele category sizes for these two alleles differ by exactly 3 bp, the difference in their reference sizes. Specifying a size for allele 6 that is 3 bp shorter than allele 7 is generally expected to be a reasonable estimate, since alleles 6 and 7 differ by a single repeat unit (3 bp).

Another example of "virtual" allele categories can be seen in the DYS385 categories. The DYS385 group contains virtual allele categories such as 14.2 and 17.2. The offset value for allele 14.2 is the same as for allele 14. In this case, since reference sizes for these two alleles differ by 2 bp, the category size used for allele 14.2 will be 2 bp longer than for allele 14. Likewise, the offset for allele 17.2 is the same as for allele 17, so the allele category size for allele 17.2 will be 2 bp longer than for allele 17.

Kazam (20% Filter) Macro

The Kazam (20% Filter) macro applies a 20% stutter filter to all loci. Use this macro:

- To apply a single filter value for all loci.
- When a high level of filtering specificity is not required, as in the typing of single source samples, such as database samples.

To view the Kazam (20% Filter) macro steps, click **Kazam (20% Filter)** in the Macro list, then select **View** > **Show Step Window**.

The Kazam (20% Filter) macro does not take into account the size (in bp) of the filtered peak relative to higher peaks. In fact, it removes labels from all peaks that are less than a specified percentage (by default, 20%) of the highest peak observed anywhere in the locus range. However, you can specify a different filter value.

To edit the filter value:

1.	Select **View** > **Show Step Window**.
2.	Click the first step of the macro ("Remove labels from peaks whose height is less than 20% of the highest peak in a category's range.")
3.	Select **Macro** > **Edit Step**. **Note:** This macro uses the second filter option (of the 4 filter options) in the Filter Labels window.
4.	Change the value, then click **Replace**.

Make Table Macros

The Yfiler Template includes three macros for making tables:

- Make Allele Table
- 310 Analyzer: Make Table
- 377 Analyzer: Make Table

For more information about the Make Table macros, refer to Chapter 10 of the *AmpFℓSTR® Profiler Plus® PCR Amplification Kit User's Manual* (PN 4303501).

Using the AmpFℓSTR Yfiler Kit Template for Automatic Genotyping

Installing the Yfiler Template

The AmpFℓSTR Yfiler Kit Template 9 is the Genotyper software template file that contains macros specifically written for use with the AmpFℓSTR® Yfiler™ PCR Amplification Kit. This template is provided in the CD that ships with this manual. This CD, AmpFℓSTR Yfiler Kit Template 9 CD (part number: 4360913), can be ordered separately if lost. The template file can also be downloaded from the following website:

http://www.appliedbiosystems.com/support/software/genotyper/templates.cfm

You must have Genotyper Software v3.7 or higher and Windows NT 4.0 with Service Pack 4 or 5 operating system to run the AmpFℓSTR Yfiler Kit Template. Install the template onto your computer following the instructions in the "READ_ME" file.

Note: The AmpFℓSTR Yfiler Kit Template file is a read-only file. When using the Template, you must save the file under a different name to ensure that the original template file is not overwritten.

Automatically Assigning Genotypes

To use the AmpFℓSTR Yfiler Kit Template:

1.	Double-click the **Yfiler** icon to launch the Genotyper software application and open the template file simultaneously.
2.	Under Edit, select **Set Preferences** to import raw data, and Blue, Green, Yellow, Red, and Orange data.
3.	Import the GeneScan Software sample files: a. Select **File** > **Import GeneScan File**(s). b. Select the project file and click **Import**.
4.	If each sample does not already have Sample Info completed in the sample sheet, enter a sample description: a. Select **Views** > **Show Dye/lanes**. b. Click the first sample row to select it. c. Click the **Sample Info** box at the top of the window and type the sample designation or description. d. Repeat steps b and c to enter a sample description for every dye/lane in the list. Enter the same sample description for all dye colors of a single sample.
5.	From the **Macro** list at the bottom left of the Main window, select **Check GS500**, then select **Macro** > **Run Macro**. In the plot window that opens, scroll through each sample to verify that each GeneScan-500 peak (from 75–400 bp) was assigned the correct size in the GeneScan Analysis Software.

To use the AmpFℓSTR Yfiler Kit Template: *(continued)*

6.	From the **Macro** list at the bottom left of the Main window, select **Kazam**, then select **Macro** > **Run Macro**. This macro may take a few minutes to run. When it is finished, a plot window opens with the blue allelic ladder (DYS456, DYS389I, DYS390, and DYS389II) and sample allele peaks labeled.
7.	Examine data, edit peaks, then print the electropherograms. a. In the Main Window, click the green **G** button at the top left. b. Select **Views** > **Show Plot Window**. c. Examine the data, edit the peaks, then print the electropherograms by selecting **File** > **Print.** "Examining and Editing Data" on page 5-47 provides more information about inspecting data. d. Repeat steps a through c for the yellow (**Y** button in the Main Window) and red (**R** button in the Main Window) data.

About the Plot Window

To zoom in and out on regions of the plot window:

1.	In the Plot window, click and drag in a region of an electropherogram to draw a box around the desired size range (the vertical size of the box is not important).
2.	Type **Ctrl+R** (hold down the Ctrl key and type the letter R) to zoom in.
3.	Type **Ctrl+H** to zoom out completely.

To view electropherograms from more than one dye color in the Plot window:

1.	Select **Views** > **Show Dye/Lanes Window**.
2.	Click the desired Dye/lane rows. **Note:** Hold down the Shift key on the keyboard to select multiple adjacent Dye/lane rows. Hold down the Ctrl key to select Dye/lane rows that are not adjacent.
3.	Select **Views** > **Show Plot Window**.

Examining and Editing Data

You examine Yfiler Kit data by checking peaks. Consider the following guidelines when examining peaks. (Refer to Figure 5-3 on page 5-55 Genotyper® software plot of the AmpFℓSTR Yfiler Allelic Ladder):

- Peaks in the allelic ladder should be labeled correctly.
  - Scroll through the samples below the allelic ladder to examine the peak labels in each electropherogram.
  - Clicking a labeled peak removes the label.
  - Clicking the same peak again defaults to the placement of bp size of that peak.
  - To customize the peak label, select **Analysis** > **Set Click Options**, type the allele designation and/or desired text, then click **OK**.
- Allele categories (which appear as dark gray bars in the Plot window) are defined to be ± 0.5 bp wide. Peaks that size within ± 0.5 bp of an allele category has a label indicating the allele designation.
- Peaks that do not size within an allele category have a label indicating "OL Allele?" (off-ladder allele).
- A sample allele peak must be recognized by GeneScan software before it can be recognized by Genotyper software. Sample allele peaks that are below the PAT that was specified in the GeneScan software Analysis Parameters cannot be labeled by Genotyper software.

  Also, because no information is imported for peaks that are not recognized by GeneScan software, such peaks will not align exactly by size relative to the x-axis size scale in the Genotyper software plot window.

- The Kazam macro, which removes labels from specific peaks, might have removed labels from peaks that should be labeled. If so, edit the macro to change the filter percentage for the locus. (Table 5-5 on page 5-39 lists the filter percentages for Yfiler kit loci.)

  The peak filtering included in the Kazam macro is intended only as a tool and guideline. You should base final conclusions on careful examination of the STR profiles. You can manually filter stutter peaks, as explained in the following procedure.

To filter stutter peaks:

1.	In the Step Window for the Kazam macro, scroll down to the line that reads "Select category: DYS390."
2.	Five rows below, select the line that reads, "Remove labels from peaks followed by an 861% higher, labeled peak within 3.25 to 4.75 bp."
3.	Select **Macro** > **Edit Step** to open the Filter Labels window. In the Filter Labels window, there are four options (check boxes) for filtering. In this example, the filtering option for DYS390 is denoted in the last check box. This filtering option includes another check box that reads "(higher by at least 861%)."

To filter stutter peaks: ***(continued)***

	3. (continued) For each labeled peak (*e.g.* peak A) in the locus size range, this filtering option examines the very next (*i.e.* greater in bp size) labeled peak (peak B). The label will be removed from peak A if peak B meets both of the specified criteria: • Peak B is higher by at least 861% • Peak B is within 3.25 to 4.75 bp The percentage value in this filtering option is calculated as follows: [(peak B – peak A / peak A] x 100 = percentage value For example, if peak A= 291 RFU and peak B = 2797 RFU, then the percentage value is calculated as follows: [(2797– 291) / 291] x 100 = 861% In this example, the label will be removed from peak A, provided that the filter option specifies a threshold of 861%, and that peak B is within 3.25 to 4.75 bp of peak A. Conventionally, percent stutter is calculated: (peak A/ peak B) x 100 = percent stutter The percentage value that is used in the Genotyper software filtering option (F) can be derived from the conventional percent stutter expression (S): F = (10,000 / S) – 100 For example, if the desired stutter percent threshold for DYS390 is 10.4%, then the percentage value that should be used in the Genotyper software filtering option is: F = (10,000 / 10.40) – 100 = 861%
4.	To use a filter value different than 861% for DYS390, enter another value, then click **Replace**.

You can manually filter plus stutter peaks, as explained in the following procedure.

To filter plus stutter peaks:

1.	In the Step Window for the Kazam macro, scroll down to the line that reads “Select category: DYS392.”
2.	Five rows below, select the line that reads, “Remove labels from peaks preceded by an 1166% higher, labeled peak within 2.25 to 3.75 bp.”
3.	Select **Macro** > **Edit Step** to open the Filter Labels window. In the Filter Labels window, there are four options (check boxes) for filtering. In this example, the filtering option for DYS392 is denoted in the third and last check box. This third option is used to filter plus stutter peaks seen at DYS392. This filtering option includes another check box that reads “(higher by at least 1166%).”

To filter plus stutter peaks: *(continued)*

	3. *(continued)* For each labeled peak (*e.g.* peak C) in the locus size range, this filtering option examines the preceding (*i.e.* smaller in bp size) labeled peak (peak B). The label will be removed from peak C if peak B meets both of the specified criteria: • Peak B is higher by at least 1166% • Peak B is within 2.25 to 3.75 bp The percentage value in this filtering option is calculated as follows: [(peak B – peak C) / peak C] x 100 = percentage value For example, if peak C = 149 RFU and peak B = 1886 RFU, then the percentage value is calculated as follows: [(1886– 149) / 149] x 100 = 1166% In this example, the label will be removed from peak C, provided that the filter option specifies a threshold of 1166%, and that peak B is within 2.25 to 3.75 bp of peak C. Conventionally, percent stutter is calculated: (peak C / peak B) x 100 = percent stutter The percentage value that is used in the Genotyper software filtering option (F) can be derived from the conventional percent stutter expression (S): F = (10,000 / S) – 100 For example, if the desired stutter percent threshold for DYS392 is 7.9%, then the percentage value that should be used in the Genotyper software filtering option is: F = (10,000 / 7.9) – 100 = 1166%
4.	To use a filter value different than 1166% for DYS392, enter another value, then click **Replace**.

Making Tables

IMPORTANT! Before making a table, examine all electropherograms and edit their peaks as described in the previous section.

To create and use tables:

1.	From the Macro list at the bottom of the Genotyper software Main Window, click one of the three table macros.
2.	Select **Macro** > **Run Macro**.
3.	Select **Views** > **Show Table Window** to view the table in full screen mode.
4.	Open and view the plot: **Note:** For all tables except the Make Allele Table, clicking in a cell of the table causes the corresponding sample electropherogram to appear in the plot window: • Click any cell in the table to display the locus region of the corresponding electropherogram in the Plot window for that sample. • Zoom out (**Ctrl+H**) to view all loci for a particular dye color for the corresponding sample.
5.	To edit the cells of the table: a. Click a cell of the table that contains an allele designation. b. Select **Edit** > **Edit Cell**. c. Type the desired information in the box and click **OK**.
6.	Print the table by selecting **File** > **Print**.
7.	Optional: Select **Table** > **Export to File** to save the table as a file that can be opened in Microsoft Excel.
8.	Select **File** > **Save** to save the template file with data.

Manual Genotyping Against the AmpFℓSTR Yfiler Kit Allelic Ladder

About the AmpFℓSTR Yfiler Kit Allelic Ladder

The AmpFℓSTR® Yfiler™ Kit Allelic Ladder contains the most common alleles for each locus.

The macro size ranges include the actual number of nucleotides contained in the smallest and largest allelic ladder alleles for each locus. The size range also includes the 3′+A nucleotide.

The AmpFℓSTR Yfiler PCR Amplification Kit is designed so that most PCR products contain the non-templated 3′+A nucleotide. Alleles have been named following the nomenclature used in the NIST Standard Reference Material 2395 for Human Y Chromosome DNA Profiling Standard. The nomenclature for DYS635 was based on Kayser et al, 2004.

The number of complete four base pair repeat units observed is designated by an integer. Variant alleles that contain a partial repeat are designated by a decimal followed by the number of bases in the partial repeat. For example, a DYS385 14.2 allele contains 14 complete four base pair repeat units and a partial repeat unit of two base pairs.

Additional variation has been seen at some loci where alleles that differ from integer allele lengths by one or three base pairs exist. For example, DYS385 allele 16.3 contains 16 complete repeat units and a partial 3-bp unit (Schoske *et al*., 2004).

The table below lists the loci included in the AmpFℓSTR Yfiler Allelic Ladder.

Table 5-6 AmpFℓSTR Yfiler Kit loci and alleles

Locus Designation	Alleles Included in Yfiler Kit Allelic Ladder[a]	Dye Label	DNA 007 Genotype
DYS456	13—18	6-FAM™	15
DYS389I	10—15		13
DYS390	18—27		24
DYS389II	24—34		29

Table 5-6 AmpFℓSTR Yfiler Kit loci and alleles *(continued)*

Locus Designation	Alleles Included in Yfiler Kit Allelic Ladder[a]	Dye Label	DNA 007 Genotype
DYS458	14—20	VIC®	17
DYS19	10—19		15
DYS385 a/b	7—25		11,14
DYS393	8—16	NED™	13
DYS391	7—13		11
DYS439	8—15		12
DYS635	20—26		24
DYS392	7—18		13
Y GATA H4	8—13	PET®	13
DYS437	13—17		15
DYS438	8—13		12
DYS448	17—24		19

a. See "About the AmpFℓSTR Yfiler Kit Allelic Ladder" on page 5-53 for more information about the Yfiler Kit allelic ladder.

A Genotyper software electropherogram of the AmpFℓSTR Yfiler Allelic Ladder listing the designation for each allele is shown in Figure 5-3. This electropherogram indicates the designation for each allele. Results were obtained on an ABI PRISM 3100 instrument.

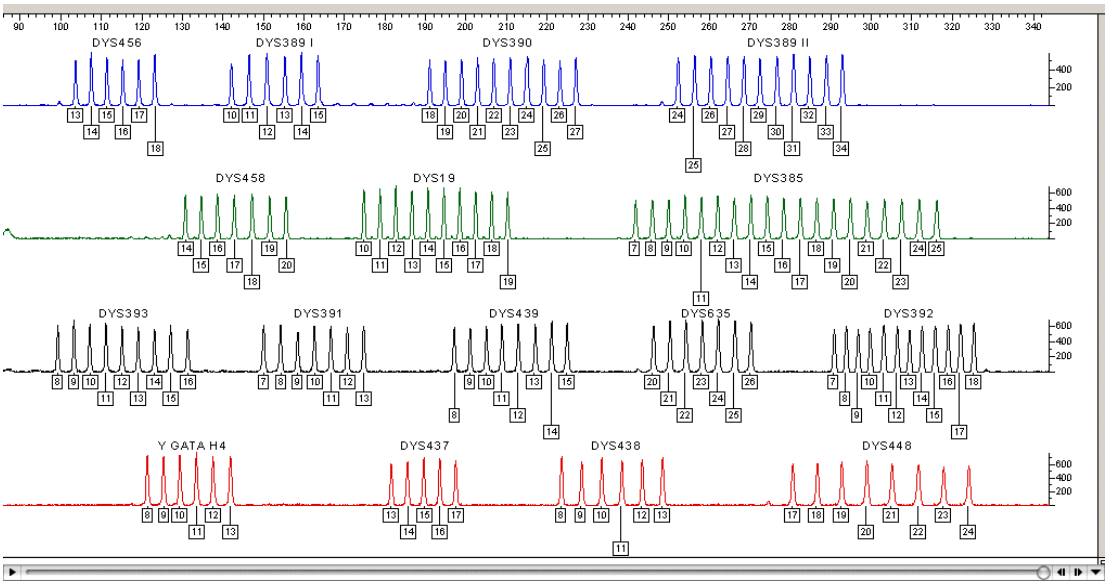


Figure 5-3 Genotyper® software plot of the AmpFℓSTR Yfiler Allelic Ladder

Genotyping Based on the AmpFℓSTR Yfiler Allelic Ladder

Genotyper software assigns genotypes to sample alleles by comparing their sizes to those obtained for the known alleles in the AmpFℓSTR Yfiler Allelic Ladder. *Genotypes*, not sizes, are used for comparison of data between runs, instruments, and laboratories.

Applied Biosystems strongly recommends that laboratories use an AmpFℓSTR Yfiler Allelic Ladder from the specific electrophoresis run to convert the allele sizes to genotypes because:

- The size values obtained for the same sample can differ between instrument platforms because of differences in the type and concentration of the gel/polymer matrices and in electrophoretic conditions.
- The size values obtained for the same sample can differ between protocols for the same instrument platform because of differences in gel or polymer concentration, run temperature, gel or capillary thickness, and well-to-read length.

- Slight procedural and reagent variations between gels or between single and multiple capillaries result in greater size variation than that found between samples on the same gel or between samples injected in the same capillary in a single run.

About the Size Standard and Sizing Method

The Genotyper software uses the internal lane size standard included in every sample (that is, both PCR products and allelic ladder samples) to normalize lane-to-lane or injection-to-injection migration differences. Running an internal size standard ensures sizing precision within a gel or within a set of capillary injections.

Because the common alleles for all AmpFℓSTR Yfiler kit loci are less than 400 base pairs, you can use the GeneScan-500 LIZ® Size Standard.

Applied Biosystems recommends that you use the Local Southern sizing method, which uses two internal lane size standard peaks larger than each allele and two smaller than each allele to be sized.

Note: When defining size standard peaks during routine analysis, you should include the 350 and 400 bp peaks of the size standard.

Manual Genotyping

The Kazam macros automatically assign allelic ladder sizes and determine sample genotype. However, you can perform both tasks manually, as explained in the following procedure.

To perform manual genotyping:

1.	Size the AmpFℓSTR Yfiler Allelic Ladder alleles. Compare the base pair sizes of one lane or injection of allelic ladder to those obtained for the other lanes or injections of allelic ladder. All corresponding peaks (peaks at the same position in the allelic ladder) should be within ± 0.5 bp of each other. If one or more corresponding peaks are not within ± 0.5 bp of each other, check the GeneScan-500 LIZ Size Standard peaks in all allelic ladder lanes or injections to confirm that all GeneScan-500 LIZ Size Standard peaks have been assigned the correct size and/or that all peaks are clearly resolved.

To perform manual genotyping: ***(continued)***

2.	Select one lane or injection of allelic ladder to use for genotyping. Applied Biosystems' studies have shown that it does not matter which lane or injection of allelic ladder is selected if the alleles in the allelic ladder samples are within ± 0.5 bp of each other.
3.	Compare the base pair size obtained for each sample allele peak to the sizes obtained for the allelic ladder peaks.
4.	Assign genotypes to those sample allele peaks falling within ± 0.5 bp of the corresponding allelic ladder peak. The allele designation for each allelic ladder peak is given in Figure 5-3 on page 5-55.

The AmpFℓSTR Yfiler Allelic Ladder contains most alleles for the DYS19, DYS385, DYS389 I, DYS389 II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 (Y GATA C4), and Y GATA H4 loci. However, alleles not found in the AmpFℓSTR Yfiler Allelic Ladder do exist. These off-ladder alleles may contain full and/or partial repeat units. An off-ladder allele should flag itself by not falling inside the ± 0.5 bp window of any known allelic ladder allele.

Note: If a sample allele peak is found to be ≥ 0.5 bp from the corresponding allelic ladder peak, the sample must be rerun to verify the result.

Section 5.5 Interpretation of Haplotype Data

This section covers:

Overview

The Yfiler Haplotype Database online search tool allows you to estimate the frequency of a given Y chromosome haplotype in specified populations. Frequency calculations are based on the haplotype data generated from the 17 loci included in the AmpFℓSTR® Yfiler™ Kit. This data was compiled from more than 2,000 samples from a range of populations.

The Yfiler Haplotype Database can be used to search complete or partial profiles generated with the Yfiler Kit and enables comparison of the discrimination capacity of the Yfiler Kit relative to other combinations of Y STR loci (e.g., European Minimal Haplotype and SWGDAM loci).

Functions of the Web-Based Search Tool

The Yfiler Kit Haplotype Database online search tool has been designed to allow searches of haplotypes generated using the AmpFℓSTR Yfiler Kit. The tool will allow the user to estimate the frequency of occurrence of a haplotype in a number of reference populations. The tool allows manual input and input of allele tables generated in GeneMapper® *ID* v3.2 and Genotyper. In addition, the user may search profiles containing microvariant alleles and display resulting matches.

Allele Representation

Table 5-7 Allele representation in the Haplotype Database

Type	Example
Singlet	‘7’ or ‘8.2’
Doublet (all except DYS385)	‘11, 12’ or ‘13, 14.5’ Each allele in the doublet must have a different value. Either or both values can be microvariants.
Doublet (DYS385)	‘11, 12’ or ‘14,14’ Alleles can have different values or they can have the same value. Either or both values can be microvariants
Triplet (DYS385 only)	‘13,15,17’

Table 5-7 Allele representation in the Haplotype Database

Type	Example
Microvariant	'7.5', > *n*, < *m* n = largest ladder allele m = smallest ladder allele
*(Wild card)	Indicates that the locus will be omitted from the search.

Searching the Database

Searching for a Haplotype by Entering Allele Information

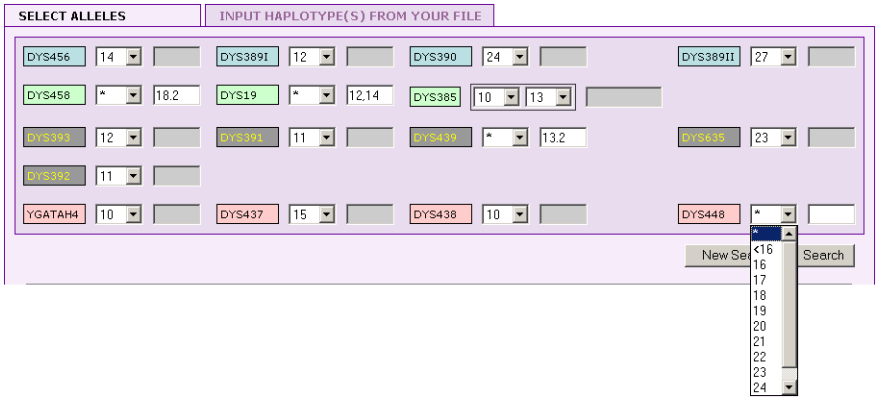
The Yfiler Kit Haplotype Database Search Tool provides two modes for searching the haplotype database:

- **Default Mode** – Allows you to specify a single allele value for each of the 17 loci included in the AmpFℓSTR Yfiler kit by selecting from a drop-down list of the most common alleles for each locus.

Table 5-8 Types of allele values for Default Mode

Drop-down List Values	Description
Integers representing the most common alleles for the loci	For example, the dropdown list includes the numbers 16 to 24 for the DYS448 locus.
* (Wildcard)	Indicates that the locus will be omitted from the search.
< *m*, where *m* is the smallest allele in the allele range	Indicates that alleles smaller than *m* will be included in the search. For example, in Figure 5-4 below, alleles smaller than 16 will be included.
> *n*, where *n* is the largest allele in the allele range	Indicates that alleles larger than *n* will be included in the search. For example, in Figure 5-4 below, alleles larger than 24 will be included.

Figure 5-4 Different Custom Entry Options



- **Custom Entry Mode** – Allows you to enter up to three comma-separated values for DYS385 and two comma-separated values for all other loci. Use the Custom Entry Mode to search for:
  - Alleles that fall within the size range for the locus but that are not included in the dropdown list (microvariants). An example would be if the size range for a locus is 14 to 20 and the allele you want to search for is 18.2.
  - Loci that have two alleles of different sizes (doublets).
  - DYS385 haplotypes containing three alleles (triplets).

Note: Occasionally three or more alleles may be detected in loci other than DYS385 and four or more alleles may be detected for DYS385. This type of variant cannot be searched and a wild type value (*) should be substituted.

To search for a haplotype:

1.	Go to the Yfiler Haplotype Database Search Tool: www.appliedbiosystems.com/yfilerdatabase

To search for a haplotype: *(continued)*

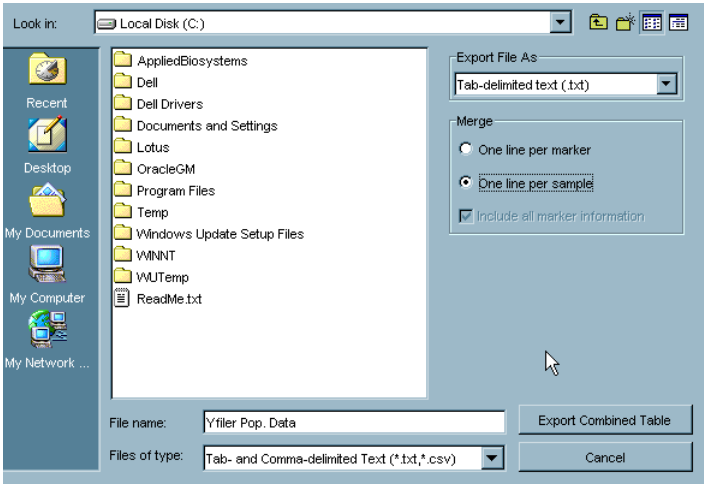
2.	Specify allele values for the loci that you want to include in the search. Use the <toggle icon> to switch between modes. • If using Default Mode, select a value for the allele, using the drop-down list (see Table 5-8 on page 5-62). • If using Custom Entry Mode, enter up to three comma-separated values (including noninteger values, for example 18.2). • You can specify values for one to up to all 17 loci. If you do not specify a value for a locus, that locus is omitted from the search. • In a single search query, you can use Default Mode for some loci and Custom Entry Mode for other loci as required by your data.
3.	Click **Search**.
4.	Review the results as explained in "Reviewing Results" on page 5-69.

Searching for a Haplotype by Uploading Analysis Files

You can upload information from allele tables created from GeneMapper *ID* and Genotyper software.

To upload haplotypes from allele tables generated in GeneMapper ID Software v3.2:

1.	Under the Tools menu, select the **Table Setting Editor.**
2.	In the Genotypes tab, select the following Column Settings: • Sample Name • Marker • Allele
3.	In the Samples tab, hide all columns. Note: The table settings may be saved by creating a new table. Using the pulldown menu adjacent to "Table Setting", select "New..." and name the table, for example, Yfiler Upload Table. Select settings and click **OK** to save.

4.	While in the Samples tab, click the File menu and select **Export Combined Table**. Select the **One line per sample** option under Merge. Name the file and save as Tab and Comma-delimited text (*.txt, *.csv). 
5.	Using a text editor such as Notepad or Wordpad, modify the text table for upload by deleting allelic ladder samples and other samples that do not need to be included in the search (such as known reference or control samples).
6.	Check the table for microvariants: • Microvariants whose sizes are within the size range for the locus are designated as “OL”. This designation may be changed based on confirmation of the microvariant designation (e.g. 17.2). • Microvariants whose sizes are outside the size range for the locus are designated as 0 (no value). This designation may be changed to < m, where m is the smallest allele in the ladder range for a locus or > n, where n is the largest allele in the ladder range for a locus.

7.	Resave the amended version of the table to be searched as a .txt file.

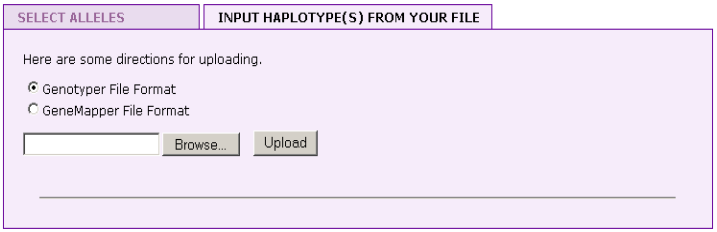
Note: Opening the text file in Excel and resaving as a .txt file will result in a format which is not compatible with the upload program.

To upload haplotypes from allele tables generated in Genotyper Software:

1.	Run the **Make Allele Table** macro.
2.	Under the Table menu, select **Export to File**. Save the file as [Name].txt.
3.	Open the file in Excel and follow the onscreen instructions to convert the tab delimited file to an Excel table. Delete the allelic ladder sample and other samples that do not need to be included in the search (such as known reference or control samples).
4.	Check the table for microvariants: • Microvariants whose sizes are within the size range for the locus are designated as "OL allele?" This designation may be changed based on confirmation of the microvariant designation (e.g. 17.2). • Microvariants whose sizes are outside the size range for the locus are designated as 0. This designation may be changed to < m, where m is the smallest allele in the ladder range for a locus or > n, where n is the largest allele in the ladder range for a locus.
5.	Resave the amended version of the table to be searched in .txt format.

To upload tables into the Search tool:

1.	In the Search tool, select **Input Haplotype(s) from your file**.

2.	Select **Genotyper File Format** or **GeneMapper File Format** and browse to find the file. 
3.	Select **Upload** to input the table haplotypes. Selection of the haplotype ID will populate the manual entry screen with the selected haplotype.
4.	Select **Search** to search the population database for matching haplotypes.

Reviewing Results

Select Allele Search Results

An example search result is shown in Figure 5-5.

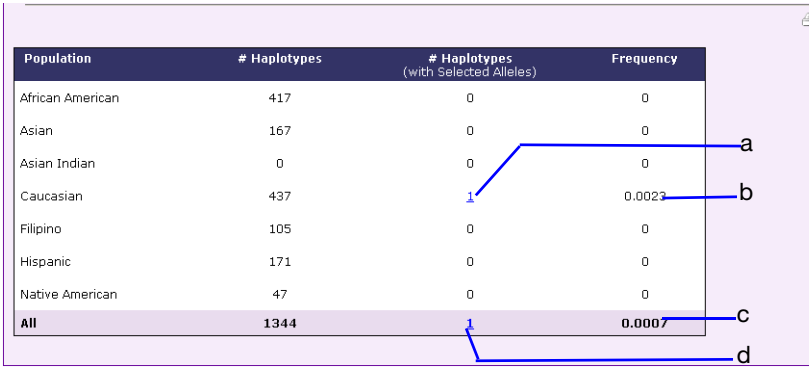


Figure 5-5 Search result example

The table provides information about the frequency of the specified haplotype within individual populations and within the entire database.

a. Indicates that, for the specified loci, one match was found in the Caucasian population.

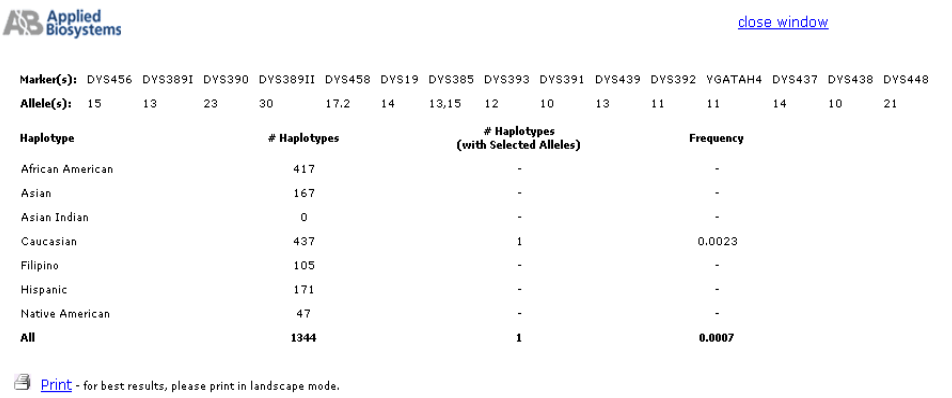
b. Indicates the frequency of the specified haplotype within the Caucasian population.

c. Indicates the frequency of the specified haplotype within the entire database.

d. Indicates that, for the specified loci, one match was found in the entire database.

Printable Search Results

Figure 5-6 illustrates the printable search result, which contains the haplotype loci specified in the search and the haplotype frequency results.



Applied Biosystems

close window

Marker(s):	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385	DYS393	DYS391	DYS439	DYS392	YGATAH4	DYS437	DYS438	DYS448
Allele(s):	15	13	23	30	17.2	14	13,15	12	10	13	11	11	14	10	21

Haplotype	# Haplotypes	# Haplotypes (with Selected Alleles)	Frequency
African American	417	-	-
Asian	167	-	-
Asian Indian	0	-	-
Caucasian	437	1	0.0023
Filipino	105	-	-
Hispanic	171	-	-
Native American	47	-	-
All	**1344**	**1**	**0.0007**

Print - for best results, please print in landscape mode.

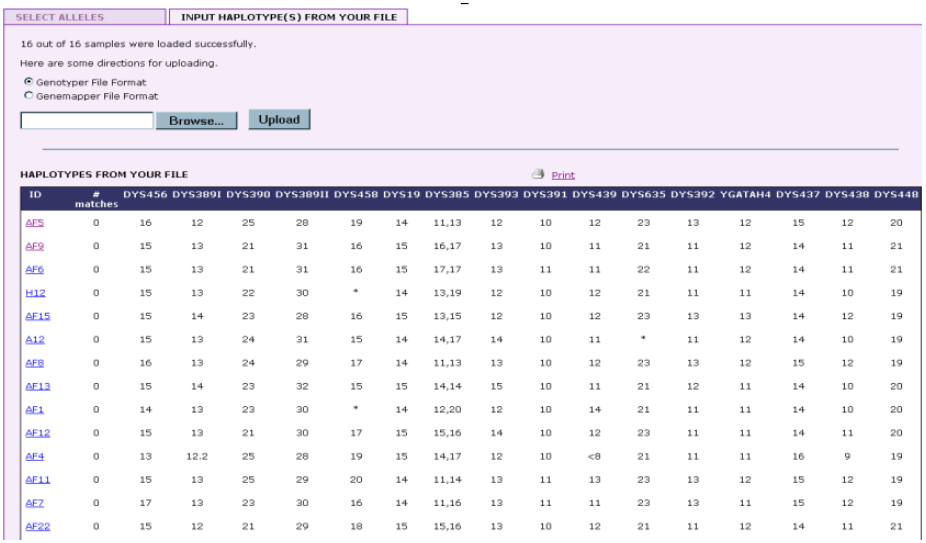
Figure 5-6 Example of a printable search result

Results for Uploaded Allele Information

When you specify an analysis file and click Search, the portal search tool compares each sample in the file against the database. Results are displayed in two stages.

First, the portal displays a table that lists the haplotypes from the uploaded file, as shown in Table 5-9

Table 5-9 Results for uploaded allele



SELECT ALLELES | INPUT HAPLOTYPE(S) FROM YOUR FILE

16 out of 16 samples were loaded successfully.

Here are some directions for uploading.

Genotyper File Format
Genemapper File Format

Browse... Upload

HAPLOTYPES FROM YOUR FILE — Print

ID	# matches	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385	DYS393	DYS391	DYS439	DYS635	DYS392	YGATAH4	DYS437	DYS438	DYS448
AF5	0	16	12	25	28	19	14	11,13	12	10	12	23	13	12	15	12	20
AF9	0	15	13	21	31	16	15	16,17	13	10	11	21	11	12	14	11	21
AF6	0	15	13	21	31	16	15	17,17	13	11	11	22	11	12	14	11	21
H12	0	15	13	22	30	*	14	13,19	12	10	12	21	11	11	14	10	19
AF15	0	15	14	23	28	16	15	13,15	12	10	12	23	13	13	14	12	19
A12	0	15	13	24	31	15	14	14,17	14	10	11	*	11	12	14	10	19
AF8	0	16	13	24	29	17	14	11,13	13	10	12	23	13	12	15	12	19
AF13	0	15	14	23	32	15	15	14,14	15	10	11	21	12	11	14	10	20
AF1	0	14	13	23	30	*	14	12,20	12	10	14	21	11	11	14	10	20
AF12	0	15	13	21	30	17	15	15,16	14	10	12	23	11	11	14	11	20
AF4	0	13	12.2	25	28	19	15	14,17	12	10	<8	21	11	11	16	9	19
AF11	0	15	13	25	29	20	14	11,14	13	11	13	23	13	12	15	12	19
AF7	0	17	13	23	30	16	14	11,16	13	11	11	23	13	11	15	12	19
AF22	0	15	12	21	29	18	15	15,16	13	10	12	21	11	12	14	11	21

The first column in Table 5-9 indicates the sample name. The second column indicates the number of haplotype matches for each specified haplotype. The succeeding columns indicate the allele values for each of the 17 AmpFℓSTR Yfiler loci for the samples.

Clicking on the sample name in the "ID" column results in population of the custom entry fields in the Select Alleles tab and provides frequency information and number of matching haplotypes as for manual database queries.

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Experiments and Results 6

This chapter covers:

Overview

Experiments Using AmpFℓSTR Yfiler PCR Amplification Kit

This chapter provides results of the developmental validation experiments performed by Applied Biosystems using the AmpFℓSTR Yfiler PCR Amplification Kit.

Importance of Validation

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

Experiments

Experiments to evaluate the performance of the AmpFℓSTR Yfiler PCR Amplification Kit were performed at Applied Biosystems. These 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. The DAB defines a laboratory as a facility in which forensic DNA testing is performed.

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 has 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., 2001).

This chapter discusses many of the experiments performed by Applied Biosystems and provides examples of results obtained. Conditions were chosen which produced maximum PCR product yield and a window in which reproducible performance characteristics were met. It is our opinion that while these experiments are not exhaustive they are appropriate for a manufacturer. Each laboratory using the AmpFℓSTR Yfiler PCR Amplification Kit should perform internal validation studies.

Developmental Validation

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 2.10.1

"The reaction conditions needed to provide the required degree of specificity and robustness must be determined. These include thermocycling parameters, the concentration of primers, magnesium chloride, DNA polymerase, and other critical reagents." (SWGDAM, July 2003)

PCR Components

The concentration of each component of the AmpFℓSTR Yfiler kit was examined. The PCR components are Tris-HCl (pH 8.3), KCl, dNTPs, primers, AmpliTaq Gold® DNA polymerase, $MgCl_2$, bovine serum albumin, and sodium azide. The concentration for a particular component was established to be in the window that meets the reproducible performance characteristics of specificity and sensitivity. Various magnesium chloride concentrations were tested on the ABI PRISM 3100 Genetic Analyzer. The amplification of 1 ng of male genomic DNA is shown in Figure 6-1. The performance of the multiplex is most robust within a ± 20% window of magnesium chloride.

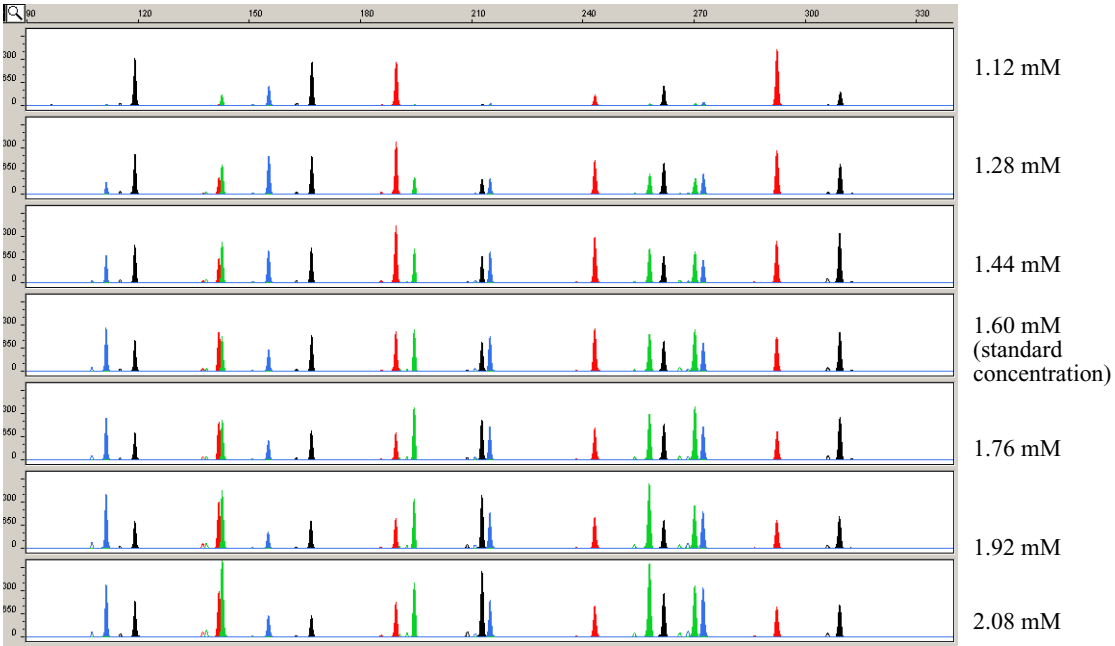

Figure 6-1 1 ng genomic DNA amplified with the AmpFℓSTR Yfiler Kit in the presence of varying concentrations of magnesium chloride: 1.12 mM, 1.28 mM, 1.44 mM, 1.6 mM, 1.76 mM, 1.92 mM and 2.08 mM, analyzed on the ABI PRISM 3100 Genetic Analyzer

Thermal Cycler Parameters

Thermal cycling parameters were established for amplification of the AmpFℓSTR Yfiler Kit. Thermal cycling times and temperatures of GeneAmp PCR systems were verified. Varying annealing and denaturation temperature windows were tested to verify that a ±1.0 °C window produced a specific PCR product with the desired sensitivity of at least 1 ng of AmpFℓSTR Control DNA 007.

The denaturation temperatures tested were 92.5 °C, 94 °C and 95.5 °C, all for 1-minute hold times on the same Silver 96-Well GeneAmp PCR System 9700. The annealing temperatures tested were 59, 60, 61, 62, and 63 °C (Figure 6-2), also for 1-minute hold times in the Silver 96-Well GeneAmp PCR System 9700. The PCR products were analyzed using the ABI PRISM 3100 Genetic Analyzer.

No preferential amplification was observed in the denaturation temperature experiments. Of the tested annealing temperatures, 59 - 62 °C produced robust profiles. At 63 °C the yield of the majority of loci was significantly reduced. Routine thermal cycler calibration is recommended when following the amplification protocol. No preferential amplification was observed at the standard annealing temperature of 61 °C.

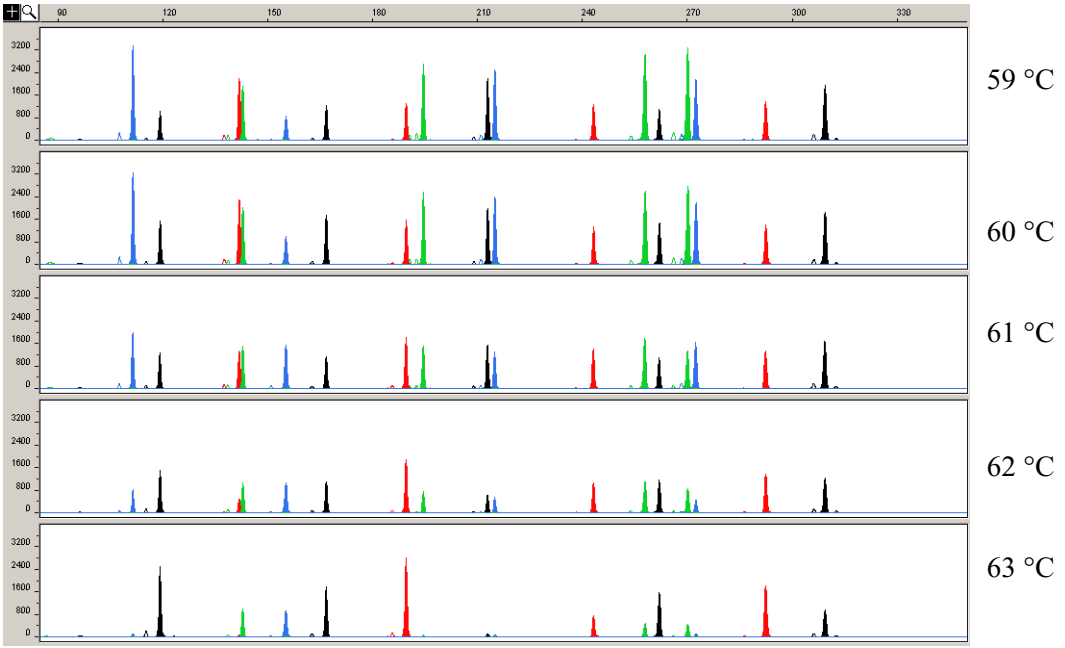

Figure 6-2 Electropherograms obtained from amplification of 1 ng genomic DNA at annealing temperatures of 59 °C, 60 °C, 61 °C, 62 °C, and 63 °C, analyzed on the ABI PRISM 3100 Genetic Analyzer

PCR Cycle Number

AmpFℓSTR Yfiler kit reactions were amplified for 28, 29, 30, 31 and 32 cycles on the Silver 96-Well GeneAmp® PCR System 9700 using 1.0 ng of three male DNA samples. As expected, PCR product increased with the number of cycles. A full profile was generated at 28 cycles; off-scale data were collected for several allele peaks at 32 cycles.

While none of the cycle numbers tested produced nonspecific peaks, 30 cycles was found to give optimal sensitivity when the amplified products were examined on ABI PRISM 3100 Genetic Analyzers. At 30 cycles, high ratios of female to male DNA amplify reliably and specifically following the conditions outlined in this user manual (Figure 6-15 on page 6-35).

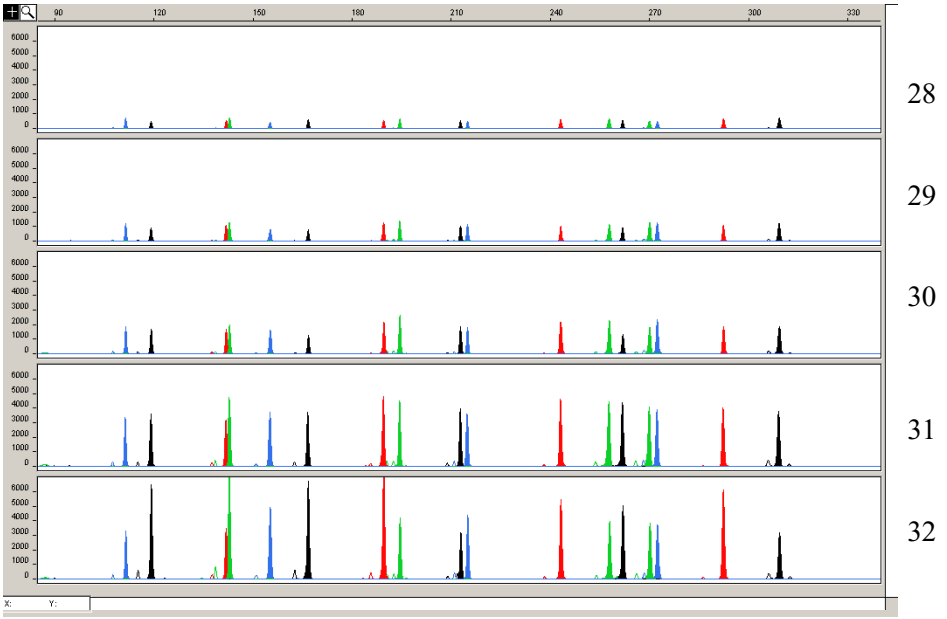

Figure 6-3 Yfiler profiles obtained from amplification of 1 ng DNA template using 28, 29, 30, 31, and 32 cycles, analyzed on the ABI PRISM 3100 Genetic Analyzer.

Accuracy, Precision, and Reproducibility

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)

Laser-induced fluorescence detection of length polymorphism at short tandem repeat loci is not a novel methodology (Holt et al., 2001; and Wallin et al., 2002). However, accuracy and reproducibility of AmpFℓSTR Yfiler kit profiles have been determined from various sample types.

Figure 6-4 illustrates the size differences that are typically observed between sample alleles and allelic ladder alleles on the ABI PRISM 3100 Genetic Analyzer with POP-4™ polymer. The x-axis in Figure 6-4 represents the nominal base pair sizes for the AmpFℓSTR Yfiler Allelic Ladder, and the dashed lines parallel to the x-axis represent the ±0.5-bp windows. The y-axis is the deviation of each sample allele size from the corresponding allelic ladder allele size. All sample alleles are within 0.5 bp of a corresponding allele in an allelic ladder.

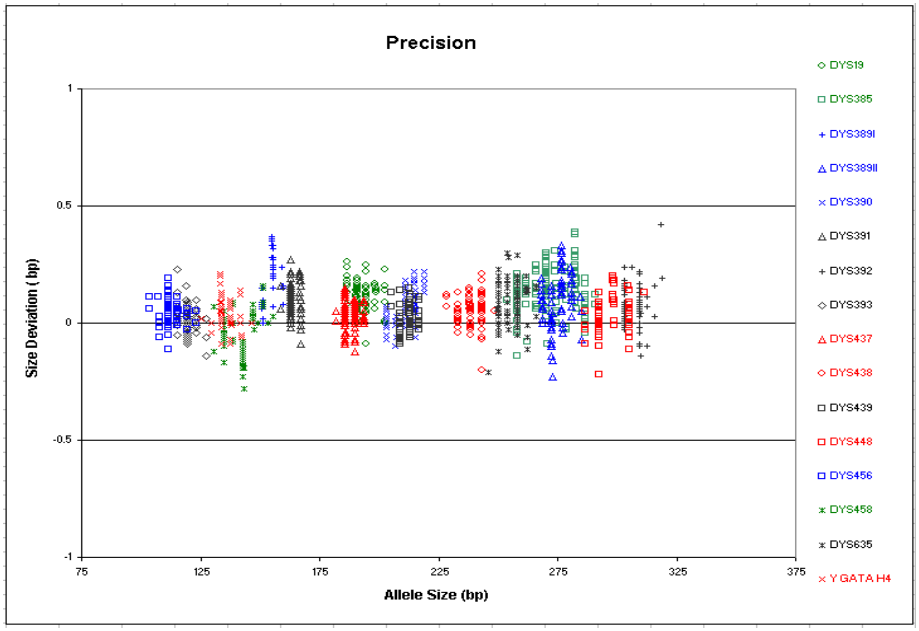

Figure 6-4 Size deviation of 78 samples analyzed on the ABI PRISM 3100 Genetic Analyzer

Precision and Size Windows

Sizing precision allows for determining accurate and reliable genotypes. Sizing precision was measured on the ABI PRISM 310 Genetic Analyzer. As indicated in the Automated Genotyping section, the recommended method for genotyping is to employ a ±0.5-bp "window" around the size obtained for each allele in the AmpFℓSTR Yfiler Allelic Ladder. A ±0.5-bp window allows for the detection and correct assignment of alleles. Any sample allele that sizes outside a window could be either of the following:

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

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

Table 6-1 indicates typical precision results obtained from the nine injections of the AmpFℓSTR Yfiler Allelic Ladder analyzed on the ABI PRISM 310 Genetic Analyzer (47-cm capillary and POP-4 polymer). The internal lane size standard used was GeneScan®500 LIZ® Size Standard. These results were obtained within a set of injections on a single capillary.

As indicated above, sample alleles may occasionally size outside of the ±0.5-bp window for a respective allelic ladder allele because of measurement error. The frequency of such an occurrence is lowest in detection systems having the smallest standard deviations in sizing. Figure 6-4 on page 6-8 illustrates the tight clustering of allele sizes obtained on the ABI PRISM 3100 Genetic Analyzer, where the standard deviation in sizing is typically less than 0.15 bp. The instance of a sample allele sizing outside of the ±0.5-bp window because of measurement error is relatively rare when the standard deviation in sizing is approximately 0.15 bp or less (Smith, 1995).

For sample alleles that do not size within a ±0.5-bp window, the PCR product must be rerun to distinguish between a true off-ladder allele 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.

GeneMapper® *ID* v3.2 and Genotyper® softwares automatically flag sample alleles that do not size within the prescribed window around an allelic ladder allele.

It is important to note that while the precision within a gel or set of capillary injections is very good, the determined allele sizes vary between platforms. Cross-platform sizing differences arise from a number of parameters, including type and concentration of polymer mixture, run temperature, and electrophoresis conditions. Variations in sizing can also be found between runs on the same instrument and between runs on different instruments because of these parameters.

We strongly recommend that the allele sizes obtained be compared to the sizes obtained for known alleles in the AmpFℓSTR Yfiler Kit Allelic Ladder from the same run and then converted to genotypes (as described in the Automated Genotyping section). Refer to Table 6-1

for the results of injections of the AmpFℓSTR Yfiler Allelic Ladder. For more information on precision and genotyping, see Lazaruk et al., 1998 and Mansfield et al.,1998.

Table 6-1 Precision results of nine injections of the AmpFℓSTR Yfiler Allelic Ladder:

ABI PRISM 310 Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
DYS456		
13	104.51	0.05
14	108.31	0.05
15	112.16	0.04
16	116.04	0.04
17	119.90	0.05
18	123.82	0.05
DYS389I		
10	142.87	0.04
11	147.28	0.04
12	151.80	0.06
13	156.43	0.07
14	160.66	0.05
15	164.81	0.07
DYS390		
18	192.26	0.05
19	195.99	0.04
20	199.93	0.05
21	203.85	0.06

Table 6-1 Precision results of nine injections of the AmpFℓSTR Yfiler Allelic Ladder: *(continued)*

ABI PRISM 310 Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
22	207.83	0.05
23	211.90	0.04
24	215.90	0.05
25	219.88	0.06
26	223.84	0.06
27	227.80	0.07
DYS389II		
24	253.05	0.05
25	257.17	0.06
26	261.19	0.07
27	265.38	0.08
28	269.42	0.08
29	273.36	0.06
30	277.63	0.07
31	281.76	0.09
32	285.78	0.07
33	289.93	0.05
34	293.94	0.06
DYS458		
14	130.98	0.05
15	134.87	0.06

Table 6-1 Precision results of nine injections of the AmpFℓSTR Yfiler Allelic Ladder: *(continued)*

ABI PRISM 310 Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
16	138.81	0.03
17	142.95	0.05
18	147.31	0.05
19	151.72	0.05
20	155.94	0.04
DYS19		
10	176.06	0.07
11	179.98	0.05
12	183.84	0.05
13	187.76	0.03
14	191.64	0.05
15	195.49	0.05
16	199.32	0.05
17	203.20	0.06
18	207.09	0.07
19	211.02	0.06
DYS385 a/b		
7	242.79	0.05
8	246.89	0.07
9	250.94	0.04
10	254.98	0.07

Table 6-1 Precision results of nine injections of the AmpFℓSTR Yfiler Allelic Ladder: *(continued)*

ABI PRISM 310 Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
11	259.04	0.08
12	263.08	0.06
13	267.24	0.05
14	271.38	0.06
15	275.47	0.10
16	279.56	0.08
17	283.70	0.07
18	287.79	0.05
19	292.06	0.06
20	296.19	0.07
21	300.42	0.06
22	305.06	0.12
23	309.50	0.07
24	313.99	0.10
25	318.39	0.05
DYS393		
8	100.26	0.05
9	104.19	0.04
10	108.05	0.04
11	112.04	0.04
12	115.98	0.04

Table 6-1 Precision results of nine injections of the AmpFℓSTR Yfiler Allelic Ladder: *(continued)*

ABI PRISM 310 Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
13	119.89	0.04
14	123.89	0.04
15	127.80	0.05
16	131.95	0.04
DYS391		
7	150.88	0.08
8	155.27	0.06
9	159.67	0.06
10	163.83	0.05
11	167.94	0.07
12	172.00	0.07
13	176.03	0.06
DYS439		
8	197.84	0.05
9	201.70	0.03
10	205.68	0.05
11	209.46	0.04
12	213.47	0.03
13	217.41	0.03
14	221.42	0.05
15	225.17	0.04

Table 6-1 Precision results of nine injections of the AmpFℓSTR Yfiler Allelic Ladder: *(continued)*

ABI PRISM 310 Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
DYS635 (Y GATA C4)		
20	246.43	0.07
21	250.49	0.06
22	254.45	0.06
23	258.49	0.03
24	262.45	0.06
25	266.56	0.06
26	270.56	0.03
DYS392		
7	291.38	0.05
8	294.39	0.07
9	297.44	0.06
10	300.30	0.06
11	303.91	0.07
12	307.44	0.07
13	310.64	0.08
14	313.74	0.07
15	317.12	0.11
16	320.45	0.08
17	323.54	0.09
18	326.79	0.10

Table 6-1 Precision results of nine injections of the AmpFℓSTR Yfiler Allelic Ladder: *(continued)*

ABI PRISM 310 Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
Y GATA H4		
8	122.01	0.06
9	125.98	0.06
10	129.97	0.07
11	134.01	0.04
12	138.09	0.03
13	142.37	0.05
DYS437		
13	182.53	0.05
14	186.45	0.07
15	190.40	0.04
16	194.25	0.04
17	198.07	0.03
DY438		
8	223.69	0.06
9	228.68	0.06
10	233.63	0.07
11	238.59	0.06
12	243.63	0.05
13	248.66	0.05

Table 6-1 Precision results of nine injections of the AmpFℓSTR Yfiler Allelic Ladder: *(continued)*

ABI PRISM 310 Genetic Analyzer		
Allele	**Mean**	**Standard Deviation**
DYS448		
17	280.49	0.04
18	286.58	0.03
19	292.70	0.05
20	298.92	0.05
21	305.51	0.04
22	312.25	0.06
23	318.60	0.10
24	324.88	0.08

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, 2001). 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). It has been reported that the DYS19 tetranucleotide repeat locus displays the typical -4 bp stutter but also a -2 bp stutter (Prinz, et al., 2001; Gusmao, et al., 1999). The DYS392 trinucleotide repeat locus displays the typical -3 bp stutter but also a smaller +3 bp stutter. Sequence analysis of this +3 bp stutter revealed that the product contains an additional repeat unit relative to the true allele peak.

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 have been measured for amplified samples at the loci used in the AmpFℓSTR Yfiler kit. All data were generated on the ABI PRISM 3100 Genetic Analyzer.

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

- For each AmpFℓSTR Yfiler kit locus, the percent stutter generally increases with allele length, as shown in Figures 6-5 to Figures 6-8.
- 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* v3.2 and Genotyper softwares. 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 Figure 6-16 on page 6-37.
- The measurement of percent stutter for peaks that are off-scale may be unusually high.

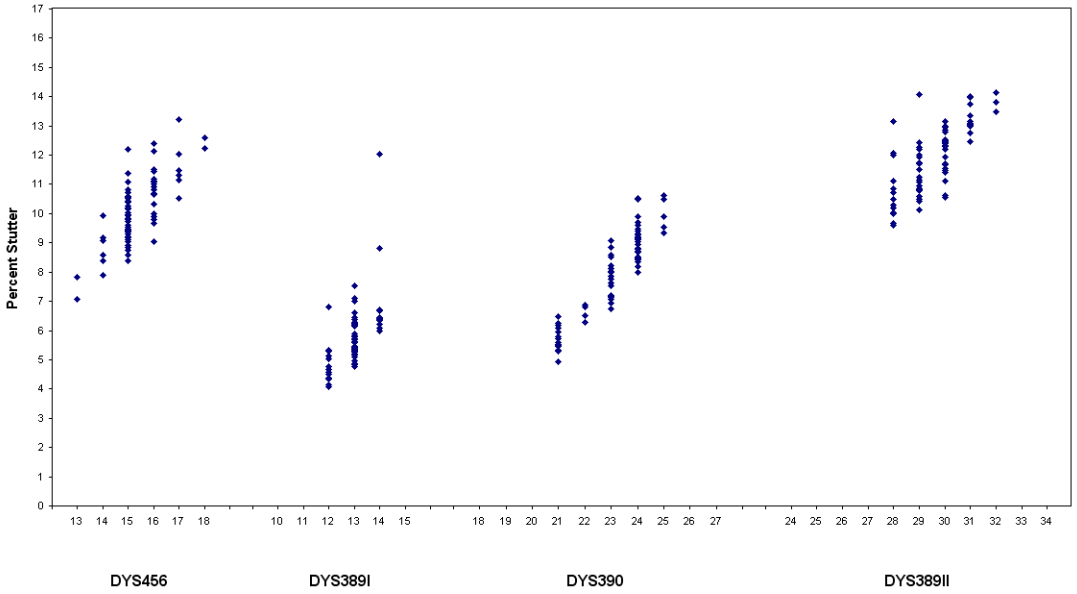

Figure 6-5 Stutter percentages for the DYS456, DYS389I, DYS390 and DYS389II loci

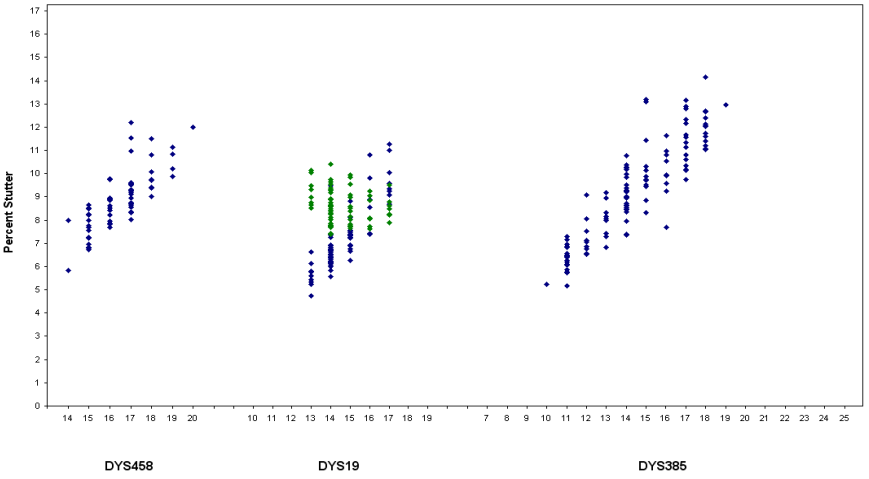

Figure 6-6 Stutter percentages for the DYS458, DYS19 and DYS385 loci. The -4 bp and -2 bp stutter percentages for DYS19 are shown in blue and green, respectively.

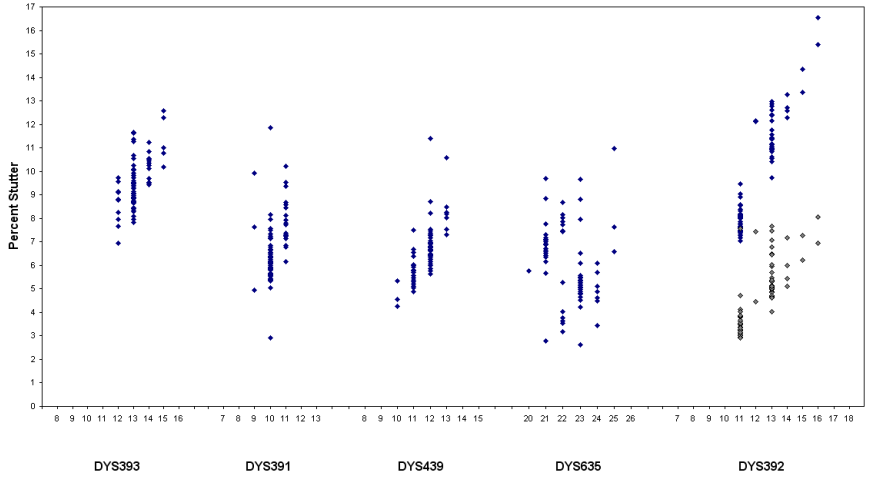

Figure 6-7 Stutter percentages for the DYS393, DYS391, DYS439, DYS635, DYS392 loci. The DYS392 (-3 bp) and (+3 bp) stutter percentages are shown in blue and grey respectively.

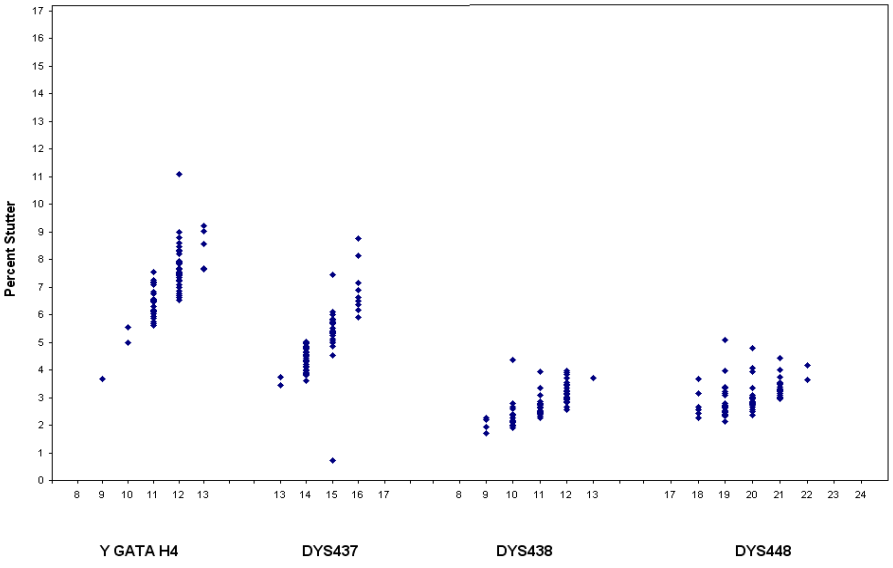

Figure 6-8 Stutter percentages for the Y GATA H4, DYS437, DYS438 and DYS448 loci

Addition of 3´ A Nucleotide

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

The efficiency of +A addition is related to the particular sequence of the DNA at the 3´ end of the PCR product. The AmpFℓSTR Yfiler 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 80 min.

This final extension step gives the AmpliTaq Gold® DNA polymerase additional time to complete +A addition to all double-stranded PCR products, especially in mixtures containing high levels of female DNA and low levels of male DNA. STR systems that have not been optimized for +A addition may have "split peaks", where each allele is represented by two peaks one base pair apart.

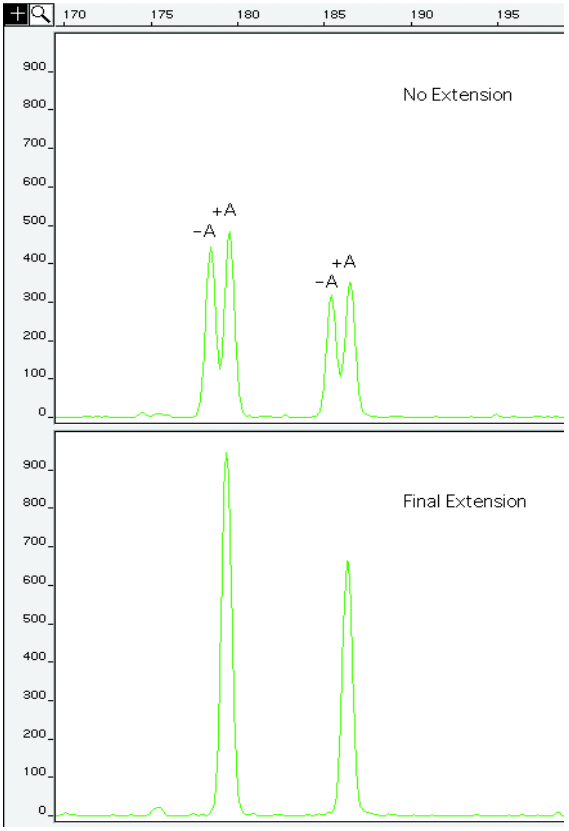

Figure 6-9 Omission of the final extension step resulted in split peaks due to incomplete A nucleotide addition. These data were generated on the ABI PRISM 310 Genetic Analyzer using another AmpFℓSTR kit

Lack of complete +A nucleotide addition may be observed in AmpFℓSTR Yfiler 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 or anomalies have been seen in data produced on genetic analyzers when using the AmpFℓSTR Yfiler kit. In amplified samples, artifacts in the non-calling region may appear in the green (88 bp), black (80 and 95 bp), and red (80 bp) dye. Low level artifacts in the calling region may or may not be reproducible.

Figure 6-10 demonstrates examples of baseline noise and artifacts in an electropherogram while using the AmpFℓSTR Yfiler kit. You should consider possible noise and artifacts when interpreting data from the AmpFℓSTR Yfiler kit on the ABI PRISM 3100 Genetic Analyzer.

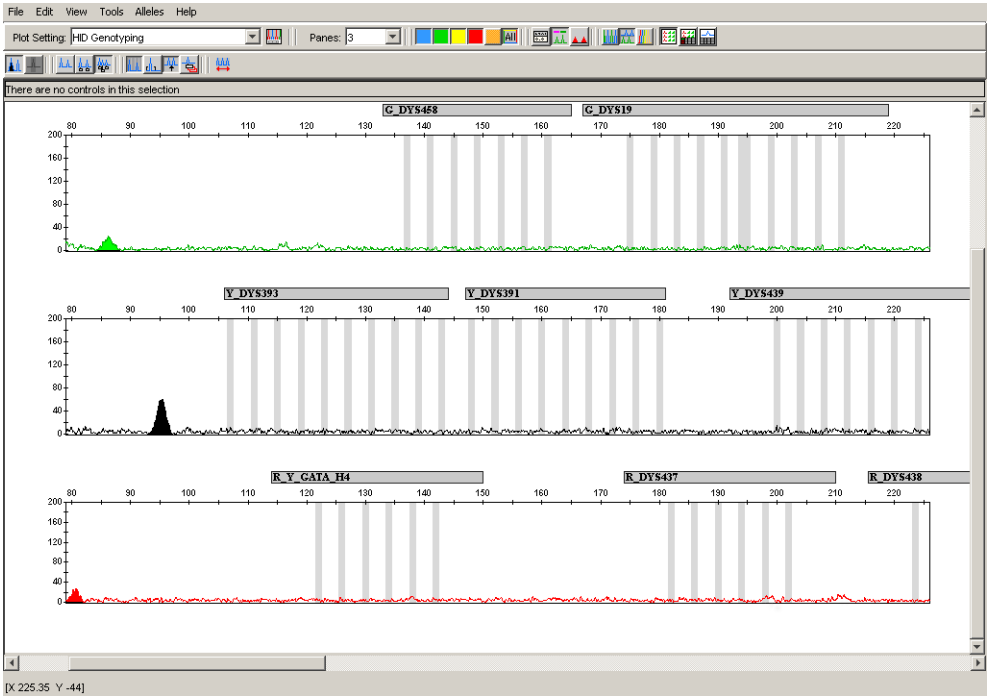

Figure 6-10 Examples of baseline noise and reproducible artifacts in data produced on the ABI PRISM 3100 Genetic Analyzer

Genotyping may result in the detection of these artifacts as off-ladder alleles, or "OL Alleles?". Note: A high degree of magnification (y-axis) is used in this figure to illustrate these artifacts (data produced on capillary electrophoresis instrument platforms).

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 17 loci that are amplified with the AmpFℓSTR Yfiler kit. These loci have been extensively characterized by other laboratories (Gusmao et al., 1999; Butler et al., 2002; Gonzalez-Neira et al., 2001; Hall and Ballantyne, 2003; Redd et al., 2002; Schoske et al., 2004).

Nature of the Polymorphisms

DYS392 is a trinucleotide repeat, DYS438 is a pentanucleotide repeat and DYS448 is a hexanucleotide repeat. Their allele differences result from differences in the number of repeat units 3-bp, 5-bp and 6-bp respectively. The remaining AmpFℓSTR Yfiler kit loci are tetranucleotide short tandem repeat (STR) loci. The length differences among alleles of these particular loci result from differences in the number of 4-bp repeat units.

All the alleles in the AmpFℓSTR Yfiler Allelic Ladder have been subjected to DNA sequencing at Applied Biosystems. In addition, other groups in the scientific community have sequenced alleles at some of these loci (Redd et al., 2002; www.cstl.nist.gov/biotech/strbase/y-strs.htm). Among the various sources of sequence data on the AmpFℓSTR Yfiler 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 39 families of Utah Mormon, French Venezuelan, and Amish descent. These DNA sets have been extensively studied all over the world and are routinely used to characterize the mode of inheritance of various DNA loci. Each family set contains three generations, generally including four grandparents, two parents, and several offspring. Consequently, the CEPH family DNA sets are ideal for studying inheritance patterns (Begovich et al., 1992).

Three CEPH family DNA sets were examined. One nanogram of DNA from each sample was amplified using the AmpFℓSTR Yfiler and AmpFℓSTR Identifiler kits, followed by analysis using an ABI PRISM® 3100 Genetic Analyzer. The families examined included #1333 (9 offspring, 7 males), #1340 (7 offspring, 5 males), and #1345 (7 offspring, 5 males), representing 23 meiotic divisions. The Identifiler results confirmed that the loci are inherited according to

Mendelian rules, as expected. The AmpFℓSTR Yfiler results confirmed that the loci were inherited according to a Y-linked (father to son) transmission. In no case was the maternal grandfather's Y-haplotype found in the offspring. In family #1345, one son (1345-7356) had a DYS458-18 allele while the rest of his male relatives had a DYS458-17 allele. In family #1340 one son (1340-7342) had a DYS458-16 allele while the rest of his male relatives had DYS458-17. Calculation of a mutation rate based on this small population size would be inaccurate due to the small sample size. The samples were reamplified and reinjected to confirm the allele call.

Mapping

The AmpFℓSTR Yfiler kit loci have been mapped and the chromosomal location on the Y-chromosome is known based on the nucleotide sequence of the Y-chromosome. The Genbank accession numbers for representative sequences are: DYS19 (X77751, AC017019), DYS385 (AC022486, Z93950), DYS389 (AC011289, AF140635), DYS390 (AC011289), DYS391 (G09613, AC011302), DYS392 (G09867, AC06152), DYS393 (G09601, AC06152), DYS437 (AC002992), DYS438 (AC002531), DYS439 (AC002992), DYS448 (AC025227.6), DYS456 (AC010106.2), DYS458 (AC010902.4), DYS635 (G42676, AC011751) and Y GATA C4 (G42673).

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 Yfiler kit provides the required degree of specificity for primates. Other species do not amplify for the loci tested.

Nonhuman Studies

Nonhuman DNA may be present in forensic casework samples. The AmpFℓSTR Yfiler kit provides the required degree of specificity for the species tested.The following experiments were conducted to investigate interpretation of AmpFℓSTR Yfiler kit results from nonhuman DNA sources.

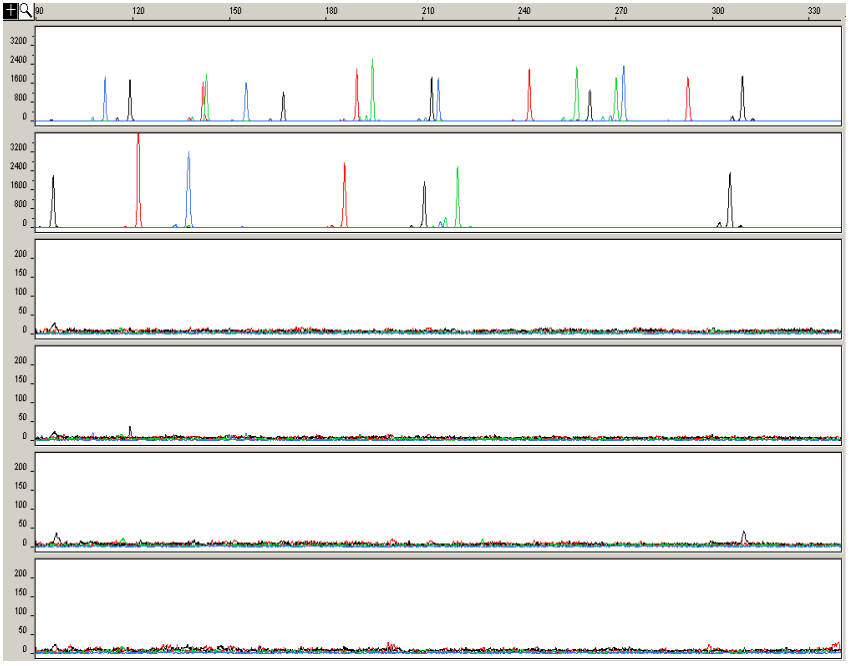

Figure 6-11 Representative electropherograms from a species specificity study including positive and non-template control (NTC).

The top panel shows a 1 ng amplification of the male control DNA 007, panel 2 chimpanzee (1 ng), panel 3 cat (10 ng), panel 4 dog (10 ng), panel 5 microbial DNA pool (5 ng each of Candida albicans, Neisseria gonorrhoeae, E coli 0157:H7, Bacillus subtilis and Lactobacillus rhamnosus) and the negative control. All samples were analyzed on an ABI PRISM 3100 Genetic Analyzer.

The extracted DNA samples were amplified with the AmpFℓSTR Yfiler kit and analyzed using the ABI PRISM 3100 Genetic Analyzer.

- Primates: gorilla, chimpanzee, orangutan, and macaque (1.0 ng each)
- Non-primates: mouse, dog, pig, cat, horse, chicken and cow (10 ng each)
- Microorganisms: *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Bacillus subtilis* and *Lactobacillus rhamnosus*.

The chimpanzee and gorilla DNA samples produced partial profiles within the 100-330 base pair region.

The remaining species tested did not yield reproducible detectable products.

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 amount of input DNA added to the AmpFℓSTR Yfiler PCR Amplification Kit should be between 0.5 and 1.0 ng. The DNA sample should be quantitated prior to amplification using a system such as the Quantifiler Human DNA and Quantifiler Y Human Male DNA Quantification Kit (P/N 4343895 and 4343906). The final DNA concentration should be in the range of 0.05-0.10 ng/µL so that 0.5-1.0 ng of DNA will be added to the PCR reaction in a volume of 10 µL. If the sample contains degraded DNA, amplification of additional DNA may be beneficial.

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 the following:

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

  Off-scale data is a problem for two reasons:

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

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

- Incomplete +A nucleotide addition

  The sample can be re-amplified using less DNA.

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

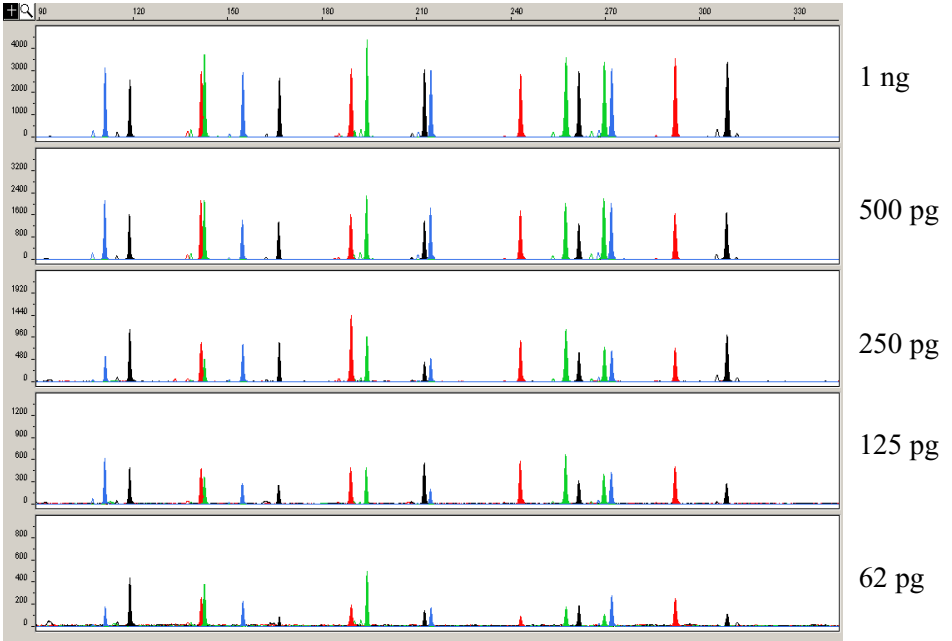

Figure 6-12 Effect of amplifying 1 ng, 500 pg, 250 pg, 125 pg and 62 pg of male control DNA 007.

Note that the y-axis scale is magnified for the lower amounts of DNA, analyzed using the ABI PRISM 3100 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)

Lack of Amplification of Some Loci

As with any multi-locus system, the possibility exists that not every locus will amplify. This situation is most often observed when the DNA sample contains PCR inhibitors or when the DNA sample has been severely degraded. Valuable information may be obtained from partial profiles.

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 DNA was incubated with the enzyme DNase I for varying amounts of time. The DNA was examined by agarose gel analysis to determine the average size of the DNA fragments at each time point.

Two nanograms of degraded DNA (or 1 ng undegraded DNA) was amplified using the AmpFℓSTR Yfiler kit. As the DNA became increasingly degraded, the loci became undetectable according to size. Preferential amplification was not observed. The loci failed to robustly amplify in the order of decreasing size as the extent of degradation progressed.

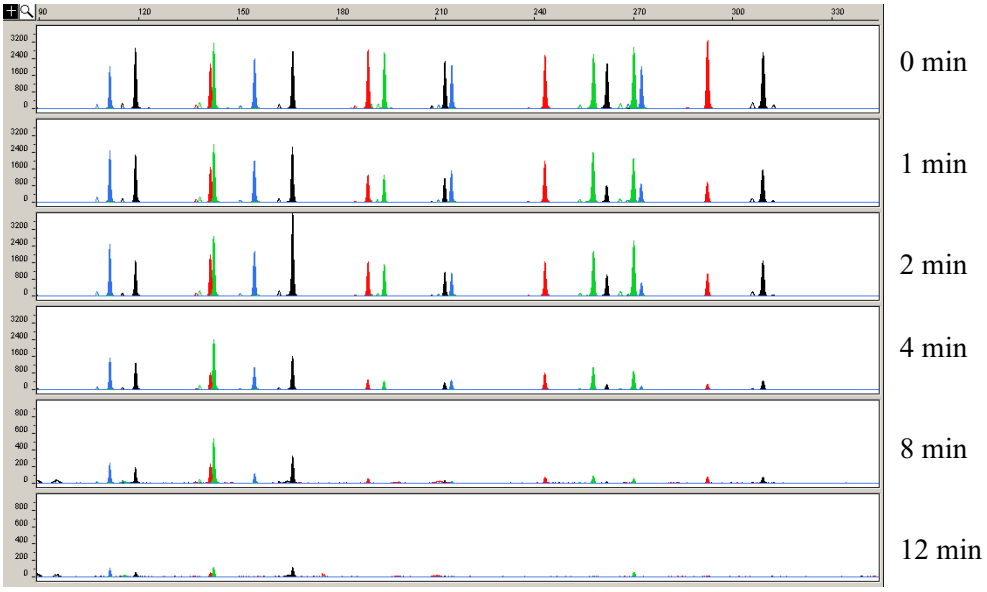

Figure 6-13 Multiplex amplification of DNA samples incubated with DNAseI. The top panel corresponds to 1 ng of DNA with no DNAseI added; panels 2-6 correspond to 2 ng of DNA incubated with DNAseI for 1, 2, 4, 8, and 12 minutes respectively.

Effect of Inhibitors

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

To examine the effects of hematin on the amplification results obtained by the AmpFℓSTR Yfiler kit, DNA samples were amplified using the AmpFℓSTR Yfiler kit reagents (including the BSA-containing PCR reaction mix) in the presence of increasing concentrations of purified hematin. The concentrations of hematin used were 0 µM, 10 µM, 12 µM, 16 µM, 20 µM, and 24 µM. No preferential amplification was observed in the presence of increasing amounts of hematin.

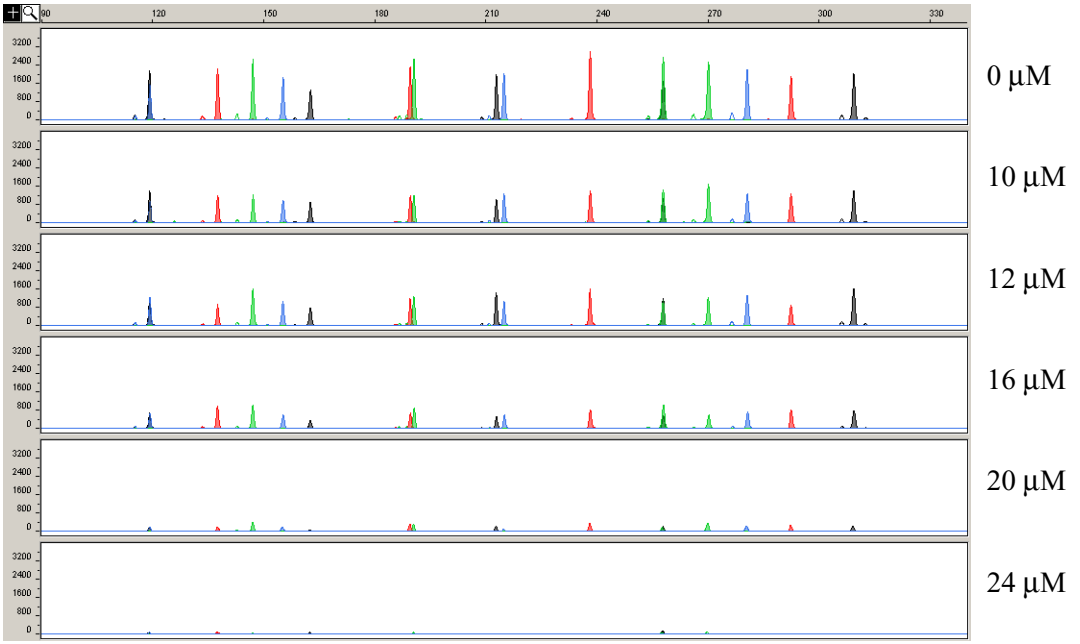

Figure 6-14 DNA amplified with the AmpFℓSTR Yfiler kit in the presence of varying concentrations of hematin: 0 µM, 10 µM, 12 µM, 16 µM, 20 µM, and 24 µM, analyzed on the ABI PRISM 3100 Genetic Analyzer

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 the results. We recommend that individual laboratories assign a minimum peak height threshold based on validation experiments performed in each laboratory to avoid typing when stochastic effects are likely to interfere with accurate interpretation of mixtures.

Male/Female 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 is able to detect DNA mixtures. In the case of Y-STRs, the female DNA component is not amplified by the Y-chromosome specific primers. Male/female mixture studies were performed up to a ratio of 1:2000 using three different female DNAs. The amount of female DNA was kept constant at 500 ng and the amount of male control DNA was changed. The female DNA did not cause any interference with the interpretation of the male Y-STR profile as shown in Figure 6-15.

Low level artifacts with female DNA have been occasionally observed in the black (136 bp) and red (291 bp) dye. In general, these artifacts peaks will not affect interpretation due to their intensity.

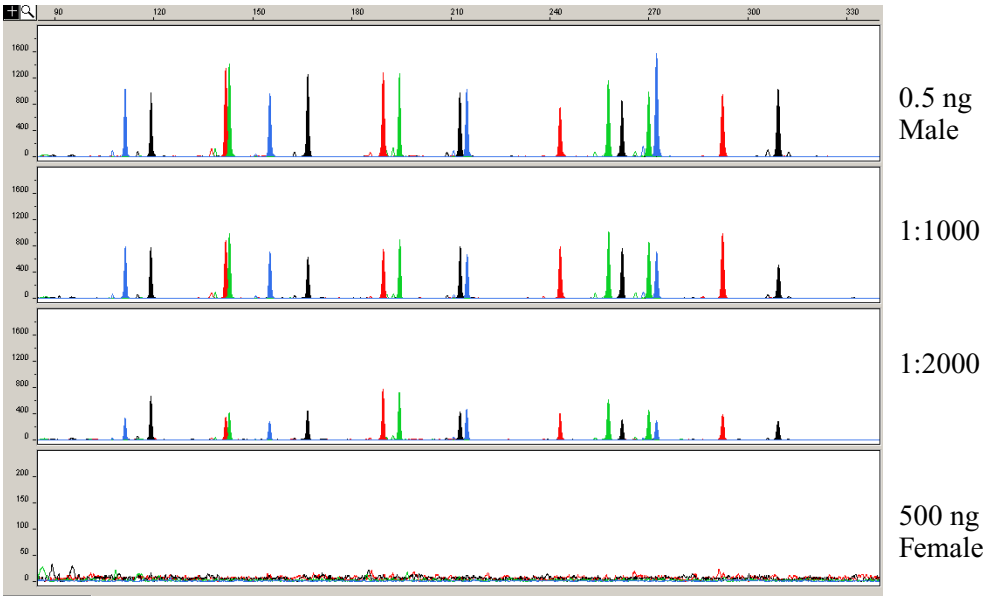

Figure 6-15 Amplification of Male Control DNA 007 in the presence of Female DNA 9947A. Profiles shown in the panels from top to bottom: 500 pg of male DNA, 500 pg male DNA with 500 ng female DNA, 250 pg male DNA with 500 ng female DNA, 500 ng female DNA.

Male/Male Mixture Studies

Forensic samples may contain body fluids or tissues originating from more than one male.

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

The samples were amplified in a Silver 96-Well GeneAmp® PCR System 9700 and were electrophoresed and detected using an ABI PRISM 3100 Genetic Analyzer.

The haplotypes of the samples in Figure 6-16 are the following:

Allele	Sample A	Sample B
DYS19	14	15
DYS385a	11	13
DYS385b	14	15
DYS389I	13	12
DYS389II	31	28
DYS390	24	23
DYS391	10	10
DYS392	13	11
DYS393	13	14
DYS437	15	16
DYS438	11	10
DYS439	12	13
DYS448	19	21
DYS456	17	15
DYS458	18	16
DYS635	23	22
Y GATA H4	12	12

The results of the mixed DNA samples are shown in Figure 6-16 where sample A and sample B were mixed according to the ratios provided.

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

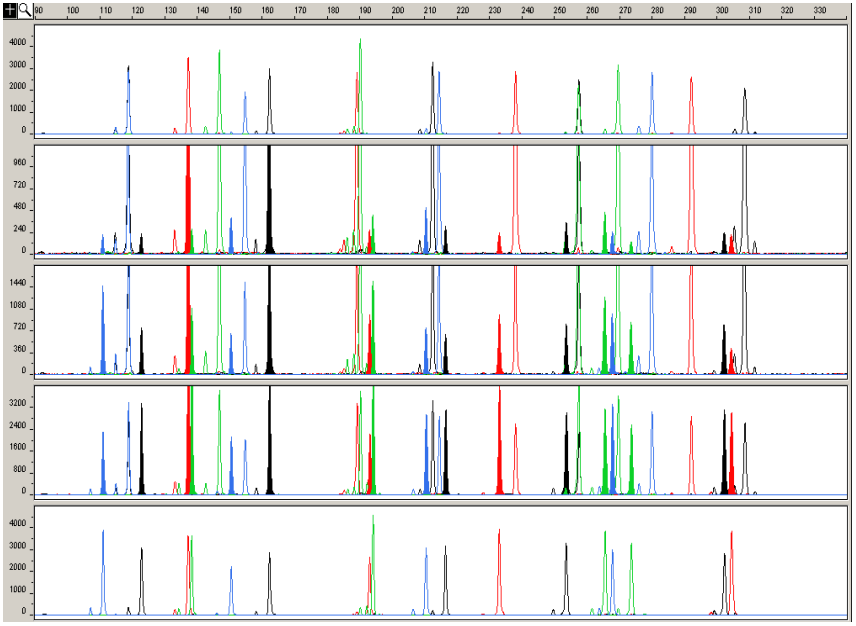

Figure 6-16 Mixtures of two male DNA samples (A and B) were amplified in various ratios using a total of 1-ng input DNA. The top panel shows Sample A and the bottom panel shows Sample B. The ratios of Sample A to Sample B (A:B ratios) shown are 10:1, 3:1 and 1:1 in panels 2, 3 and 4, respectively. For the mixture samples, the alleles attributable to the minor component, even when the major component shares an allele, are highlighted.

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, it is necessary to know the population distribution of alleles at each locus in question. If the genotype of the relevant evidence sample is different from the genotype of the suspects's reference sample, then the suspect is "excluded" as the donor of the biological evidence tested. An exclusion is independent of the frequency of the two genotypes in the population.

If the suspect and evidence samples have the same genotype, then the suspect is "included" as a possible source of the evidence sample. The probability that another, unrelated, individual would also match the evidence sample is estimated by the frequency of that genotype in the relevant population(s).

Population Samples Used in These Studies

The AmpFℓSTR Yfiler 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.

African-American - 333 samples were provided.
U.S. Caucasian - 254 samples were provided.
Hispanic - 175 samples were provided.

AmpFℓSTR Yfiler Kit Gene Diversity Values

Table 6-2 AmpFℓSTR Yfiler Kit gene diversity across three different U.S. populations

Locus[1]	U.S. African-American (N=333)	U.S. Caucasian (N=254)	U.S. Hispanic (N=175)
DYS456	0.598	0.703	0.663
DYS389I	0.538	0.575	0.492
DYS390	0.635	0.713	0.694
DYS389II	0.744	0.703	0.727

Table 6-2 AmpFℓSTR Yfiler Kit gene diversity across three different U.S. populations *(continued)*

Locus[1]	U.S. African-American (N=333)	U.S. Caucasian (N=254)	U.S. Hispanic (N=175)
DYS458	0.755	0.808	0.77
DYS19	0.748	0.541	0.645
DYS385a/b	0.951	0.855	0.931
DYS393	0.619	0.412	0.507
DYS391	0.423	0.54	0.52
DYS439	0.629	0.663	0.665
DYS635	0.701	0.682	0.71
DYS392	0.419	0.615	0.671
Y GATA H4	0.599	0.604	0.575
DYS437	0.495	0.624	0.583
DYS438	0.528	0.622	0.712
DYS448	0.685	0.651	0.726

[1]The gene diversity (D) for each locus was computed using the formula:

$$D = \frac{n(1 - \Sigma p_i^2)}{n - 1}$$

where n represents the sample size and pi is the allele frequency (Johnson et al., 2003).

Analyzing the Population Data

In addition to the alleles that were observed and recorded in the Applied Biosystems databases, other known alleles have been published or reported to us by other laboratories. Some of these alleles occur at a low frequency and include several microvariants (Furedi et al., 1999; Schoske et al., 2004).

Discriminatory Capacity of Haplotypes

Table 6-3 shows the discriminatory capacity (DC) and the number of unique haplotypes (UH) for each Y-STR marker combination listed. The discriminatory capacity was determined by dividing the number of different haplotypes by the number of samples in that population (Schoske et al., 2004). A unique haplotype is defined as one that occurs only once in a given population. The number of unique haplotypes is usually less than the number of different haplotypes in any given population

Table 6-3 Discriminatory capacity and number of unique haplotypes for three U. S. populations

Y-STR marker combination	African-American (N=333)		U.S. Caucasian (N-254)		U.S. Hispanics (N=175)	
	DC (%)	UH	DC (%)	UH	DC (%)	UH
"Minimal haplotype"[a]	84.6	249	74.8	162	85.1	136
"U.S. haplotype"[b]	91.3	286	83.8	196	90.3	146
"U.S. haplotype + DYS437"	91.9	286	85.8	202	91.4	148
"Yfiler haplotype"	99.1	327	98.8	248	98.3	169

[a] The "minimal haplotype" includes the markers DYS19, DYS385 a/b, DYS389 I/II, DYS390, DYS391, DYS392, DYS393.
[b] The "U.S. haplotype" includes the minimal haplotype loci plus DYS438 and DYS439.

Mutation Rate

Estimating Germline Mutations

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

In a previous study, the haplotypes for 8 loci amplified by the AmpFℓSTR Yfiler PCR Amplification Kit were determined for a total of 4999 parent-son (Kayser and Sajantila, 2001). Fourteen mutations were identified and an overall average mutation rate was estimated at 2.80×10^{-3}. In two confirmed father/son pairs mutation at two Y-STRs were observed.

Additional studies need to be performed for other loci in order to estimate their average mutation rate.

Troubleshooting

A

In This Appendix

Follow the recommended actions for the observations described in this appendix to understand and eliminate problems you experience during analysis.

Troubleshooting

Table A-1 Troubleshooting causes and recommended actions

Observation	Possible Causes	Recommended Actions
Faint or no signal from both the AmpFℓSTR® Control DNA 007 and the DNA test samples at all loci	Incorrect volume or absence of either AmpFℓSTR® PCR Reaction Mix, AmpFℓSTR Yfiler™ 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.
	PCR Master Mix not vortexed thoroughly before aliquoting	Vortex PCR Master Mix thoroughly.
	AmpFℓSTR Yfiler Primer Set exposed to too much light	Store 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 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 causes and recommended actions *(continued)*

Observation	Possible Causes	Recommended Actions
Faint or no signal from both the AmpFℓSTR® Control DNA 007 and the DNA test samples at all loci. *(continued)*	Insufficient PCR product electrokinetically injected	**For ABI PRISM® 310 runs:** Mix 1.5 µL of PCR product and 25 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 causes and recommended actions *(continued)*

Observation	Possible Causes	Recommended Actions
Positive signal from AmpFℓSTR Control DNA 007 but no signal from DNA test samples	Quantity of test DNA sample is below assay sensitivity	Quantitate DNA and add 0.5–1.0 ng of DNA. Repeat test.
	Test sample contains PCR inhibitor (*e.g.*, 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 degraded	If possible, evaluate the quality of DNA sample by running an agarose gel. If DNA is degraded, re-amplify with an increased amount of DNA.
	Dilution of test sample DNA in H_2O or wrong buffer (*e.g.*, wrong EDTA concentration)	Re-dilute DNA using TE Buffer (with 0.1-mM EDTA).
More than one allele present at a locus (except for DYS385 a/b)	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 (0.5–1.0 ng).
	Mixed sample	See "Stutter Products" on page 6-18
	Amplification of stutter product (n-4 bp position)	
	Incomplete 3´ A base addition (n-1 bp position)	See "Addition of 3´ A Nucleotide" on page 6-21. Be sure to include the final extension step of 60 °C for 80 min in the PCR.
	Signal exceeds dynamic range of instrument (off-scale data)	Quantitate DNA and re-amplify sample, adding 0.5–1.0 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 causes and recommended actions *(continued)*

Observation	Possible Causes	Recommended Actions
Some but not all loci visible on electropherogram	Test sample DNA is degraded	If possible, evaluate the quality of DNA sample by running an agarose gel. If DNA is degraded, re-amplify with an increased amount of DNA.
	Test sample contains PCR inhibitor (*e.g.*, heme compounds, certain dyes)	Quantitate DNA and add minimum necessary volume. Repeat test.
		Wash the sample in a Centricon-100. Repeat test.

Troubleshooting Automated Genotyping

To Troubleshoot Automated Genotyping:

Observation	Probable Cause	Recommended Action
Warning message: "Could not complete 'Run Macro' command because no dye/lanes are selected."	The word "ladder" is not in Sample Info for the lane or injection of allelic ladder.	Type the word **ladder** in the Sample Info column for each dye color (Blue, Green, Yellow, and Red) for the AmpFℓSTR Yfiler Allelic Ladder sample.
Warning message: "Could not complete 'Run Macro' command because the labeled peak could not be found."	One or more peaks in the allelic ladder are below the Peak Amplitude Threshold that was specified in the GeneScan software Analysis Parameters.	Use another allelic ladder in the project, or 1. In the GeneScan Analysis Software, lower the Peak Amplitude Threshold values for Blue, Green, Yellow, and Red dye colors in the Analysis Parameters. 2. Reanalyze the sample file(s) containing the allelic ladder. 3. Import all sample files into a new Genotyper software project, and run the Kazam macro again.

Bibliography

Akane, A., Matsubara, K., Nakamura, H., Takahashi, S., and Kimura, K. 1994. Identification of the heme compound copurified with deoxyribonucleic acid (DNA) from bloodstains, a major inhibitor of polymerase chain reaction (PCR) amplification. *J. Forensic Sci.* 39:362–372.

Begovich A.B., McClure G.R., Suraj V.C., Helmuth R.C., Fildes N., Bugawan T.L., Erlich H.A., Klitz W. 1992. Polymorphism, recombination, and linkage disequilibrium within the HLA class II region. *J Immunol.* 148:249-58.

Butler J.M., Schoske R., Vallone P.M., Kline M.C., Redd A.J., Hammer M.F. 2002. A novel multiplex for simultaneous amplification of 20 Y chromosome STR markers. *Forensic Sci Int.* 129:10-24.

Butler, J.M. 2001. Forensic DNA Typing. San Diego, CA: Academic Press.

Clark J.M. 1988. Novel non-templated nucleotide addition reactions catalyzed by procaryotic and eucaryotic DNA polymerases. *Nucleic Acids Res.* 16:9677-86.

DeFranchis, R., Cross, N.C.P., Foulkes, N.S., and Cox, T.M. 1988. A potent inhibitor of Taq DNA polymerase copurifies with human genomic DNA. *Nucleic Acids Res.* 16:10355.

DNA Advisory Board, Federal Bureau of Investigation, U.S. Department of Justice. 1998. Quality assurance standards for forensic DNA testing laboratories.

Frank, W., Llewellyn, B., Fish, P., *et al.* 2001. Validation of the AmpFℓSTR® Profiler Plus™ PCR Amplification Kit for use in forensic casework. *J. Forensic Sci.* 46(3):642–646.

Furedi, S., Woller, J., Padar, Z., Angyal, M. 1999. Y-STR haplotyping in two Hungarian populations. *Int J Legal Med.* 113:38-42.

Gonzalez-Neira, A., Elmoznino, M., Lareu, M.V., Sanchez-Diz, P., Gusmao, L., Prinz, M., Carracedo, A. 2001. Sequence structure of 12 novel Y chromosome microsatellites and PCR amplification strategies. *Forensic Sci Int.* 122:19-26.

Gusmao, L., Gonzalez-Neira, A., Pestoni, C., Brion, M., Lareu, M.V., Carracedo, A. 1999. Robustness of the Y STRs DYS19, DYS389 I and II, DYS390 and DYS393: optimization of a PCR pentaplex. *Forensic Sci Int.* 106:163-72.

Hall, A. and Ballantyne, J. 2003. The development of an 18-locus Y-STR system for forensic casework. *Anal Bioanal Chem.* 376:1234-46.

Holt, C., Stauffer, C., Wallin, J., *et al.* 2000. Practical applications of genotypic surveys for forensic STR testing. *Forensic Sci. Int.* 112(2,3):91–109.

Johnson, C.L., Warren, J.H., Giles, R.C., Staub, R.W. 2003. Validation and uses of a Y-chromosome STR 10-plex for forensic and paternity laboratories. *J Forensic Sci.* 2003. 48(6):1260-8.

Kayser, M., Kittler, R., Erler, A., Hedman, M., Lee, A.C., Mohyuddin, A., Mehdi, S.Q., Rosser, Z., Stoneking, M., Jobling, M.A., Sajantila, A., Tyler-Smith, C. 2004. A comprehensive survey of human Y-chromosomal microsatellites. *Am J Hum Genet.* 2004 74:1183-97.

Kayser, M., Sajantila, A. Mutations at Y-STR loci: implications for paternity testing and forensic analysis. 2001. *Forensic Sci Int.* 118:116-21.

Kwok, S., and Higuchi, R. 1989. Avoiding false positives with PCR. *Nature* 339:237–238.

Lazaruk, K., Walsh, P.S., Oaks, F., Gilbert, D., Rosenblum, B.B., Menchen, S., Scheibler, D., Wenz, H.M., Holt, C., Wallin, J. 1998. Genotyping of forensic short tandem repeat (STR) systems based on sizing precision in a capillary electrophoresis instrument. *Electrophoresis.* 19:86-93.

Magnuson, V.L., Ally, D.S., Nylund, S.J., Karanjawala, Z.E., Rayman, J.B., Knapp, J.I., Lowe, A.L., Ghosh, S., Collins, F.S. 1996. Substrate nucleotide-determined non-templated addition of adenine by Taq DNA polymerase: implications for PCR-based genotyping and cloning. *Biotechniques*. 21:700-9.

Mansfield, E.S., Robertson, J.M., Vainer, M., Isenberg, A.R., Frazier, R.R., Ferguson, K., Chow, S., Harris, D.W., Barker, D.L., Gill, P.D., Budowle, B., McCord, B.R. 1998. Analysis of multiplexed short tandem repeat (STR) systems using capillary array electrophoresis. *Electrophoresis*. 1998 19:101-7.

Moretti, T., Baumstark, A., Defenbaugh, D., Keys, K., Smerick, J., and Budowle, B. 2001. Validation of short tandem repeats (STRs) for forensic usage: Performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. *J. Forensic Sci.* 46(3):647–660.

Prinz, M., Ishii, A., Coleman, A., Baum, H.J., Shaler, R.C. 2001. Validation and casework application of a Y chromosome specific STR multiplex. *Forensic Sci Int*. 120:177-88.

Redd, A.J., Agellon, A.B., Kearney, V.A., Contreras, V.A., Karafet, T., Park, H., de Knijff, P., Butler, J.M., Hammer, M.F. 2002. Forensic value of 14 novel STRs on the human Y chromosome. *Forensic Sci Int*. 130:97-111.

Revised Validation Guidelines-Scientific Working Group on DNA Analysis Methods (SWGDAM). *Forensic Science Communications* (July 2004)-Volume 6 (3). Available at http://www.fbi.gov/hq/lab/fsc/current/standards/2004_03_standards02.htm

Schoske, R., Vallone, P.M., Kline, M.C., Redman, J.W., Butler, J.M. 2004. High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays. *Forensic Sci Int.*139:107-21.

Smith, R.N. 1995. Accurate size comparison of short tandem repeat alleles amplified by PCR. *Biotechniques.* 18:122-128.

Sparkes, R., Kimpton, C., Watson, S., Oldroyd, N., Clayton, T., Barnett, L., Arnold, J., Thompson, C., Hale, R., Chapman, J., Urquhart, A., and Gill, P. 1996a. The validation of a 7-locus multiplex STR test for use in forensic casework. (I). Mixtures, ageing, degradation and species studies. *Int. J. Legal Med.* 109:186-94.

Sparkes, R., Kimpton, C., Gilbard, S., Carne, P., Andersen, J., Oldroyd, N., Thomas, D., Urquhart, A., and Gill, P. 1996b. The validation of a 7-locus multiplex STR test for use in forensic casework. (II), Artifacts, casework studies and success rates. *Int. J. Legal Med*. 109:195-204.

Wallin, J.M., Holt, C.L., Lazaruk, K.D., Nguyen, T.H., Walsh, P.S. (2002) Constructing universal multiplex PCR systems for comparative genotyping. *J. Forensic Sci*. 47:52-65.

Wallin, J.M., Buoncristiani, M.R., Lazaruk, K.D., Fildes, N., Holt, C.L., Walsh, P.S. 1998. SWGDAM validation of the AmpFlSTR blue PCR amplification kit for forensic casework analysis. *J. Forensic Sci.* 43:854-70.

Walsh, P.S., Fildes, N.J., Reynolds, R. 1996. Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. *Nucleic Acids Res.* 24:2807-12.

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Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

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