USER GUIDE

applied biosystems by life technologies™

AmpFℓSTR® Yfiler® Direct PCR Amplification Kit

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life technologies™

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TRADEMARKS

Contents

About This Guide

IMPORTANT! Before using this product, read and understand the information in Appendix D, "Safety" on page 85 in this document.

Revision history

Revision	Date	Description
B	September 2012	Additional virtual bins added to the Yfiler® allelic ladder bin set in Chapter 4, "Analyze Data"starting on page 25.
A	July 2012	New document

Purpose

The *AmpFℓSTR® Yfiler® Direct PCR Amplification Kit User Guide* (Pub. no. 4479446) provides information about the Life Technologies instruments, chemistries, and software associated with the AmpFℓSTR® Yfiler® Direct PCR Amplification Kit.

1 Overview

Product overview

Purpose

The AmpF*l*STR® Yfiler® Direct PCR Amplification Kit is a short tandem repeat (STR) multiplex assay optimized to allow direct amplification of single-source samples:

- Blood samples collected on treated paper or untreated paper substrates without the need for sample purification.
- Buccal samples collected on treated or untreated paper substrates without the need for sample purification.

The loci amplified by the kit are:

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

Substrate examples

- Treated paper: COPAN® NUCLEIC-CARD™ system or Whatman FTA® cards
- Untreated paper: 903 paper, Bode Buccal DNA Collector™

Product description

The Yfiler® Direct Kit contains all the necessary reagents for the direct amplification of human male-specific genomic DNA.

The reagents are designed for use with the following Life Technologies instruments:

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

About the primers

Non-nucleotide linkers are used in primer synthesis for the DYS438 and DYS456 loci. For these primers, non-nucleotide linkers are placed between the primers and the fluorescent dye during oligonucleotide synthesis (Butler, 2005, Grossman et al. 1994, and Baron et al., 1996). Non-nucleotide linkers enable reproducible positioning of the alleles to facilitate interlocus spacing. The combination of a five-dye fluorescent system and the inclusion of non-nucleotide linkers allows for simultaneous amplification and efficient separation of the 17 Y-STR loci during automated DNA fragment analysis.

Loci amplified by the kit

The following table shows the loci amplified, their chromosomal locations, and the corresponding fluorescent marker dyes. The AmpFℓSTR® Yfiler® Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the allelic ladder and the genotype of the AmpFℓSTR® DNA Control 007 are also listed in the table.

Table 1 Yfiler® Direct Kit loci and alleles

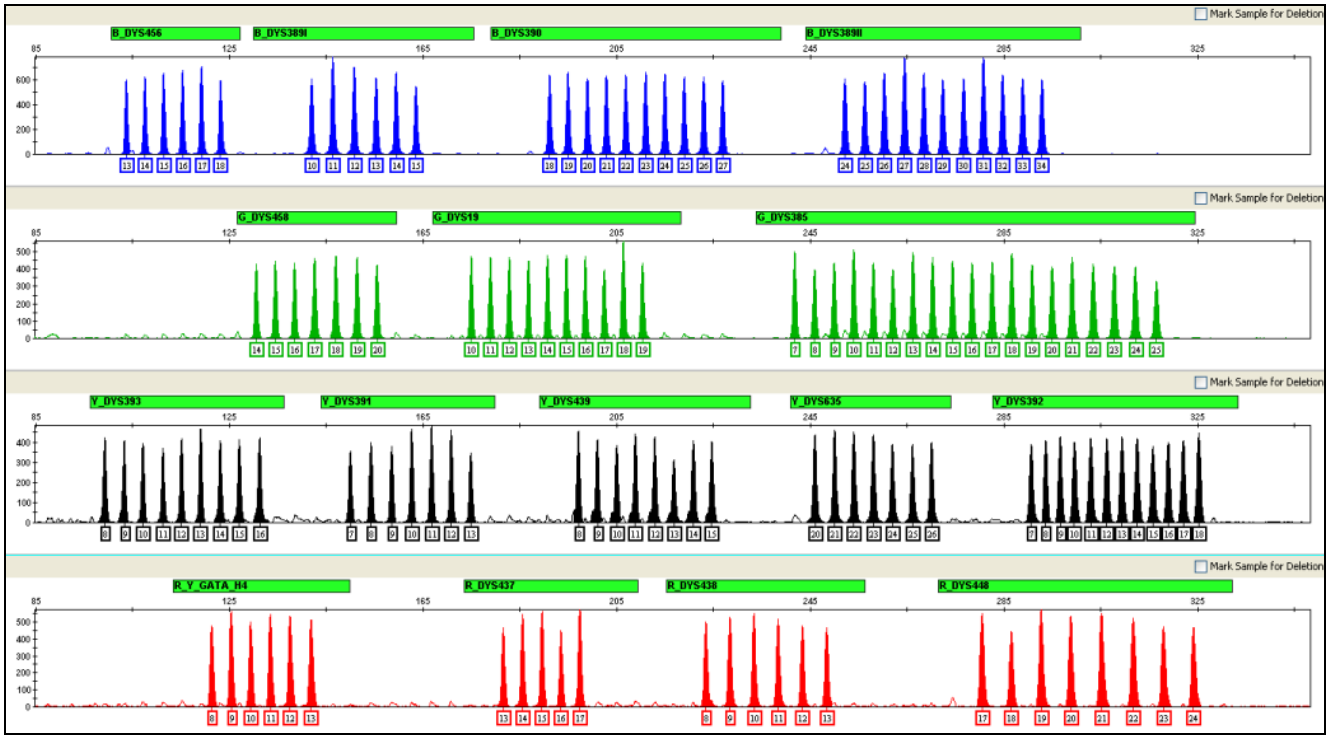
Locus designation	Alleles included in AmpFℓSTR® Yfiler® Allelic Ladder	Dye label	DNA Control 007
DYS456	13, 14, 15, 16, 17, 18	6-FAM™	15
DYS389 I	10, 11, 12, 13, 14, 15		13
DYS390	18, 19, 20, 21, 22, 23, 24, 25, 26, 27		24
DYS389 II	24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34		29
DYS458	14, 15, 16, 17, 18, 19, 20	VIC®	17
DYS19	10, 11, 12, 13, 14, 15, 16, 17, 18, 19		15
DYS385 a/b	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25		11,14
DYS393	8, 9, 10, 11, 12, 13, 14, 15, 16	NED™	13
DYS391	7, 8, 9, 10, 11, 12, 13		11
DYS439	8, 9, 10, 11, 12, 13, 14, 15		12
DYS635	20, 21, 22, 23, 24, 25, 26		24
DYS392	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18		13
Y GATA H4	8, 9, 10, 11, 12, 13	PET®	13
DYS437	13, 14, 15, 16, 17		15
DYS438	8, 9, 10, 11, 12, 13		12
DYS448	17, 18, 19, 20, 21, 22, 23, 24		19

Note: Additional virtual bins have been added to the Yfiler® allelic ladder bin set to increase the number of peaks that can be assigned an allele label. Refer to Chapter 4, "Analyze Data" on page 29 for additional information.

Allelic ladder profile

Figure 1 shows the allelic ladder for the Yfiler® Direct Kit. See "Allelic ladder requirements" on page 23 for information on genotyping accuracy.

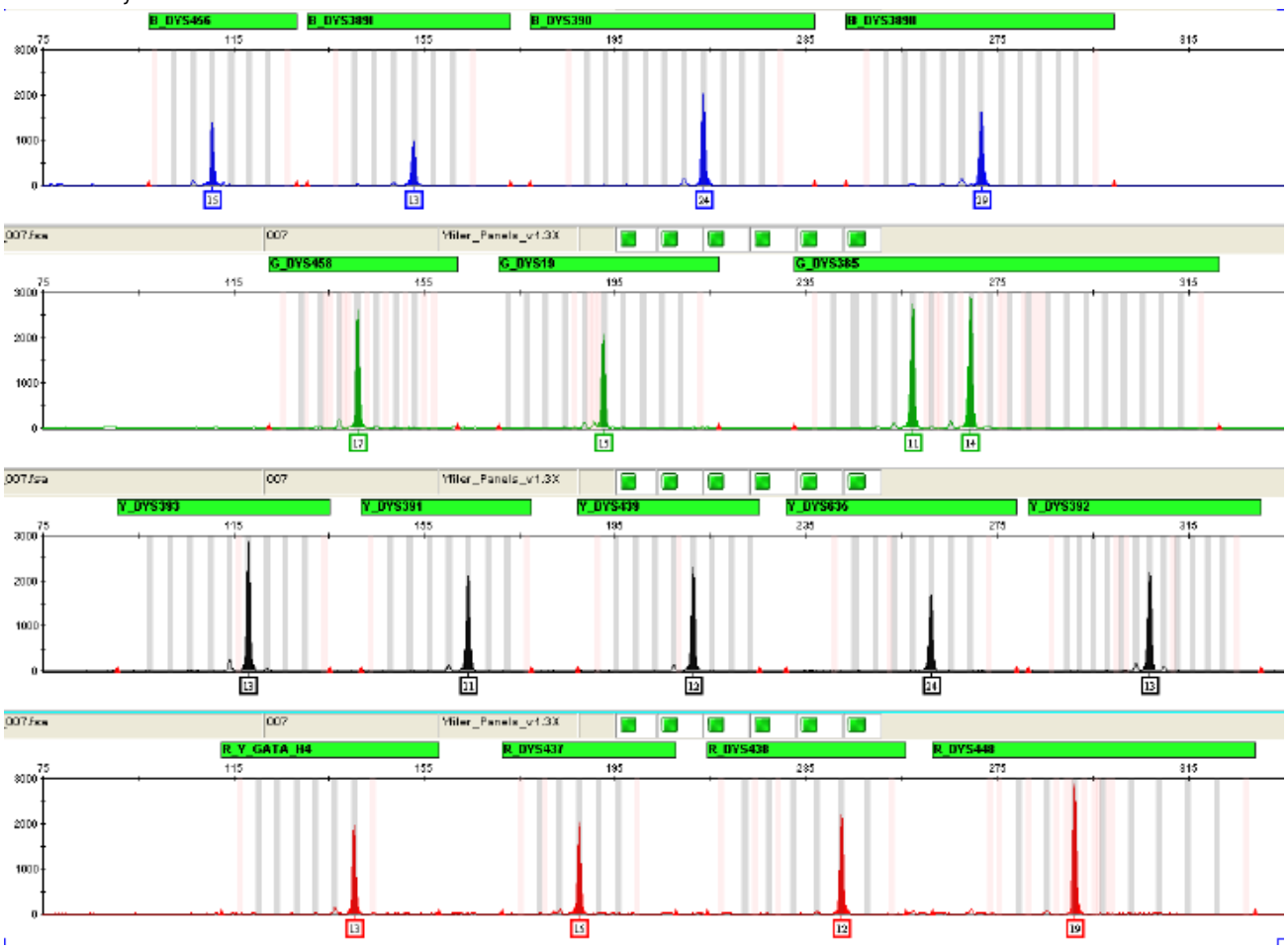
Figure 1 GeneMapper® *ID-X* Software plot of the AmpFℓSTR® Yfiler® Allelic Ladder



DNA Control 007 profile

Figure 2 shows amplification of DNA Control 007 using the Yfiler® Direct Kit.

Figure 2 DNA Control 007 (4 ng) amplified with the Yfiler® Direct Kit and analyzed on the Applied Biosystems® 3130*xl* Genetic Analyzer



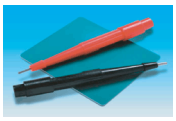
Workflow

Prepare samples

Obtain samples — Treated paper substrates

Obtain samples — Untreated paper substrates

Prepare samples



Harris Manual Punch

or

BSD600 Semi-Automated Dried Sample Punch Instrument

Perform PCR

Prepare reactions — AmpF*l*STR® Yfiler® Direct PCR Amplification Kit

Perform PCR

GeneAmp® PCR System 9700 Cycler

Veriti® 96-Well Thermal Cycler

Perform electro-phoresis

3100/3100-*Avant* Genetic Analyzer

3130/3130*xl* Genetic Analyzer

3500/3500xL Genetic Analyzer

Analyze data

GeneMapper® *ID-X* Software

GeneMapper® *ID* Software

Instrument and software overview

This section provides information about the Data Collection Software versions required to run the Yfiler® Direct Kit on specific instruments.

Data Collection and GeneMapper® *ID* or *ID-X* Software

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

Instrument and software compatibility

Table 2 Software specific to each instrument

Instrument	Operating System	Data Collection Software	Analysis Software
3500/3500xL	• Windows® XP • Windows Vista®	3500 Series Data Collection Software v1.0	GeneMapper® *ID-X* Software v1.2 or higher
3130/3130*xl*†	Windows® XP	3.0	• GeneMapper® *ID* Software v3.2.1 *and* • GeneMapper® *ID-X* Software v1.0.1 or higher
3100/3100-*Avant*	Windows® NT	1.1 (3100) 1.0 (3100-*Avant*)	
	Windows® 2000	2.0	

† Validation studies were conducted using these configurations.

About multicomponent analysis

Life Technologies 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.

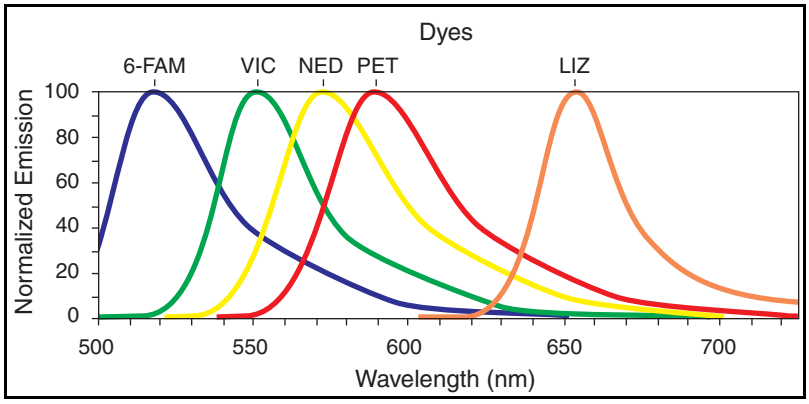
Multicomponent analysis is the process that separates the five different fluorescent dye colors into distinct spectral components. The four dyes used in the Yfiler® Direct Kit to label samples are 6-FAM™, VIC®, NED™, and PET® dyes. The fifth dye, LIZ®dye, is used to label the GeneScan™ 600 LIZ® Size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

How multicomponent analysis works

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

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

Figure 3 Emission spectra of the five dyes used in the Yfiler® Direct Kit



Materials and equipment

Kit contents and storage

The Yfiler® Direct Kit (Cat. no. 4477555) contains materials sufficient to perform 250 amplifications at 25 µL/amplification.

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

Component	Description	250× Volume	Storage
AmpFℓSTR® Yfiler® Direct Master Mix	Contains enzyme, salts, dNTPs, carrier protein, and 0.04% sodium azide.	2 tubes, 1.25 mL	–15°C to –25°C on receipt, 2°C to 8°C after initial use
AmpFℓSTR® Yfiler® Direct Primer Set	Contains forward and reverse primers to amplify human male DNA target.	2 tubes, 0.55 mL	
AmpFℓSTR® Yfiler® Allelic Ladder	Contains amplified alleles. See Table 1 on page 10 for a list of alleles included in the allelic ladder.	1 tube, 50.0 µL	
AmpFℓSTR® DNA Control 007	Contains 2 ng/µL human male DNA in 0.05% sodium azide and buffer†. See Table 1 on page 10 for profile.	1 tube, 50.0 µL	
AmpFℓSTR® PCR Enhancer	Contains proprietary biochemical reagents.	2 tubes, 1.44 mL	

† The AmpFℓSTR® DNA Control 007 is included at a concentration appropriate to its intended use as an amplification control (i.e., to provide confirmation of the capability of the kit reagents to generate a profile of expected genotype). The AmpFℓSTR® DNA Control 007 is not designed to be used as a DNA quantitation control, and you may see variation from the labelled concentration when quantitating aliquots of the AmpFℓSTR® DNA Control 007.

Standards for samples

For the Yfiler® Direct Kit, the panel of standards needed for PCR amplification, PCR product sizing, and genotyping are:

- **AmpFℓSTR® Control DNA 007** – A positive control for evaluating the efficiency of the amplification step and STR genotyping using the AmpFℓSTR® Yfiler® Allelic Ladder.
- **GeneScan™ 500 LIZ® Size Standard or GeneScan™ 600 LIZ® Size Standard v2.0** – Used for obtaining sizing results. These standards, which have been evaluated as internal size standards, yield precise sizing results for Yfiler® Direct Kit PCR products. Order the GeneScan™ 500 LIZ® Size Standard (Cat. no. 4322682) or the GeneScan™ 600 LIZ® Size Standard v2.0 (Cat. no. 4408399) separately.
- **AmpFℓSTR® Yfiler® Allelic Ladder** – Allelic ladder developed by Life Technologies for accurate characterization of the alleles amplified by the Yfiler® Direct Kit. The AmpFℓSTR® Yfiler® Allelic Ladder contains most of the alleles reported for the 17 loci. Refer to Table 1 on page 10 for a list of the alleles included in the AmpFℓSTR® Yfiler® Allelic Ladder.

2 Perform PCR

Optimize PCR cycle number

Before using the Yfiler® Direct Kit for the first time, perform a single initial sensitivity experiment to determine the appropriate cycle number to use during internal validation studies and operational use of the Yfiler® Direct Kit. This experiment accounts for instrument-to-instrument and sample-to-sample variations. If you are processing multiple sample type and substrate combinations (for example, buccal samples on treated paper and blood samples on untreated paper), perform separate sensitivity experiments for each sample type and substrate to be used for testing.

The Yfiler® Direct Kit is optimized to amplify unpurified:

- Single-source blood samples on treated paper or untreated paper substrates
- Buccal samples on treated or untreated paper substrates

When amplifying single-source, unpurified samples using the Yfiler® Direct Kit, you should expect to see greater variation in peak height from sample to sample than is expected with purified samples. Careful optimization of the cycle number will help to minimize the impact of this variation.

Select samples and prepare plates

1. Select 26 of each sample and substrate type. Ensure the selected samples represent a "typical" range of samples analyzed in your laboratory.

   IMPORTANT! The number of samples recommended for this study has been chosen to allow you to complete electrophoresis using a single 96-well plate, thus minimizing the impact of run-to-run variation on the results.

2. Prepare the samples and the reactions as described in the protocols later in this chapter. Prepare sufficient PCR reagents to complete amplification of three replicate plates.
3. Create three identical PCR plates.

4. Amplify each plate using a different cycle number to determine the optimum conditions for use in your laboratory. Suggested cycle numbers for different sample type and substrate combinations are listed below:

Sample type	Substrate	
	Treated paper	Untreated paper
Blood	25, 26, 27 cycles	25, 26, 27 cycles
Buccal	26, 27, 28 cycles	26, 27, 28 cycles

Note: To minimize the effect of instrument-to-instrument variation, use the same thermal cycler to amplify all three plates. To maximize result quality, prepare and amplify Plate 1 then repeat for Plates 2 and 3. Do not prepare all three plates simultaneously.

Determine optimum conditions

1. Run the PCR products on the appropriate CE platform using the recommended protocol; see Chapter 3, "Perform Electrophoresis" on page 23.
2. Based on the results of the sensitivity study, select the appropriate PCR cycle number for future experiments.

Our studies indicate the optimum PCR cycle number should generate profiles with the following heterozygote peak heights, with no instances of allelic dropout and minimal occurrence of off-scale allele peaks.

Instrument	Heterozygous peak height
31xx	1500–3000 RFU
3500 Series	3000–6,000 RFU

Prepare the reactions

Sample prep guidelines

- Do not add water to the wells on the reaction plate before adding the punches. If your laboratory is experiencing static issues with the paper discs, you may prepare and dispense the 25 µL reaction mix into the wells of the reaction plate before adding the punches.
- For samples collected using the Bode Buccal DNA Collector™, add 2 µL of Prep-n-Go™ buffer (Cat. no. 4467079) to the sample and negative control wells in a 96-well plate. Do not add Prep-n-Go™ Buffer to the positive control wells.
- Make the punch as close as possible to the center of the sample to ensure optimum peak intensity. If you are using a Bode Buccal DNA Collector™, make the punch as close as possible to the tip of the DNA collector to ensure optimum peak intensity. Increasing the size of the punch may cause inhibition during PCR amplification.

- For manual punching: Place the tip of a 1.2 mm Harris Micro-Punch on the card, hold the barrel of the Harris Micro-Punch (do not touch the plunger), gently press and twist 1/4-turn, then eject the punch into the appropriate well on the reaction plate.
- For automated punching: Please refer to the User Guide of your automated or semi-automated disc punch instrument for proper guidance.

Prepare the reactions

1. Add samples to the reaction plate:

Well(s)	Add the following to wells of a MicroAmp® Optical 96-Well Reaction Plate	
Negative control	1.2 mm blank disc	
Test samples	1.2 mm sample disc	
Positive control **IMPORTANT!** Do not add a blank disc to the positive control well.	• For 25 and 26 cycles	3 µL of Control DNA 007
	• For 27 cycles	2 µL of Control DNA 007
Note: The volumes of positive control are suggested amounts and may be adjusted if peak heights are too high or too low for your optimized cycle number.		

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

Reaction component	Volume per reaction
Master Mix	10.0 µL
Primer Set	3.5 µL
PCR Enhancer	11.5 µL

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

IMPORTANT! This kit has been optimized for a 25-µL PCR reaction volume to overcome the PCR inhibition expected when amplifying unpurified samples. Using a lower PCR reaction volume may reduce the ability of the kit chemistry to generate full STR profiles.

3. Prepare reagents. Thaw the Master Mix, Primer Set, and PCR Enhancer, then vortex for 3 seconds. Centrifuge briefly before opening the tubes or bottles.

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

4. Pipet the required volumes of components into an appropriately sized polypropylene tube.
5. Vortex the reaction mix for 3 seconds, then centrifuge briefly.
6. Dispense 25 µL of the reaction mix into each reaction well of a MicroAmp® Optical 96-Well Reaction Plate.

7. Seal the plate with MicroAmp® Clear Adhesive Film or MicroAmp® Optical Adhesive Film.

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

8. Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders.

Perform PCR

1. Program the thermal cycling conditions.

IMPORTANT! When using the GeneAmp® PCR System 9700 or the Veriti® Thermal Cycler, select **9600 emulation mode**.

Initial incubation step	Denature	Anneal	Extend	Final hold
HOLD	CYCLE (25–28)			HOLD
95°C 1 min	94°C 11 sec	58°C 1 min 15 sec	61°C 2 min 30 sec	4°C ∞

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

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

3. Start the run.

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

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

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

3 Perform Electrophoresis

Allelic ladder requirements

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

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

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

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

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

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

Reagents and parts

"Ordering Information" on page 79 lists the required materials not supplied with the Yfiler® Direct Kit.

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

Electrophoresis software setup and reference documents

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

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

† We conducted validation studies using these configurations.

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

Prepare the samples for electrophoresis immediately before loading.

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

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

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

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

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

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

Section 3.2 3500/3500xL Series instruments

Set up the 3500/3500xL instrument for electrophoresis

Reagents and parts

"Ordering Information" on page 79 lists the required materials not supplied with the Yfiler® Direct Kit.

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

Electrophoresis software setup and reference documents

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

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

Prepare samples for electrophoresis on the 3500/3500xL instrument

Prepare the samples for electrophoresis immediately before loading.

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

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

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

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

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

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

4 Analyze Data

Section 4.1 GeneMapper® *ID* Software

Overview of GeneMapper® *ID* Software

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

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

Instruments

Refer to "Instrument and software overview" on page 14 for a list of compatible instruments.

Before you start

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

- HID analysis requires at least one allelic ladder sample per run folder. Your laboratory can use multiple ladder samples in an analysis, provided individual laboratories conduct the appropriate validation studies.

  For multiple ladder samples, the GeneMapper® *ID* Software calculates allelic bin offsets by using an average of all ladders that use the same panel within a run folder.
- Allelic ladder samples in an individual run folder are considered to be from a single run.

  When the software imports multiple run folders into a project, only the ladder(s) within their respective run folders are used for calculating allelic bin offsets and subsequent genotyping.
- Allelic ladder samples must be labeled as "Allelic Ladder" in the Sample Type column in a project. Failure to apply this setting for ladder samples results in failed analysis.
- Injections containing the allelic ladder must be analyzed with the same analysis method and parameter values that are used for samples to ensure proper allele calling.
- Alleles that are not in the AmpFℓSTR® Allelic Ladders do exist. Off-ladder (OL) alleles may contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the ± 0.5-nt bin window of any known allelic ladder allele or virtual bin.

  Note: If a sample allele peak is called as an off-ladder allele, the sample result needs to be verified according to the laboratory's protocol.

Set up GeneMapper® *ID* Software for data analysis

File names

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

Before using the software for the first time

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

- Import panels and bins into the Panel Manager, as explained in "Import panels and bins" on page 31.
- Create an analysis method, as explained in , "Create an analysis method" on page 34.
- Create a size standard, as explained in "Designate size standard" on page 40.
- Define custom views of analysis tables.

  Refer to Chapter 1 of the *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (Pub. no. 4335523) for more information.
- Define custom views of plots.

  Refer to Chapter 1 of the *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (Pub. no. 4335523) for more information.

Import panels and bins

To download the most recent versions of the Yfiler® panel and bin set go to **www.lifetechnologies.com/support** ▸ **Software, Patches & Updates** ▸ GeneMapper® *ID* Software:

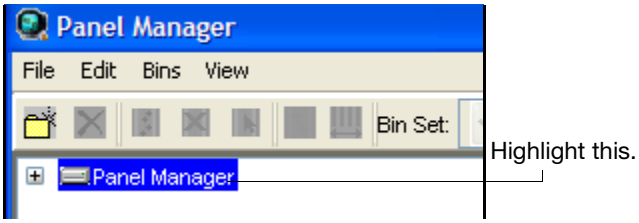
1. Start the GeneMapper® *ID* Software, then log in with the appropriate user name and password.

   IMPORTANT! If you need login instructions, refer to pages 2–7 of the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (Pub. no. 4338775).

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

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

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

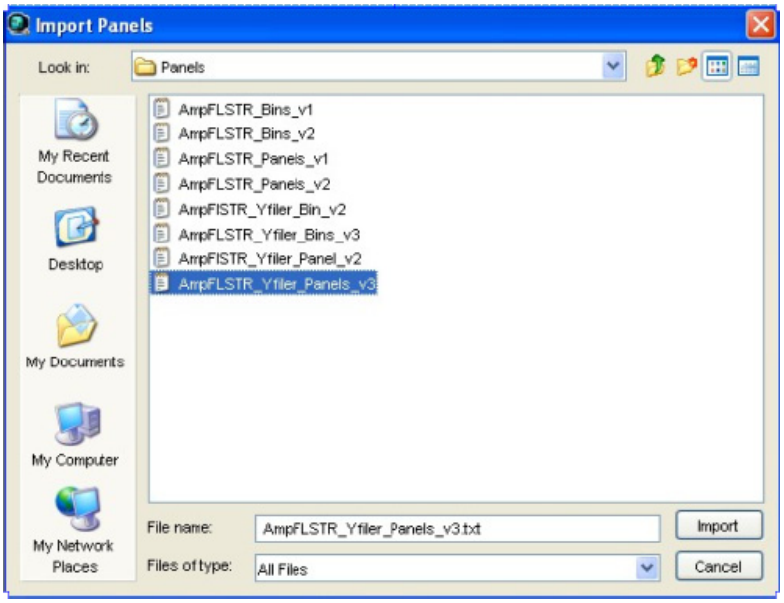


   b. Select **File** ▸ **Import Panels** to open the Import Panels dialog box.

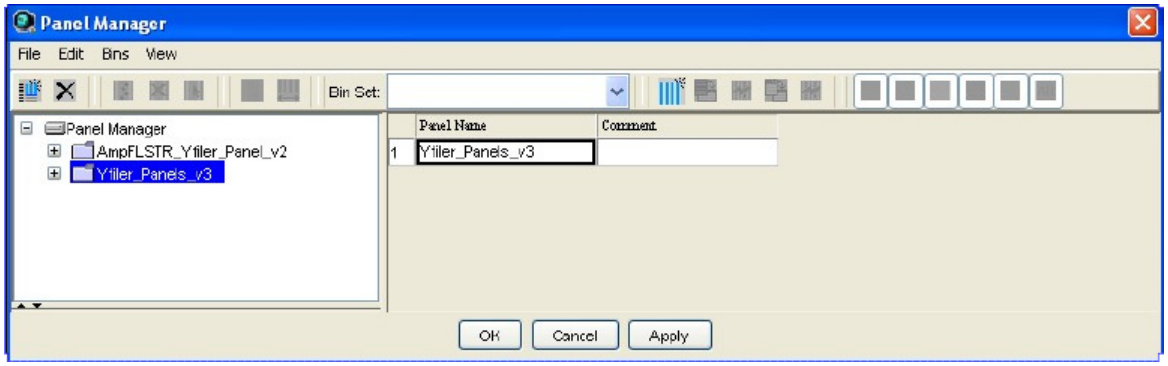
   c. Navigate to, then open the ***x***:\Applied Biosystems\GeneMapper\Panels folder, where *x* is the drive on which the GeneMapper® *ID* Software is installed.

4. Select **AmpFLSTR_Yfiler_Panels_v3.txt**, then click **Import**.

   Note: Importing this file creates a new folder in the navigation pane of the Panel Manager, Yfiler_Panels_v3. This folder contains the panels and associated markers.

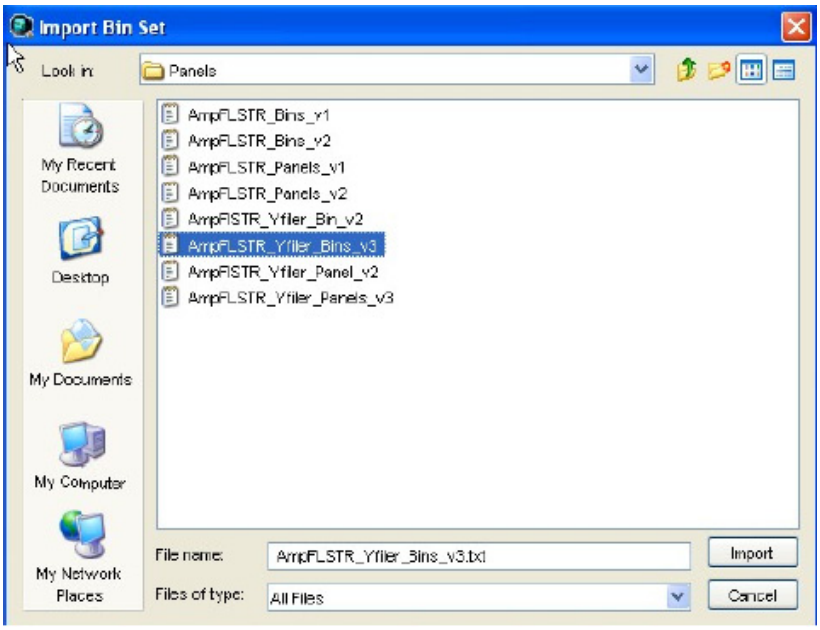


5. Import **AmpFLSTR_Yfiler_Bins_v3**:
   a. Select the **Yfiler_Panels_v3** folder in the navigation pane.



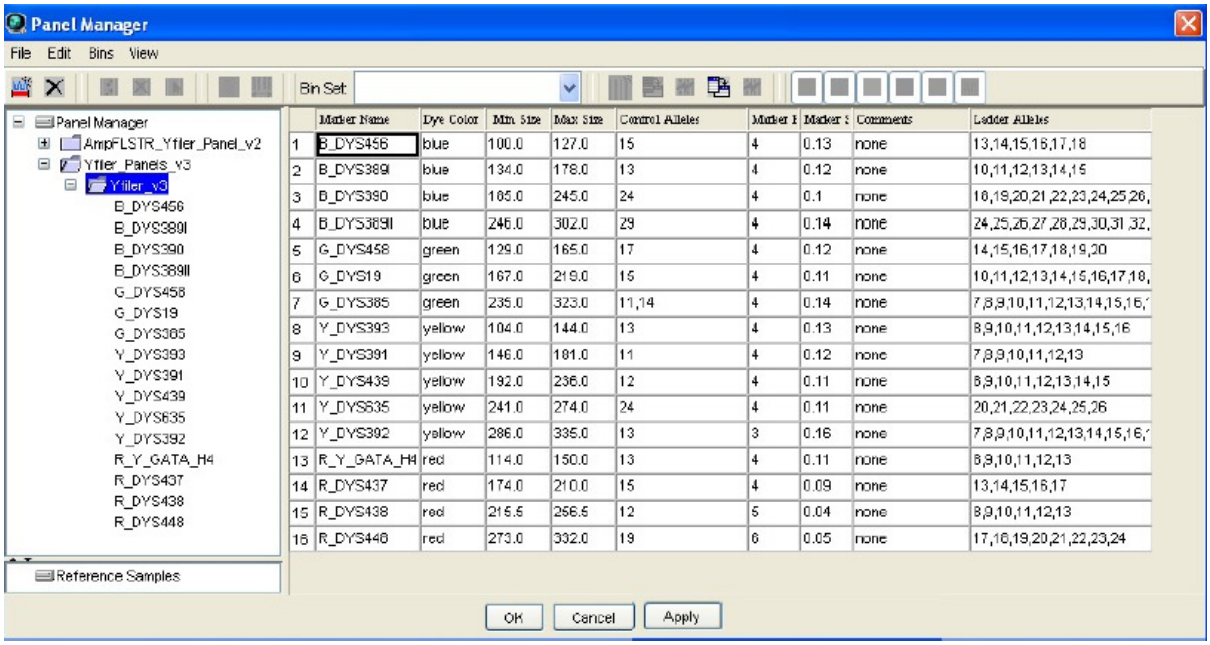
   b. Select **File ▸ Import Bin Set** to open the Import Bin Set dialog box.
   c. Navigate to, then open the ***x***:\Applied Biosystems\GeneMapper\Panels folder.
   d. Select **AmpFLSTR_Yfiler_Bins_v3**, then click **Import**.

   Note: Importing this file associates the bin set with the panels in the Yfiler_Panels_v3 folder.



6. View the imported panels in the navigation pane:
   a. Double-click the **Yfiler_Panels_v3** folder.

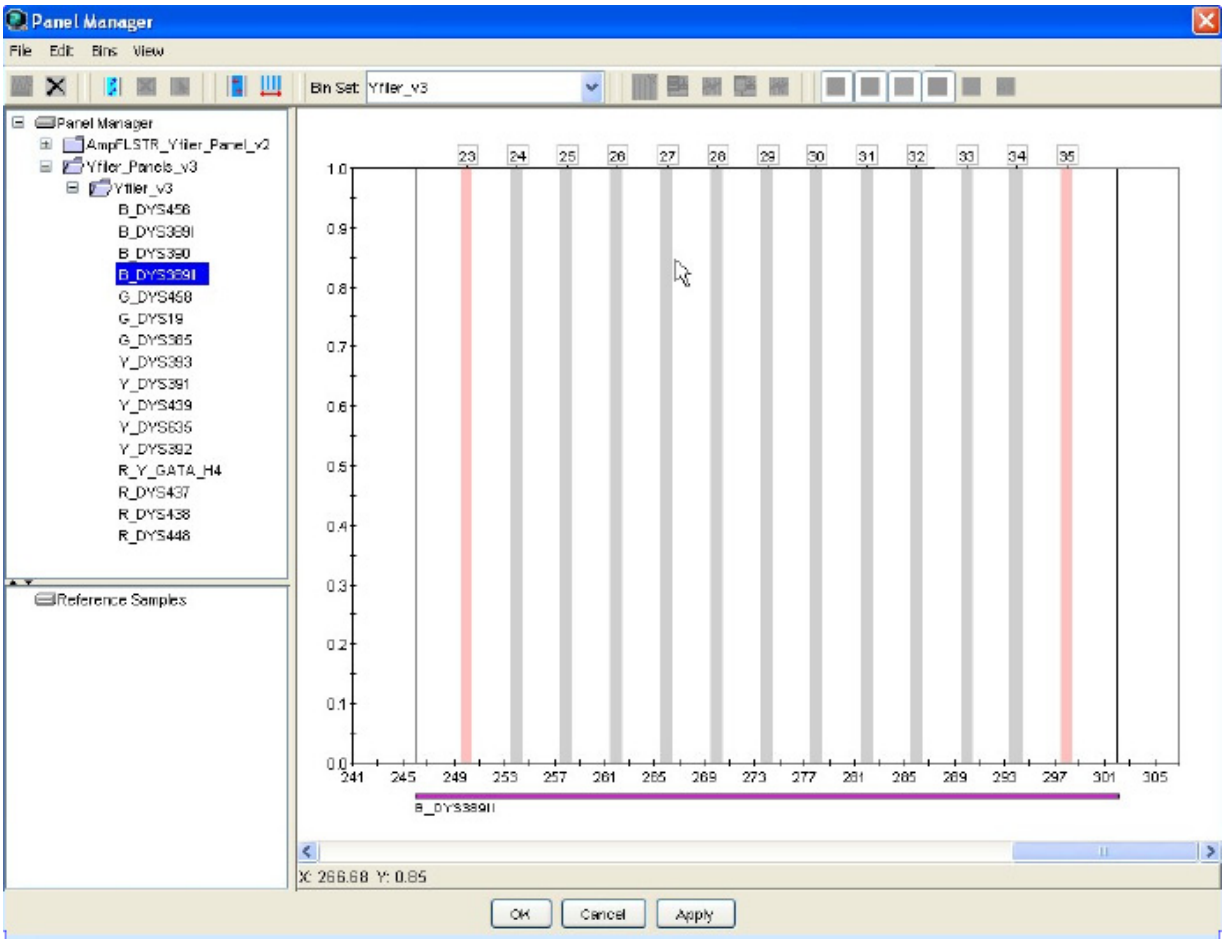
b. Double-click the **Yfiler_v3** folder to display the panel information in the right pane and the markers below it.



	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker I	Marker S	Comments	Ladder Alleles
1	B_DYS456	blue	100.0	127.0	15	4	0.13	none	13,14,15,16,17,18
2	B_DYS389I	blue	134.0	178.0	13	4	0.12	none	10,11,12,13,14,15
3	B_DYS390	blue	185.0	245.0	24	4	0.1	none	16,19,20,21,22,23,24,25,26,
4	B_DYS389II	blue	246.0	302.0	29	4	0.14	none	24,25,26,27,28,29,30,31,32,
5	G_DYS458	green	129.0	165.0	17	4	0.12	none	14,15,16,17,18,19,20
6	G_DYS19	green	167.0	219.0	15	4	0.11	none	10,11,12,13,14,15,16,17,18,
7	G_DYS385	green	235.0	323.0	11,14	4	0.14	none	7,8,9,10,11,12,13,14,15,16,
8	Y_DYS393	yellow	104.0	144.0	13	4	0.13	none	8,9,10,11,12,13,14,15,16
9	Y_DYS391	yellow	146.0	181.0	11	4	0.12	none	7,8,9,10,11,12,13
10	Y_DYS439	yellow	192.0	236.0	12	4	0.11	none	8,9,10,11,12,13,14,15
11	Y_DYS635	yellow	241.0	274.0	24	4	0.11	none	20,21,22,23,24,25,26
12	Y_DYS392	yellow	286.0	335.0	13	3	0.16	none	7,8,9,10,11,12,13,14,15,16,
13	R_Y_GATA_H4	red	114.0	150.0	13	4	0.11	none	8,9,10,11,12,13
14	R_DYS437	red	174.0	210.0	15	4	0.09	none	13,14,15,16,17
15	R_DYS438	red	215.5	256.5	12	5	0.04	none	8,9,10,11,12,13
16	R_DYS448	red	273.0	332.0	19	6	0.05	none	17,18,19,20,21,22,23,24

7. View the markers and display the Bin view in the navigation pane:
   a. Select the **Yfiler_Panels_v3** folder to display its list of kits in the right pane.
   b. Double-click the **Yfiler_v3** folder to display its list of markers below it.

c. Select **DYS389II** to display the Bin view for the marker in the right pane.



Note: Two additional virtual bins, 23 and 35, have now been added. In total, 53 additional virtual bins were added to Yfiler_Bins_v3.txt.

8. Click **Apply**, then **OK** to add the AmpFℓSTR® panel and bin set to the GeneMapper® *ID* Software database.

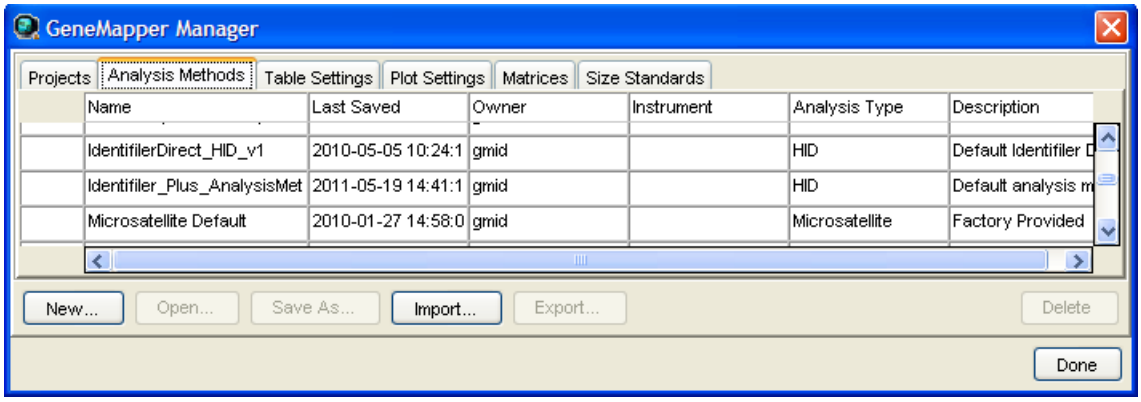
IMPORTANT! If you close the Panel Manager without clicking OK, the panels and bins are not imported into the GeneMapper® *ID* Software database.

Create an analysis method

The HID Advanced analysis method for the Yfiler® Direct Kit uses the Yfiler_Bins_v3 file described in step 5 on page 32.

Use the following procedure to create a HID analysis method for the Yfiler® Direct Kit.

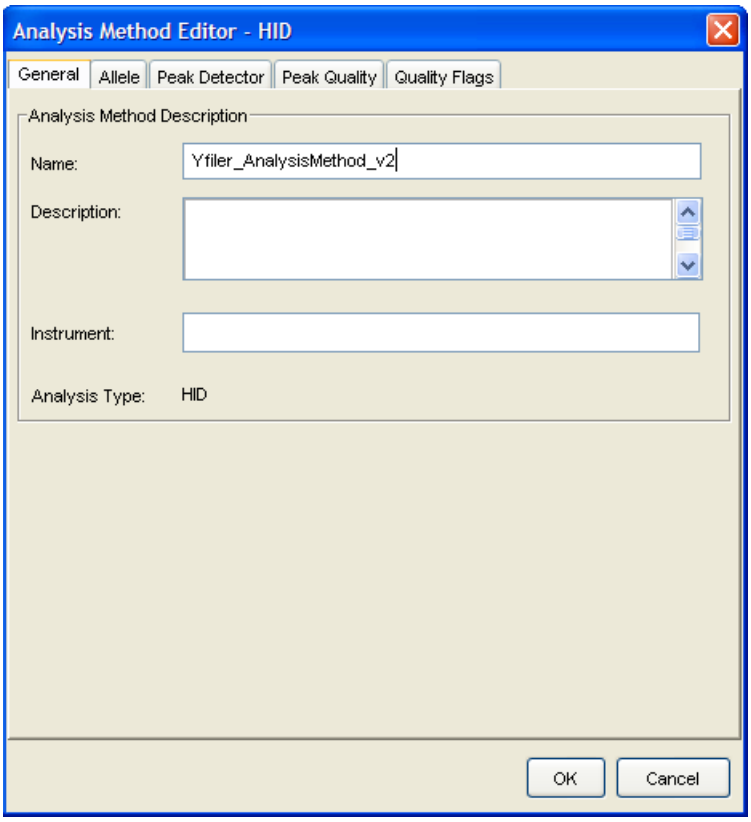
1. Select **Tools** ▸ **GeneMapper Manager** to open the GeneMapper Manager.



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

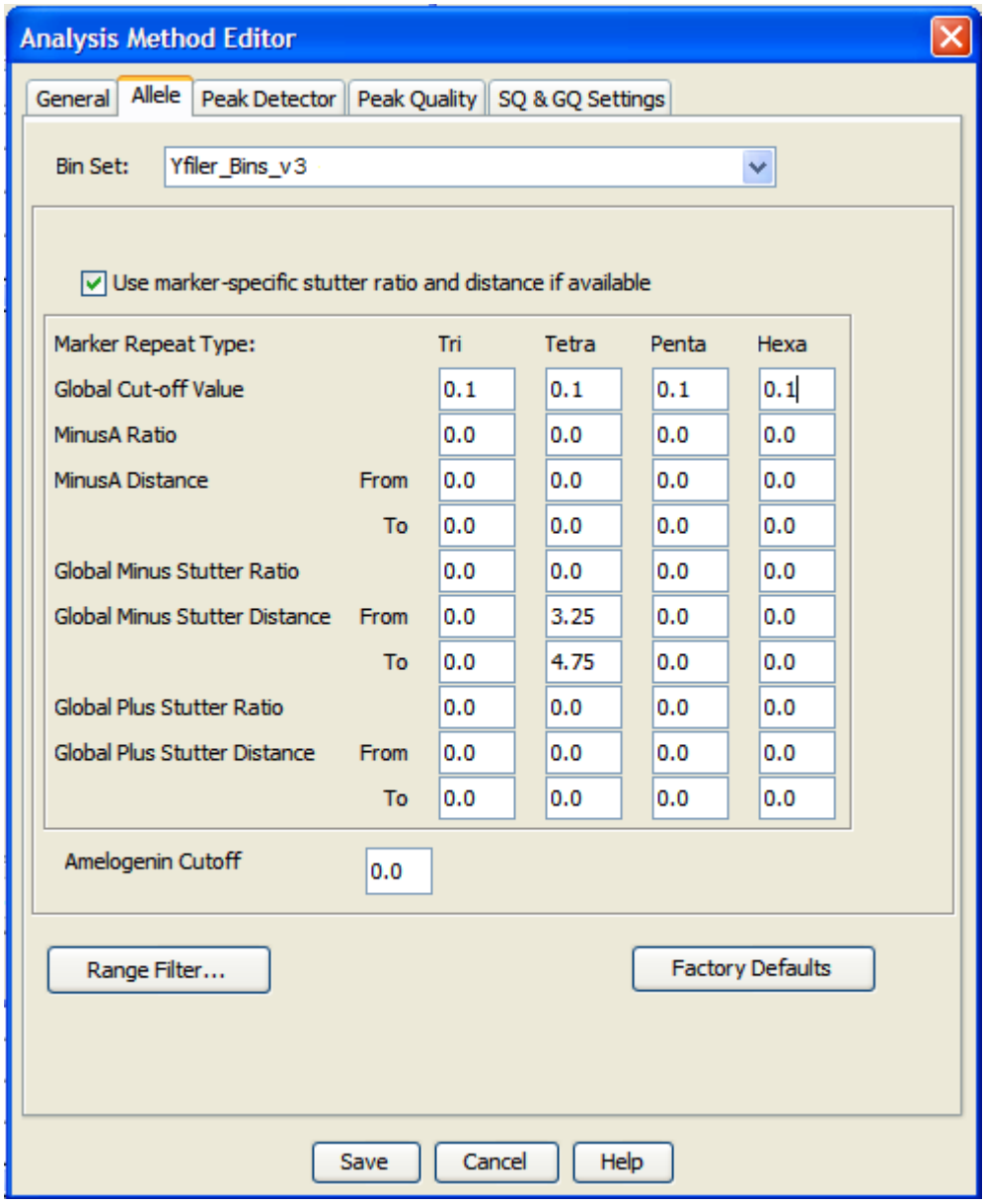
   Note: The Analysis Method Editor closes when you save your settings. To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.
5. After you enter settings in all tabs, click **Save**.

General tab settings



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

Allele tab settings

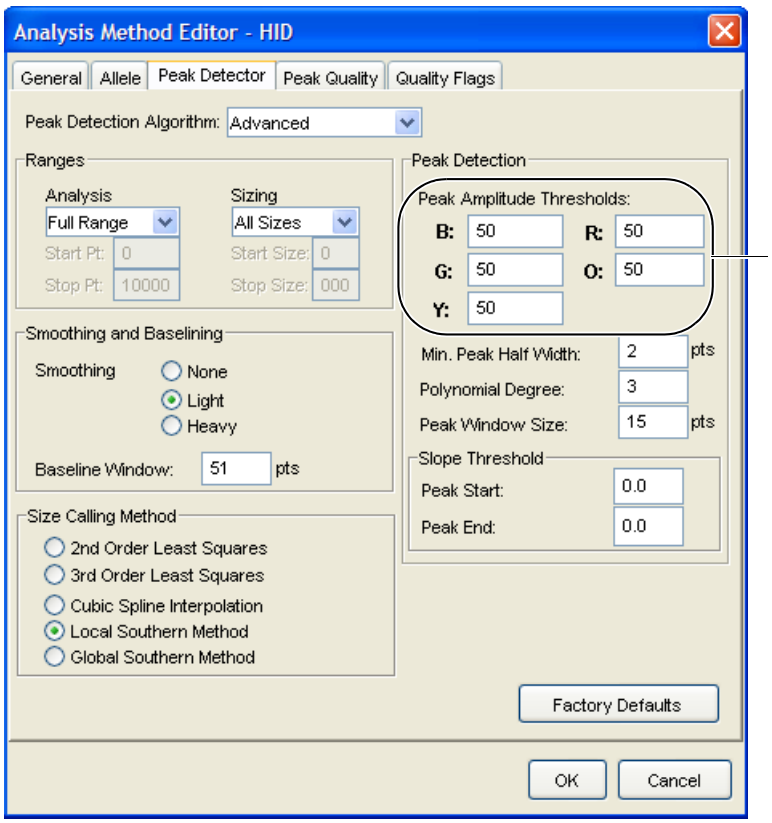


- In the Bin Set field, select the **Yfiler_Bins_v3** bin set imported previously and configure the stutter distance parameters as shown.
- GeneMapper® *ID* Software v3.2.1 allows you to specify four types of marker repeat motifs: tri, tetra, penta, and hexa. You can enter parameter values for each type of repeat in the appropriate column.
- Specify the stutter ratio:
  - To apply the stutter ratios listed in the Allele tab for single-source data, deselect the "Use marker-specific stutter ratio if available" check box (selected by default). Perform appropriate internal validation studies to determine the appropriate filter setting to use.

    Note: Applying global stutter ratios may reduce the editing required for single-source sample data.
  - To apply the stutter ratios contained in the AmpFLSTR_Yfiler_Panels_v3.txt

file, select the "Use marker-specific stutter ratio if available" check box (selected by default). Perform appropriate internal validation studies to determine the appropriate filter setting to use.

Peak Detector tab settings

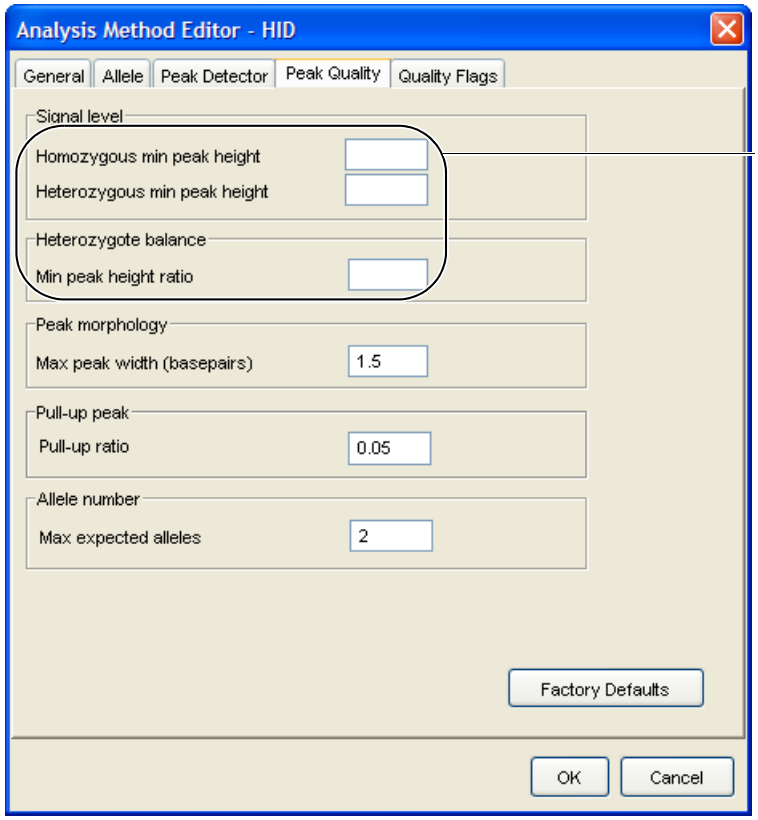


IMPORTANT! Perform the appropriate internal validation studies to determine the peak amplitude thresholds for interpretation of Yfiler® Direct Kit data.

Fields include:

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

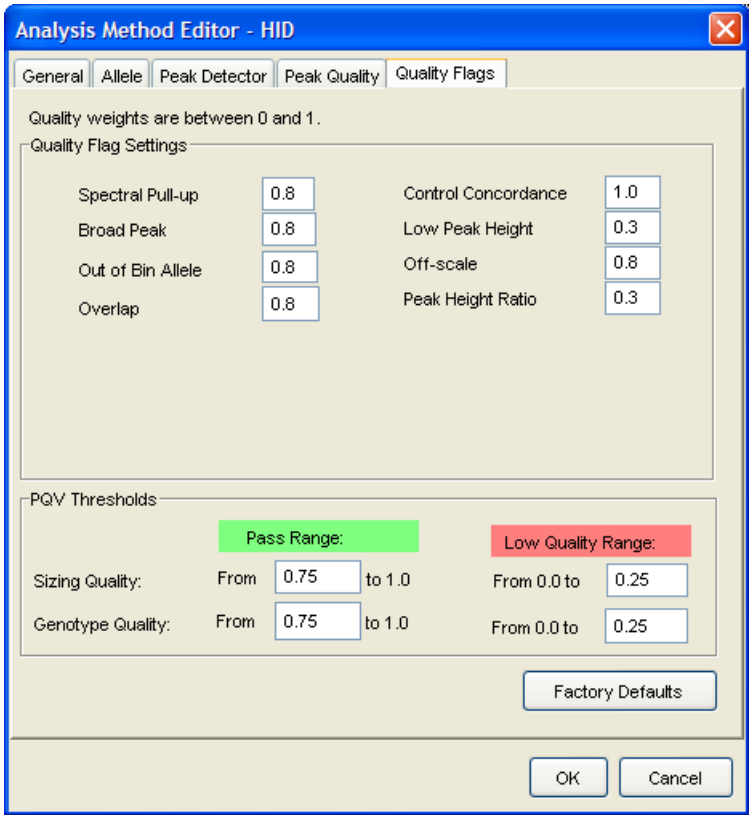
Peak Quality tab settings



Perform internal validation studies to determine settings

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

Quality Flags tab settings



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

Designate size standard

For the Yfiler® Direct Kit , use the following size standard peaks:

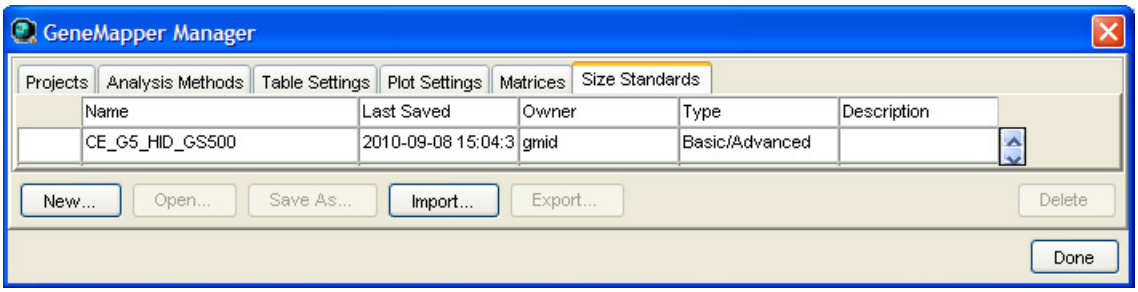
GeneScan™ 500 LIZ® Size Standard peak sizes	GeneScan™ 600 LIZ® Size Standard v2.0 peak sizes
75, 100, 139, 150, 160, 200, 300, 340, 350, 400, and 450	80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440 and 460

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

Use the following procedure to create the appropriate size standard:

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

2. Select the **Size Standards** tab, click **Open**, select the CE_G5_HID_GS500 size standard, then click **Done**.



Analyze and edit sample files with GeneMapper® *ID* Software

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

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	YfilerDirect_AnalysisMethod_v1 (or the name of the analysis method you created)
Panel	Yfiler_v3
Size Standard	CE_G5_HID_GS500† (or the name of the size standard you selected)

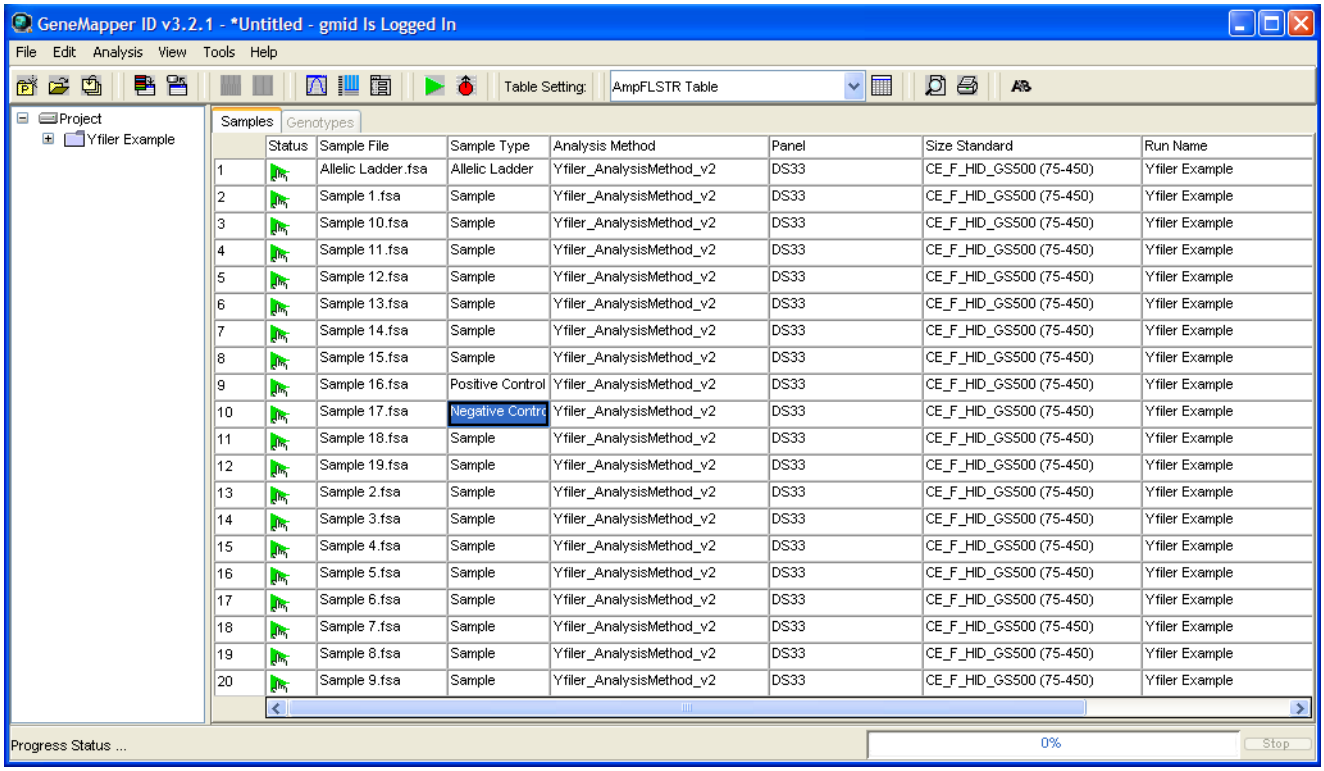
† The Yfiler® Direct Kit was originally validated using the GeneScan™ 600 LIZ® Size Standard v2.0. If you use the GeneScan™ 500 LIZ® Size Standard as an alternative, perform the appropriate internal validation studies to support the use of this size standard with the Yfiler® Direct Kit.

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

3. Click (Analyze), enter a name for the project (in the Save Project dialog box), then click **OK** to start analysis.
   - The status bar displays the progress of analysis:
     - As a completion bar extending to the right with the percentage indicated
     - With text messages on the left

- The table displays the row of the sample currently being analyzed in green (or red if analysis failed for the sample).
- The Genotypes tab becomes available after analysis (see the figure on the next page).

Project window after analysis



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

Examine and edit a project

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

For more information

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

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

Section 4.2 GeneMapper® *ID-X* Software

Overview of GeneMapper® *ID-X* Software

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

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

Instruments

Refer to "Instrument and software overview" on page 14 for a list of compatible instruments.

Before you start

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

- HID analysis requires at least one allelic ladder sample per run folder. Your laboratory can use multiple ladder samples in an analysis, provided individual laboratories conduct the appropriate validation studies.

  For multiple ladder samples, the GeneMapper® *ID-X* Software calculates allelic bin offsets by using an average of all ladders that use the same panel within a run folder.
- Allelic ladder samples in an individual run folder are considered to be from a single run.

  When the software imports multiple run folders into a project, only the ladder(s) within their respective run folders are used for calculating allelic bin offsets and subsequent genotyping.
- Allelic ladder samples must be labeled as "Allelic Ladder" in the Sample Type column in a project. Failure to apply this setting for ladder samples results in failed analysis.
- Injections containing the allelic ladder must be analyzed with the same analysis method and parameter values that are used for samples to ensure proper allele calling.
- Alleles that are not in the AmpFℓSTR® Allelic Ladders do exist. Off-ladder (OL) alleles may contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the ±0.5-nt bin window of any known allelic ladder allele or virtual bin.

  Note: If a sample allele peak is called as an off-ladder allele, the sample result needs to be verified according to the laboratory's protocol.

Set up GeneMapper® *ID-X* Software for data analysis

Panel, bin, and stutter file version

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

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

Before using the software for the first time

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

1. Check the version of panel, bin, and stutter files installed with the GeneMapper® *ID-X* Software as explained in "Check panel, bin, and stutter file version" below.
2. Check **www.lifetechnologies.com/support ▸ Software, Patches & Updates ▸** GeneMapper® *ID-X* Software to determine if newer files are available.
3. If updated files are available, download and import the files into the GeneMapper® *ID-X* Software, as explained in "Import panels, bins, and marker stutter" on page 46.

   Note: When downloading new versions of analysis files, refer to the associated Read Me file for details of changes between software file versions. If you have validated previous file versions for data analysis, conduct the appropriate internal verification studies before using new file versions for operational analysis.
4. Create an analysis method, as explained in "Create an analysis method" on page 50.
5. Define custom views of analysis tables.

   Refer to Chapter 1 of the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574) for more information.
6. Define custom views of plots.

   Refer to Chapter 1 of the *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574) for more information.

For more information

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

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

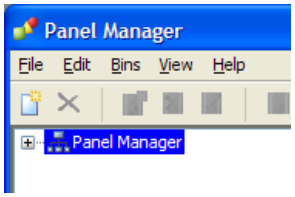
- *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574)
- *GeneMapper® ID-X Software Version 1.0 Quick Reference Guide* (Pub. no. 4375670)
- *GeneMapper® ID-X Software Version 1.0 Reference Guide* (Pub. no. 4375671)

Check panel, bin, and stutter file version

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

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

2. Select **Tools** ▸ **Panel Manager**.
3. Check the version of files imported into the Panel Manager:

   a. Select **Panel Manager** in the navigation pane.
   b. Expand the Panel Manager folder and any sub-folders to identify the analysis file version already installed for your kit choice.
4. Check the version of files available for import into the Panel Manager:
   a. Select **Panel Manager**, then select **File** ▸ **Import Panels** to open the Import Panels dialog box.
   b. Navigate to, then open the Panels folder and check the version of panel, bin, and stutter files installed.
5. If newer versions are available on the website, download and import as described below.

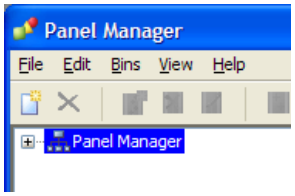
Import panels, bins, and marker stutter

To import the latest version of the Yfiler® panel, bin set, and marker stutter from our web site into the GeneMapper® *ID-X* Software database:

1. Download and open the file containing panels, bins, and marker stutter:
   a. Go to **www.lifetechnologies.com/support** ▸ **Software, Patches & Updates** ▸ GeneMapper® *ID-X* Software. Download the file Yfiler v1.3X PBS.
   b. Unzip the file.
2. Start the GeneMapper® *ID-X* Software, then log in with the appropriate user name and password.

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

3. Select **Tools** ▸ **Panel Manager**.
4. Find, then open the folder containing the panels, bins, and marker stutter:
   a. Select **Panel Manager** in the navigation pane.

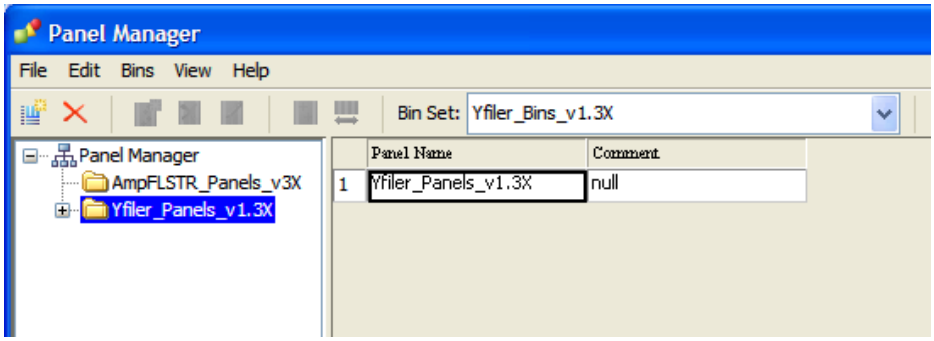
   b. Select **File** ▸ **Import Panels** to open the Import Panels dialog box.
   c. Navigate to, then open the **Yfiler v1.3X PBS** folder that you unzipped in step 1 on page 46.

5. Select **Yfiler_Panels_v1.3X** (or the version you installed), then click **Import**.

   Note: This folder contains the panel and associated markers.

6. Import Yfiler_Bins_v1.3X.txt:

   a. Select the **Yfiler_Panels_v1.3X** folder in the navigation pane.

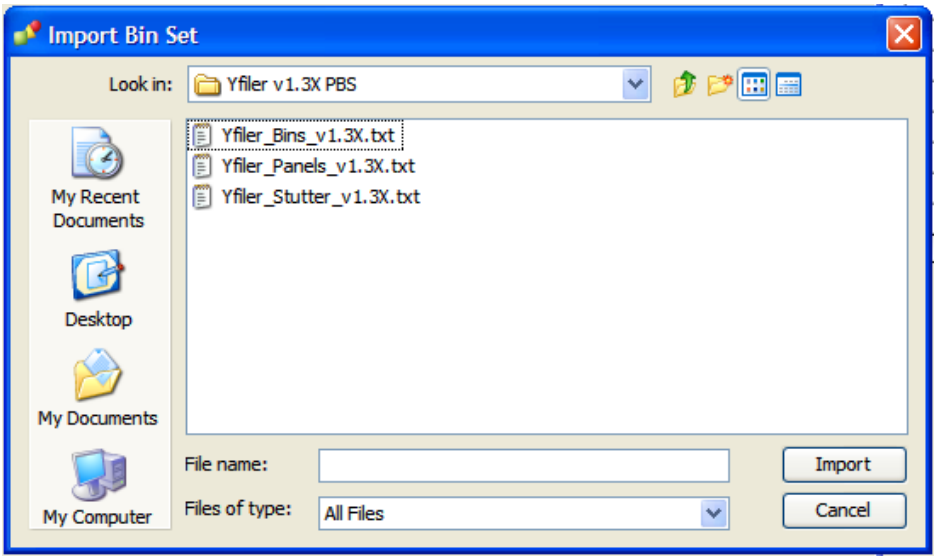


   b. Select **File** ▸ **Import Bin Set** to open the Import Bin Set dialog box.

   c. Navigate to, then open the **Yfiler v1.3X PBS** folder.

   d. Select **Yfiler_Bins_v1.3X.txt**, then click **Import**.

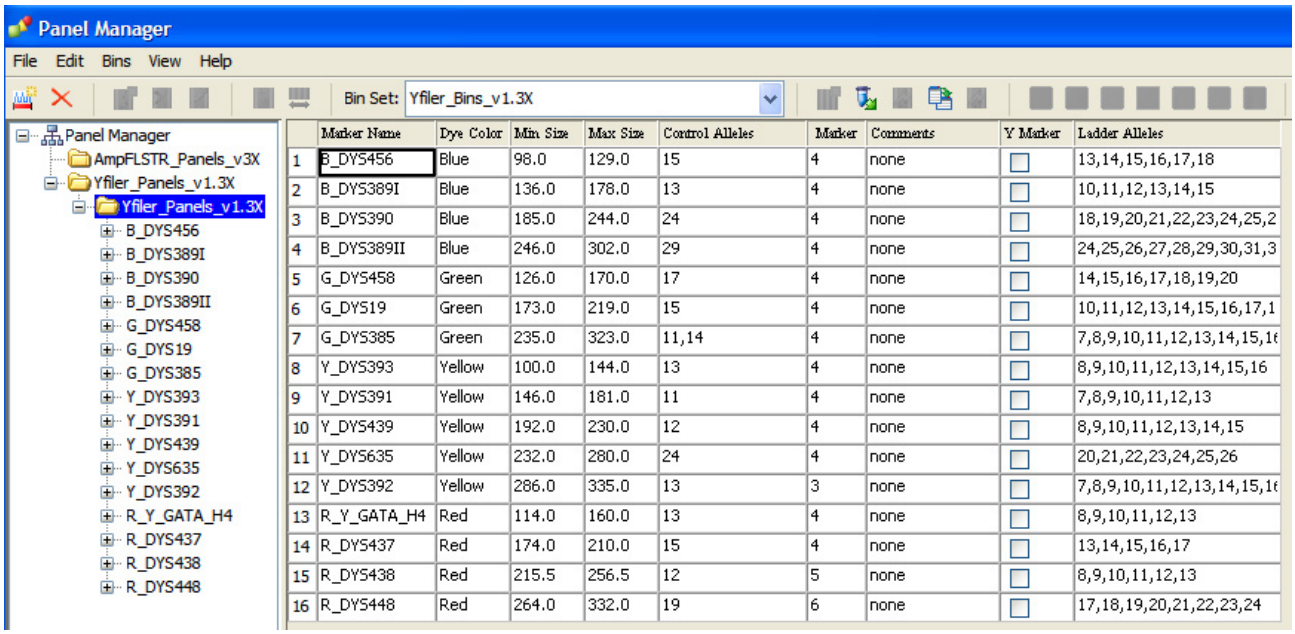
   Note: Importing this file associates the bin set with the panels in the Yfiler_Panels_v1.3X folder.



7. View the imported panels in the navigation pane:

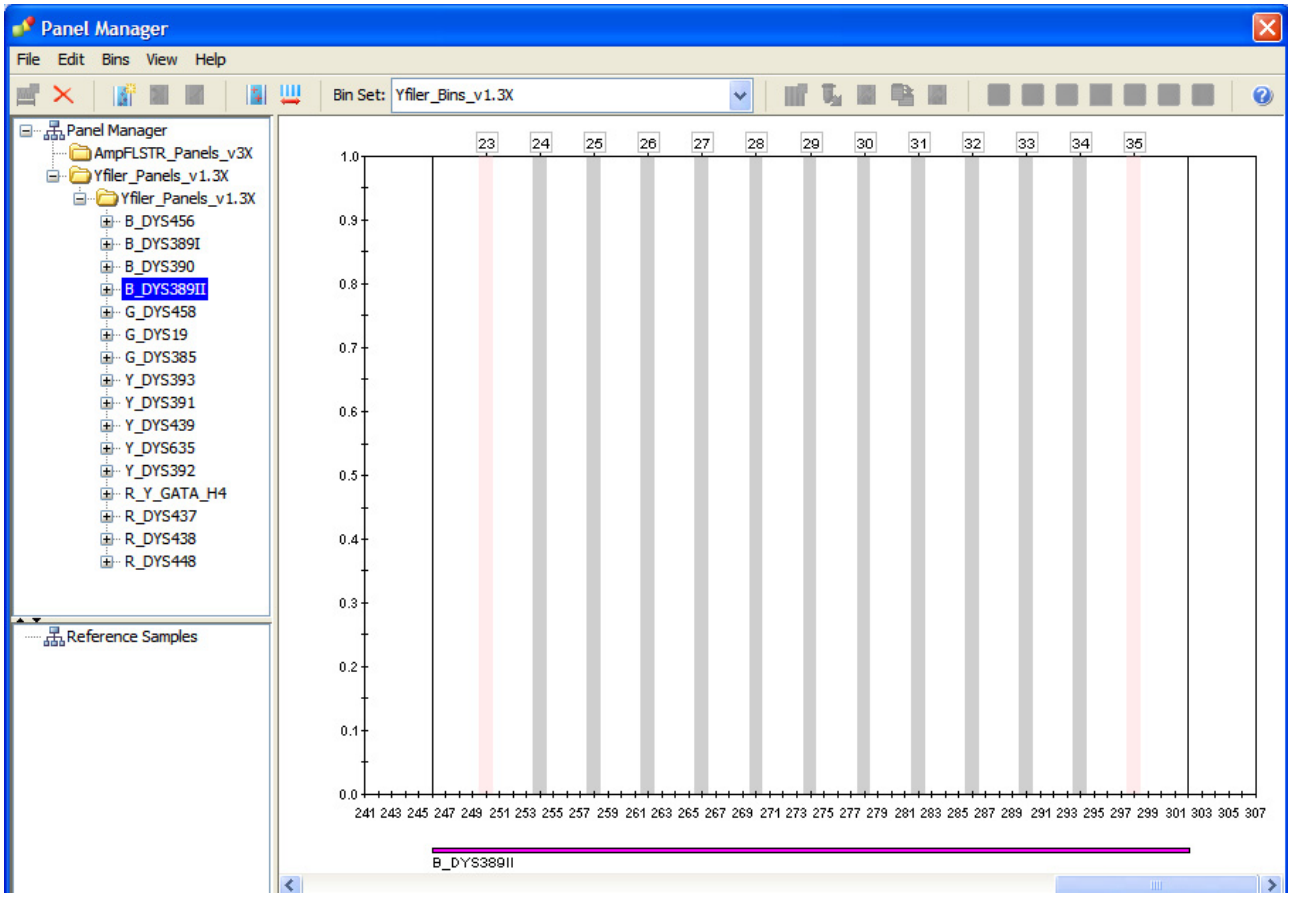
   a. Double-click the **Yfiler_Panels_v1.3X** folder.

b. Double-click the **Yfiler_v1.3X** folder to display the panel information in the right pane.

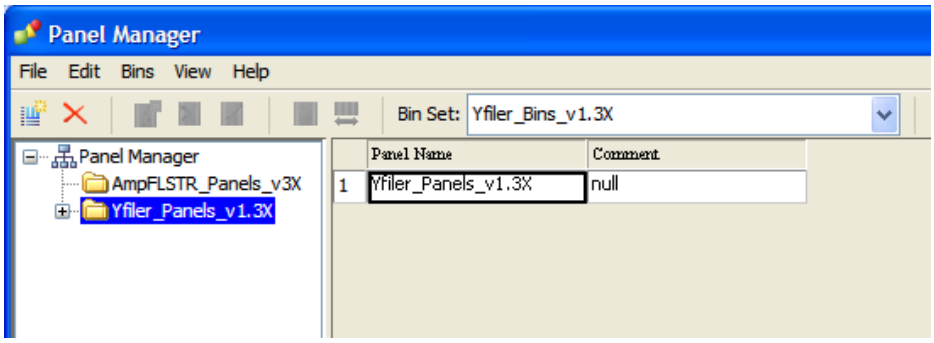


	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker	Comments	Y Marker	Ladder Alleles
1	B_DYS456	Blue	98.0	129.0	15	4	none		13,14,15,16,17,18
2	B_DYS389I	Blue	136.0	178.0	13	4	none		10,11,12,13,14,15
3	B_DYS390	Blue	185.0	244.0	24	4	none		18,19,20,21,22,23,24,25,2
4	B_DYS389II	Blue	246.0	302.0	29	4	none		24,25,26,27,28,29,30,31,3
5	G_DYS458	Green	126.0	170.0	17	4	none		14,15,16,17,18,19,20
6	G_DY519	Green	173.0	219.0	15	4	none		10,11,12,13,14,15,16,17,1
7	G_DYS385	Green	235.0	323.0	11,14	4	none		7,8,9,10,11,12,13,14,15,1
8	Y_DYS393	Yellow	100.0	144.0	13	4	none		8,9,10,11,12,13,14,15,16
9	Y_DYS391	Yellow	146.0	181.0	11	4	none		7,8,9,10,11,12,13
10	Y_DYS439	Yellow	192.0	230.0	12	4	none		8,9,10,11,12,13,14,15
11	Y_DYS635	Yellow	232.0	280.0	24	4	none		20,21,22,23,24,25,26
12	Y_DYS392	Yellow	286.0	335.0	13	3	none		7,8,9,10,11,12,13,14,15,1
13	R_Y_GATA_H4	Red	114.0	160.0	13	4	none		8,9,10,11,12,13
14	R_DYS437	Red	174.0	210.0	15	4	none		13,14,15,16,17
15	R_DYS438	Red	215.5	256.5	12	5	none		8,9,10,11,12,13
16	R_DYS448	Red	264.0	332.0	19	6	none		17,18,19,20,21,22,23,24

8. Select and expand **Yfiler_v1.3X** in the navigation pane, then select **DYS389II** to display the Bin view for the marker in the right pane.

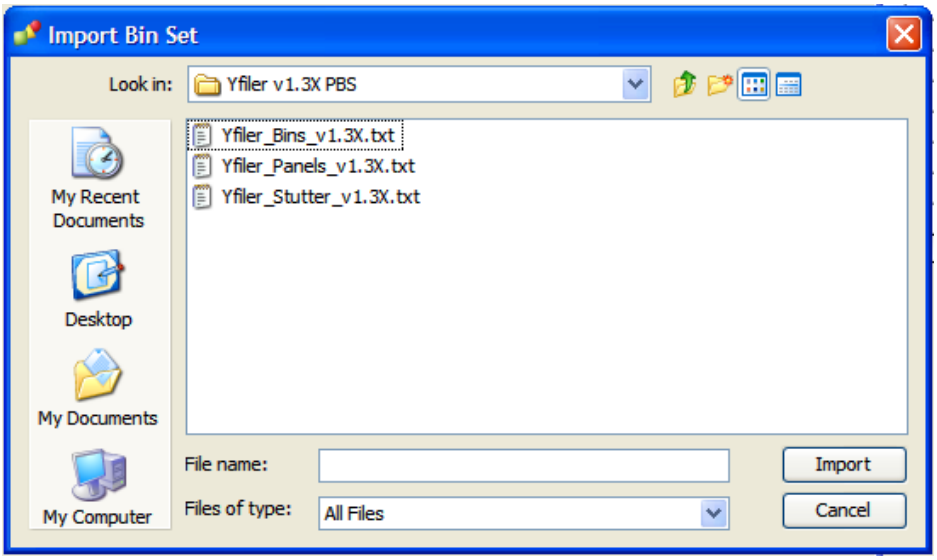


9. Import AmpFLSTR_Stutter_v2X:
   a. Select the **Yfiler_Panels_v1.3X** folder in the navigation panel.



   b. Select File ▸ **Import Marker Stutter** to open the Import Marker Stutter dialog box.
   c. Navigate to, then open the **Yfiler v1.3X PBS** folder.
   d. Select **AmpFLSTR_Stutter_v2X**, then click **Import**.

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



10. View the imported marker stutters in the navigation pane:
    a. Double-click the **Yfiler_Panels_v1.3X** folder to display its list of kits in the right pane.
    b. Double-click the **Yfiler_v1.3X** folder to display its list of markers below it.
11. Click **Apply**, then **OK** to add the Yfiler® panel, bin set, and marker stutter to the GeneMapper® *ID-X* Software database.

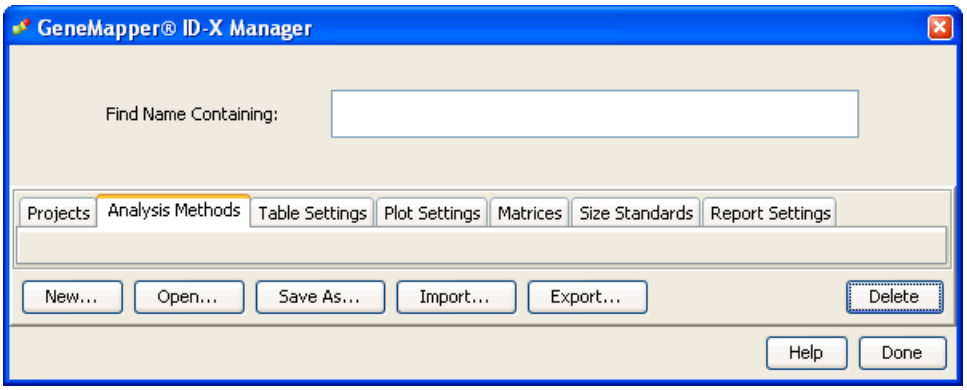
IMPORTANT! If you close the Panel Manager without clicking **Apply**, the panels, bin sets, and marker stutter will not be imported into the GeneMapper® *ID-X* Software database.

Create an analysis method

Use the following procedure to create an analysis method for the Yfiler® Direct Kit.

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

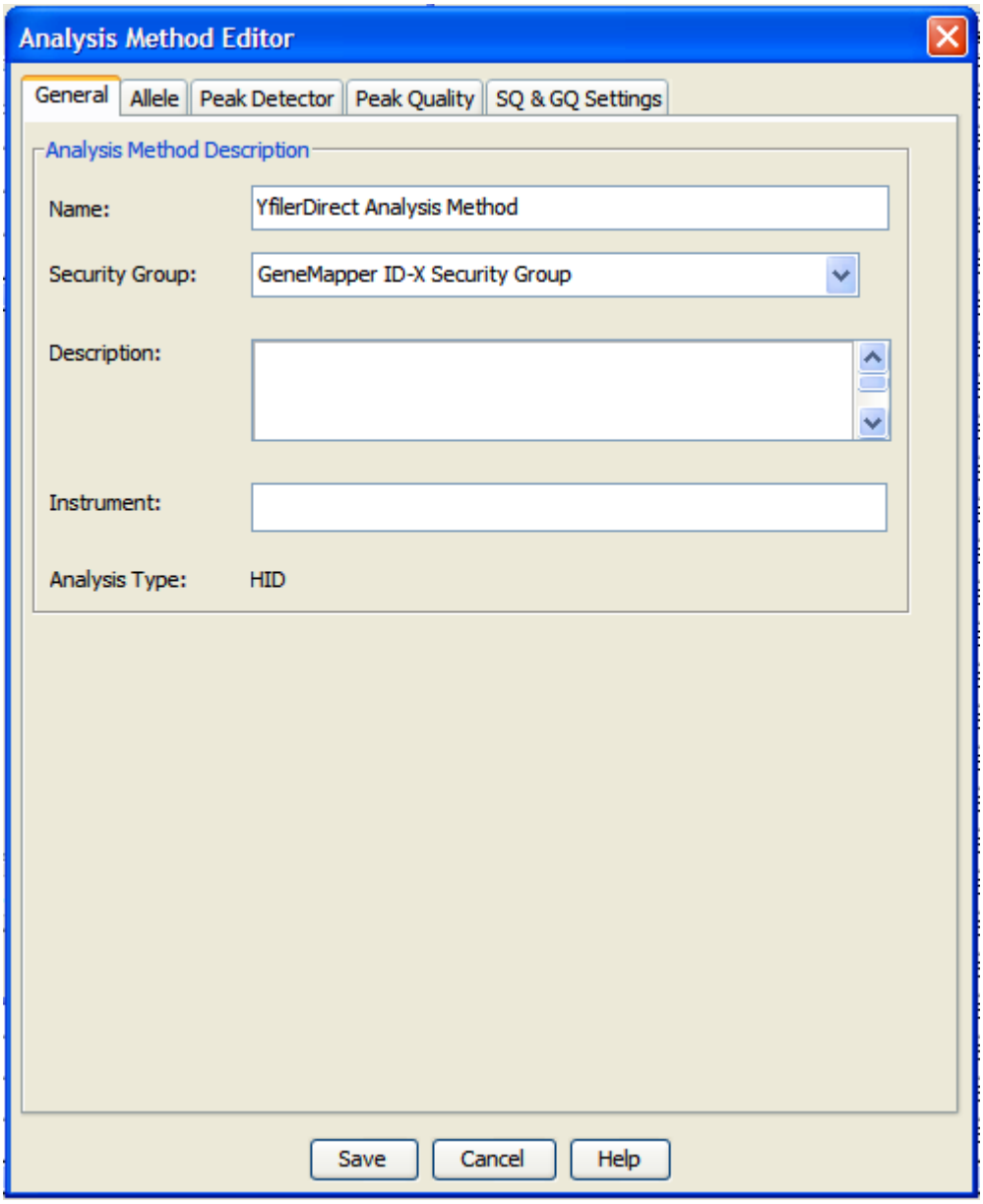
1. Select **Tools** ▸ **GeneMapper® ID-X Manager** to open the GeneMapper® *ID-X* Manager.



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

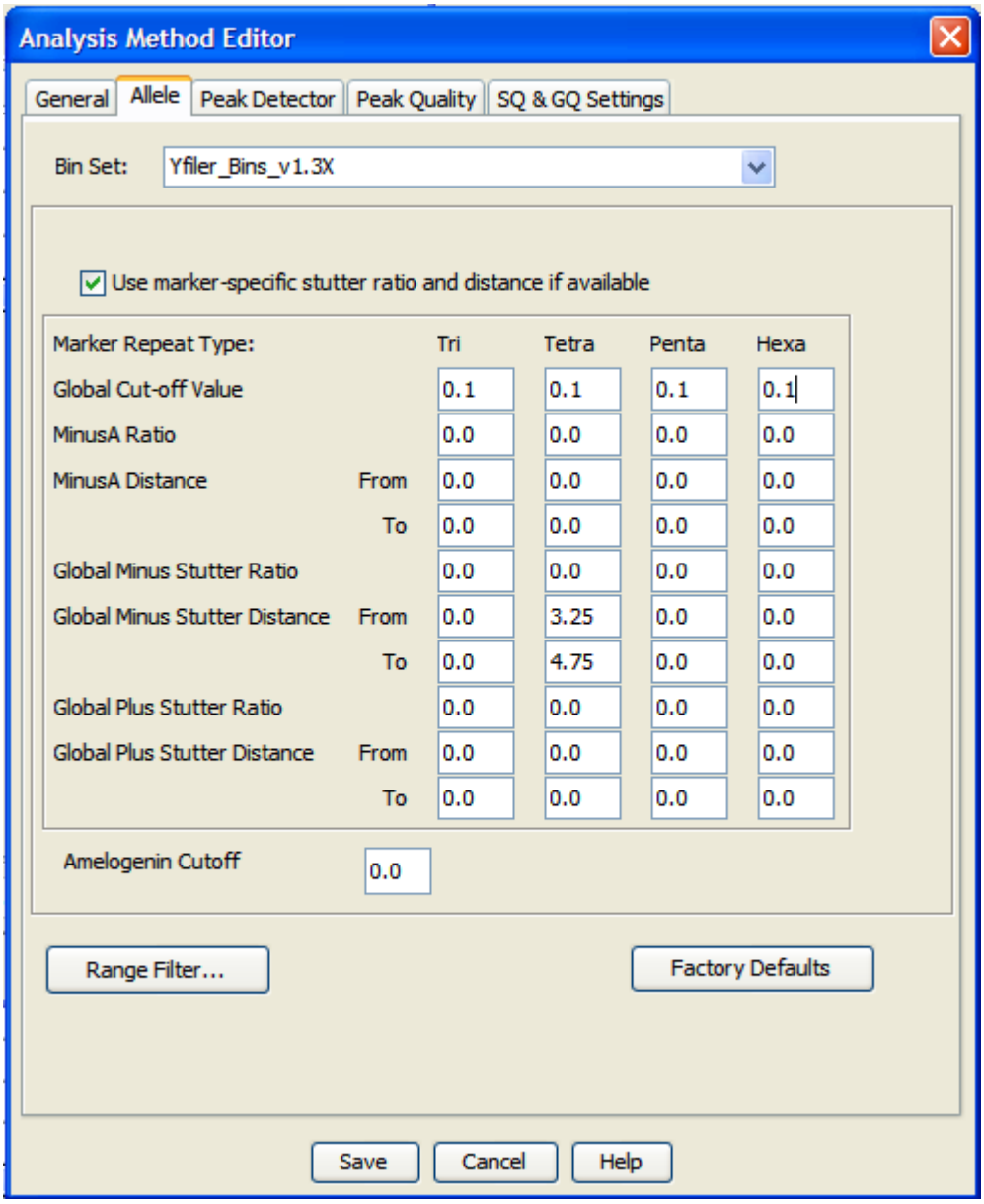
   Note: The Analysis Method Editor closes when you save your settings. To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.
4. After you enter settings in all tabs, click **Save**.

General tab settings



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

Allele tab settings

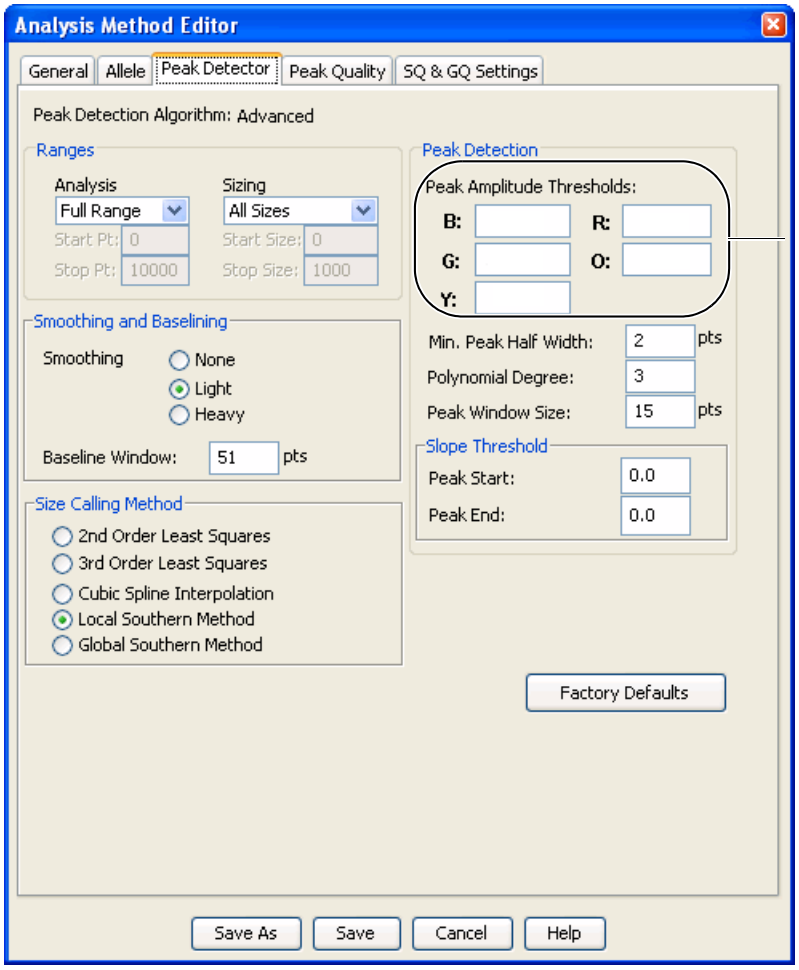


- In the Bin Set field, select the **Yfiler_Bins_v1.3X** bin set and configure the Global cutoff value as shown. These settings were used during developmental validation of the Yfiler® Direct Kit.

  Note: Applying global stutter ratios may reduce the editing required for single-source sample data. Perform internal validation studies to determine the appropriate filter setting to use.

- To apply the stutter ratios contained in the Yfiler_Panel_v1.3X.txt file, select the "Use marker-specific stutter ratio if available" check box (selected by default). Perform internal validation studies to determine the appropriate filter setting to use.

Peak Detector tab settings



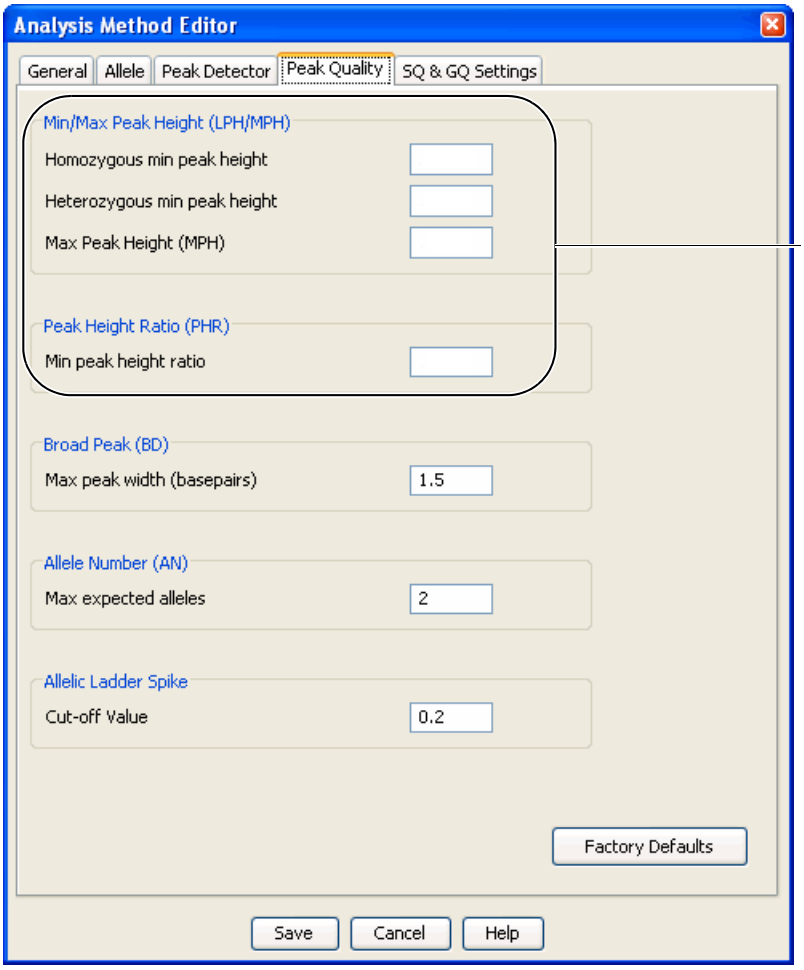
Perform internal validation studies to determine settings

IMPORTANT! Perform the appropriate internal validation studies to determine the appropriate peak amplitude thresholds for interpretation of Yfiler® Direct Kit data.

Fields include:

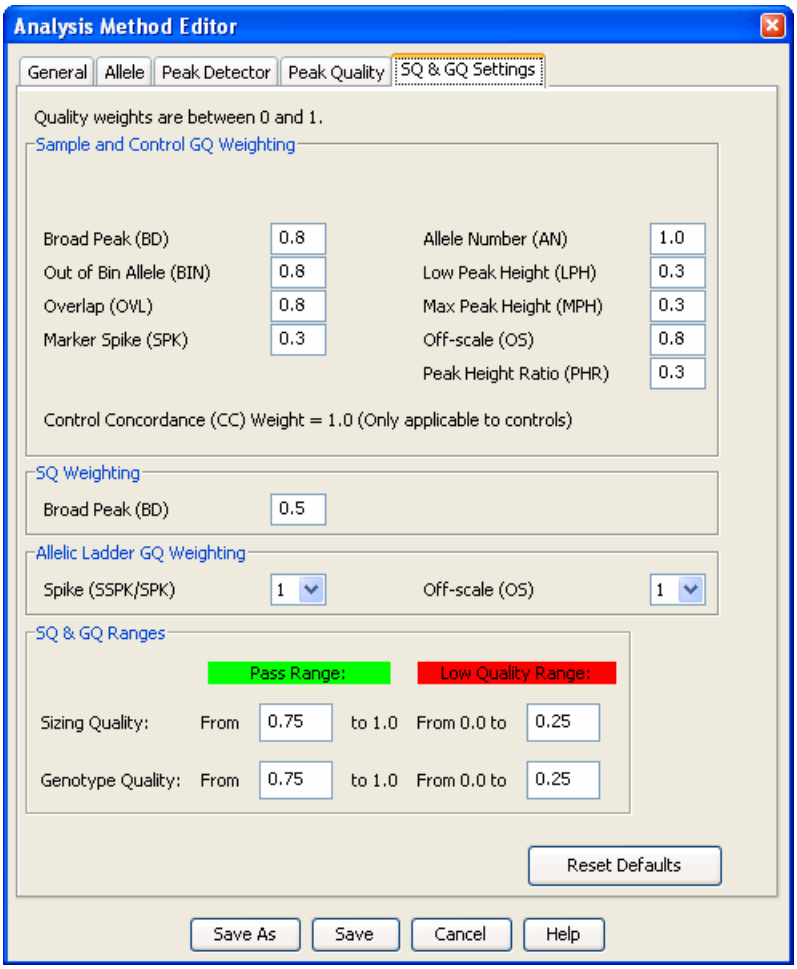
- **Peak amplitude thresholds** – The software uses these parameters to specify the minimum peak height to limit the number of detected peaks. Although GeneMapper® *ID-X* Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the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

Peak Quality tab settings



IMPORTANT! Perform internal validation studies to determine the minimum heterozygous and homozygous minimum peak height thresholds, maximum peak height threshold and the minimum peak height ratio threshold for interpretation of Yfiler® Direct Kit data.

SQ & GQ tab settings



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

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

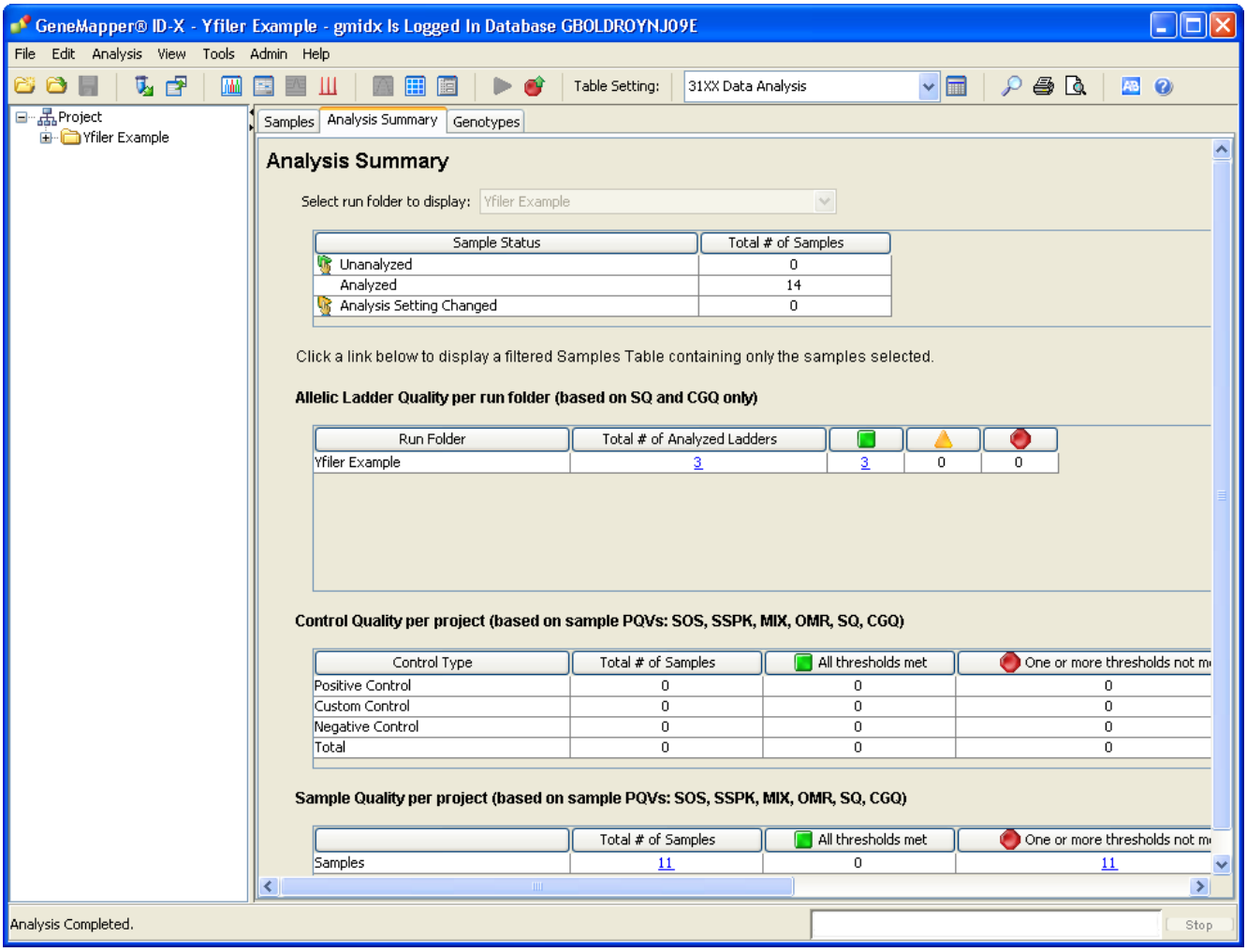
1. In the Project window, select **File ▸ Add Samples to Project**, then navigate to the disk or directory containing the sample files.
2. Apply analysis settings to the samples in the project.

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	YfilerDirect_AnalysisMethod_v2X (or the name of the analysis method you created)
Panel	Yfiler_v1.3X
Size Standard	GS600_LIZ_(80-400)

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

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

Analysis summary window after analysis



Examine and edit a project

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

For more information

For more information, refer to:

- *GeneMapper® ID-X Software Version 1.0 Getting Started Guide* (Pub. no. 4375574)
- *GeneMapper® ID-X Software Version 1.0 Quick Reference Guide* (Pub. no. 4375670)
- *GeneMapper® ID-X Software Version 1.0 Reference Guide* (Pub. no. 4375671)
- *GeneMapper® ID-X Software Version 1.1(Mixture Analysis) Getting Started Guide* (Pub. no. 4396773)
- *GeneMapper® ID-X Software Version 1.2 Reference Guide* (Pub. no. 4426481)
- *GeneMapper® ID-X Software Version 1.2 Quick Reference Guide* (Pub. no. 4426482)
- *GeneScan® Analysis Software for the Windows® NT Operating System Overview of the Analysis Parameters and Size Caller User Bulletin* (Pub. no. 4335617).

5 Experiments and Results

Overview

This chapter provides results of the developmental validation experiments we performed using the Yfiler® Direct Kit.

Importance of validation

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

Experiment conditions

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

We performed additional studies according to the revised guidelines from the Scientific Working Group on DNA Analysis Methods (SWGDAM, July 10, 2003). This DNA methodology is not novel (Moretti et al., 2001; Frank et al., 2001; Wallin et al., 2002; and Holt et al., 2001).

Based on these standards, we conducted experiments which comply with Standards 1.0 and 2.0 and its associated subsections. Whereas this DNA methodology is not novel, Standard 8.1.2 and its related subsections have been addressed (Holt et al., 2001 and Wallin et al., 2001). This chapter will discuss many of the experiments we performed and examples of the results we obtained. We used conditions that produced

maximum PCR product yield and a window in which reproducible performance characteristics were met. These experiments, while not exhaustive, are appropriate for a manufacturer, in our opinion. Each laboratory using the Yfiler® Direct Kit should perform appropriate validation studies.

Note: The Yfiler® Direct Kit is intended for use on unpurified, single source blood samples on FTA® treated or untreated paper, or buccal samples on FTA® treated paper only. We did not perform mixture or inhibition studies during the developmental validation of the Yfiler® Direct Kit, as the results would bear no relevance to the intended use of this chemistry.

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).

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

SWGDAM Guideline 2.1.0.1

"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 theYfiler® Direct Primer Mix, Yfiler® Direct Master Mix and PCR Enhancer were evaluated. The concentration for a particular component was established to be in the window that meets the reproducible performance characteristics of specificity and sensitivity.

Thermal cycler parameters

Thermal cycling parameters were established for amplification of the Yfiler® Direct Kit in the GeneAmp® PCR System 9700. Thermal cycling times and temperatures of GeneAmp PCR systems were also verified on the Veriti® thermal cycler. Annealing and denaturation temperature windows were tested around each stipend 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 effects of denaturation and annealing temperatures on the amplification of Yfiler® Direct Kit loci were examined using AmpFℓSTR® Control DNA 007 and two DNA samples.

Denaturation times of 10, 15, and 20 seconds were tested at a denaturing temperature of 94°C on the GeneAmp® PCR System 9700. The annealing temperatures tested were 57, 58, 59, and 61°C for 30-, 60-, 90-, 120-, and 180-second hold times in the GeneAmp® PCR System 9700 with the silver 96-well block. The PCR products were analyzed using the 3130*xl* and 3500 Genetic Analyzers.

Based on multifactorial analysis, denaturation conditions of 11 seconds at 94°C and annealing for 75 seconds at 58°C yielded the most robust profiles with regard to peak height and intracolor balance.

PCR cycle number

The Yfiler® Direct Kit reactions were amplified for 26 and 27 cycles on the GeneAmp® PCR System 9700 using blood samples on FTA®, buccal samples on FTA®, and 007 male control DNA. As expected, PCR product increased with the number of cycles.

To determine the cycle number that gives optimal sensitivity, we recommend analysts perform a sensitivity study amplifying their sample types for 25 to 27 cycles. Select the cycle number that generates average peak heights of 1000 to 3000 RFU on a 3130/3130*xl* platform with minimal off-scale peaks. See "Optimize PCR cycle number" on page 17.

Accuracy, precision, and reproducibility

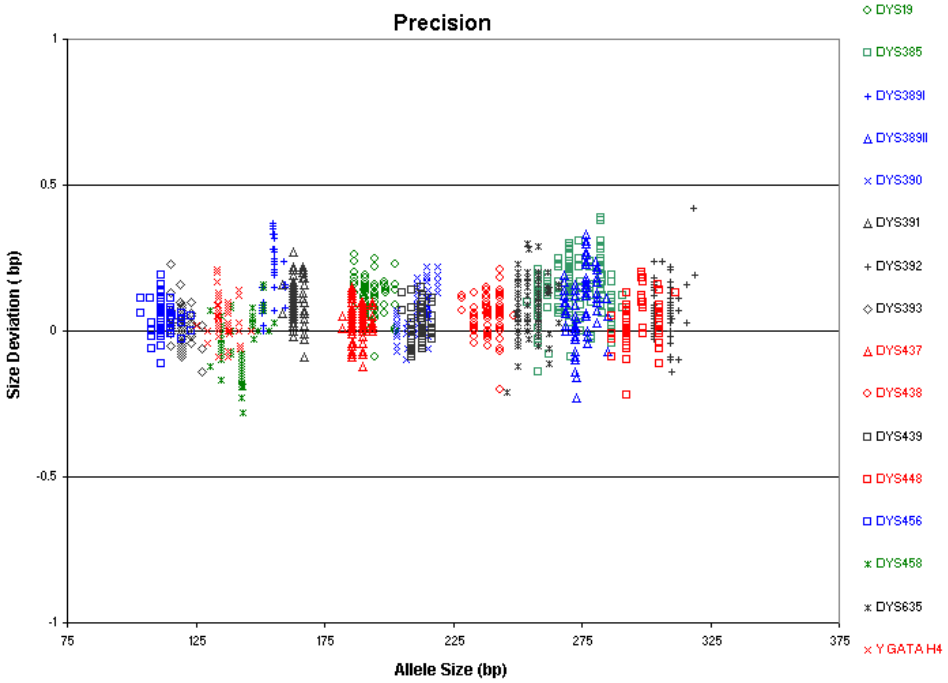
SWGDAM Guideline 2.9

"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 systems of length polymorphism at short tandem repeat loci is not a novel methodology (Holt et al., 2001 and Wallin et al., 2001). However, accuracy and reproducibility of Yfiler® Direct Kit profiles have been determined from various sample types.

Figure 4 illustrates the size differences that are typically observed between sample alleles and allelic ladder alleles on the 3100 Genetic Analyzer with POP-4® polymer. The x-axis in Figure 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 4 Size deviation of 78 samples analyzed on the 3100 Genetic Analyzer



Precision and size windows

Sizing precision allows for determining accurate and reliable genotypes. Sizing precision was measured on the 3100 Genetic Analyzer. The recommended method for genotyping is to use a ± 0.5-bp "window" around the size obtained for each allele in the AmpFℓSTR® 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, for example, 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 in one capillary run. Table 3 on page 62 indicates typical precision results obtained from the seven injections of the AmpFℓSTR® Yfiler® Allelic Ladder analyzed on the 3100 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 4 illustrates the tight clustering of allele sizes obtained on the 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 vs. measurement error of a sample allele that corresponds with an allele in the allelic ladder. Repeat analysis, when necessary, provides an added level of confidence to the final allele assignment. GeneMapper® *ID* Software and GeneMapper® *ID-X* Software automatically flags sample alleles that do not size within the prescribed window around an allelic ladder allele.

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

Table 3 Example of precision results of nine injections of the AmpFℓSTR® Yfiler® Allelic Ladder run on the 3100 Genetic Analyzer

Allele	Mean	Standard Deviation
DYS456		
13	104.51	0.05

Allele	Mean	Standard Deviation
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
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

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
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

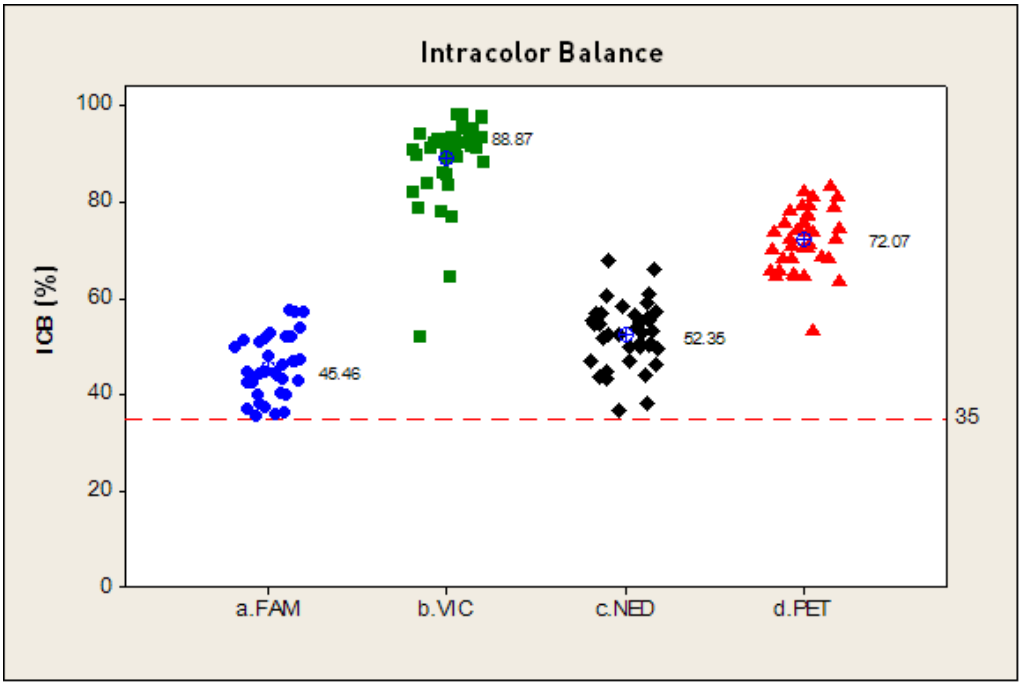
Allele	Mean	Standard Deviation
9	104.19	0.04
10	108.05	0.04
11	112.04	0.04
12	115.98	0.04
13	119.89	0.04
14	123.89	0.04
15	127.80	0.05
16	131.95	0.04
DYS391		
7	150.88	0.08
8	155.27	0.06
9	159.67	0.06
10	163.83	0.05
11	167.94	0.07
12	172.00	0.07
13	176.03	0.06
DYS439		
8	197.84	0.05
9	201.70	0.03
10	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
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

Allele	Mean	Standard Deviation
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
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
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

Reproducibility

1.2 mm punches from 40 male buccal samples on FTA® paper were amplified for 26 cycles with the Yfiler® Direct Kit on the GeneAmp® PCR System 9700. One sample yielded a partial profile. All other samples yielded complete profiles with >40% average intracolor balance in all 4 dye channels.

Figure 5 Intracolor balance for Yfiler® Direct profiles generated from 39 male buccal samples on FTA® paper.



1.2 mm punches from the same 40 male buccal samples and 72 male blood samples on FTA® paper were also amplified using the Yfiler® Direct Kit on the Veriti® Thermal Cycler. All samples had been previously amplified with Yfiler® Direct on the 9700 and genotyped. Results generated from amplification on the Veriti® Thermal Cycler were 100% concordant to results previously generated following amplification on the 9700.

Extra Peaks in the electropherogram

Causes of extra peaks

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

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 (stutter percent) is measured by dividing the height of the stutter peak by the height of the main allele peak. The Yfiler® Direct Kit stutter percentages were calculated from data generated from 86 samples (57 blood samples on FTA® paper and 29 buccal samples on FTA® paper). The mean stutter percentage plus three standard deviations were calculated for each locus and compared to the stutter percentages generated previously with the Yfiler® Kit.

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

- For each Yfiler® Direct Kit locus, the stutter percentage generally increases with allele length. Smaller alleles display a lower level of stutter relative to the longer alleles within each locus.
- Each allele within a locus displays percent stutter that is reproducible.
- The stutter value for each locus was determined by taking the mean plus three times the standard deviation. For all loci these values are lower than the stutter filter percentages determined using the original Yfiler® Kit. Therefore, the original Yfiler® Kit stutter values were used during the filtering step in GeneMapper® *ID* Software or GeneMapper® *ID-X* Software. Peaks in the stutter position that are above the stutter filter percentage will not be filtered. Peaks in the stutter position that have not been filtered and remain labeled can be further evaluated.
- The measurement of percent stutter may be unusually high for main peaks that are off-scale.

Note: The stutter values observed for the Yfiler® Direct Kit were consistent with those obtained with the Yfiler® Kit. In situations where a global cutoff filter is used, locus-specific stutter filters will be applied when the stutter percentage exceeds the global cutoff filter value.

Addition of 3’ A

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. For the Yfiler® Direct Kit the primer sequences are optimized to promote A addition.

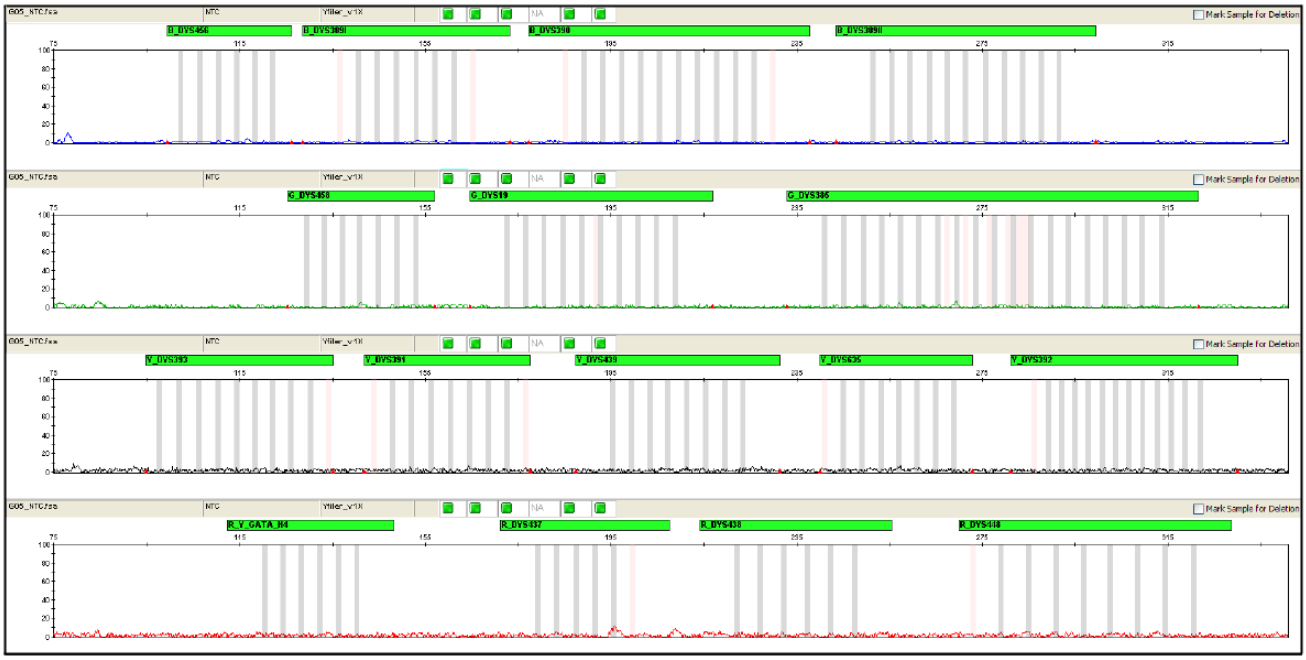
Lack of full A nucleotide addition may be observed in Yfiler® Direct Kit results when the amount of input DNA is greater than recommended protocols. This is because more time is needed for AmpliTaq Gold® DNA Polymerase to add the A nucleotide to all molecules as more PCR product is generated. Amplification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

Artifacts

Developmental validation studies for the Yfiler® Kit were performed using a 10% global cut-off value which provides effective filtering of low-level artifacts and streamlines the analysis of single source samples. If a global cut-off value is not used low-level artifacts, or anomalies may be observed above the detection threshold.

Figure 6 demonstrates reproducible artifacts while using the Yfiler® Direct Kit. Consider these artifacts when interpreting data.

Figure 6 Examples of baseline noise and reproducible artifacts in data produced on the 3130*xl* 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 Yfiler® Direct 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 Yfiler® Direct 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.

We have sequenced all the alleles in the AmpFℓSTR® Yfiler® Allelic Ladder. 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 Yfiler® Direct 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. 1 ng of DNA from each sample was amplified using the Yfiler® Kit and the Identifiler® Kit, followed by analysis using a 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® Kit results confirmed that the loci are inherited according to Mendelian rules, as has been reported in the literature (Nakahori et al., 1991; Edwards et al., 1992; Kimpton et al., 1992; Mills et al., 1992; Sharma and Litt, 1992; Li et al., 1993; Straub et al., 1993). The Yfiler® Kit 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 Yfiler® Direct 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 Yfiler® Direct Kit provides the required degree of specificity specific to primates. Other species do not amplify for the loci tested.

Nonhuman Studies

The Yfiler® Direct Kit provides the required degree of specificity for the species tested (Figure 7 on page 72).

The following experiments were conducted to investigate interpretation of Yfiler® Direct Kit results from nonhuman DNA sources.

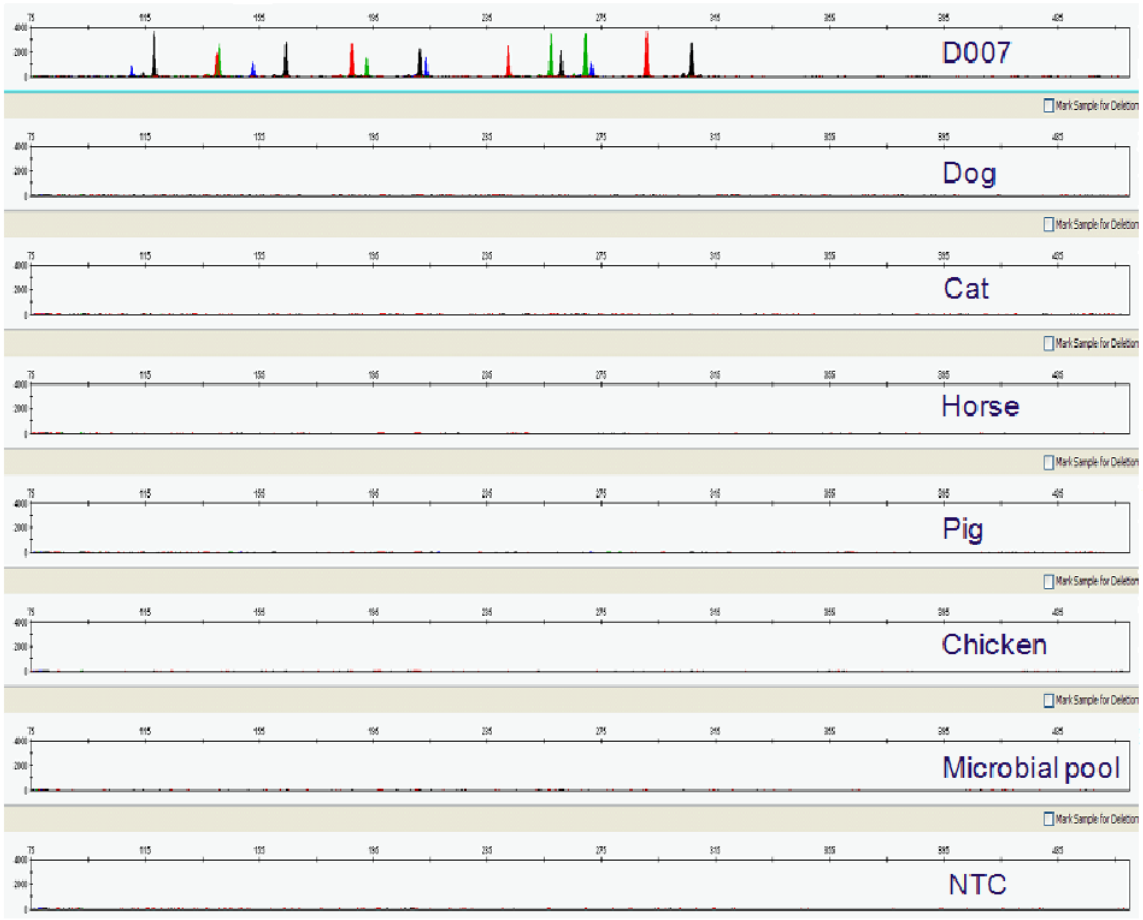
The extracted DNA samples were amplified in Yfiler® Direct Kit reactions and analyzed using the 3130*xl* Genetic Analyzer.

Non-primates – Dog, pig, cat, horse, and chicken (10 ng each).

Microorganisms – *Candida albicans*, *Neisseria gonorrhoeae*, *Escherichia coli* 0157:H7, *Bacillus subtilis*, *Staphylococcus aureus*, and *Lactobacillus rhamnosus* (10 ng total from a pool of these microorganisms).

The non-human species tested did not yield reproducible, detectable results.

Figure 7 Representative electropherograms from a species specificity study, including positive and negative controls.



Sensitivity

SWGDAM Guideline 2.3

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

Blood on FTA® cards or Copan® NUCLEIC-CARD™ system

The Yfiler® Direct Kit has been optimized at 25 µL PCR reaction volume to overcome the PCR inhibition expected when amplifying blood samples directly from unpurified 1.2 mm FTA® or Copan® NUCLEIC-CARD™ discs. Depending on the volume of blood spotted onto the FTA® card, DNA quantities present on the 1.2 mm disc may vary between laboratories. It is essential for your laboratory to optimize the PCR conditions based on the types of blood samples received or based on your standard operating protocol used in the spotting of blood onto FTA® cards or Copan® NUCLEIC-CARD™ system. Refer to page 17 for instructions on PCR optimization.

Buccal cells on FTA® cards or Copan® NUCLEIC-CARD™ system

The Yfiler® Direct Kit has been optimized at 25 µL PCR reaction volume to overcome the PCR inhibition expected when amplifying buccal cells directly from unpurified 1.2 mm FTA® or Copan® NUCLEIC-CARD™ discs. Depending on the collecting devices used, the collection methods applied, and the swab-to-treated paper transfer protocol employed, DNA quantities present on the 1.2 mm disc may vary between samples and different laboratories. It is essential for your laboratory to optimize the PCR conditions based on the types of buccal samples received or based on your standard operating protocol used in transferring saliva from a buccal swab onto an FTA® card or Copan® NUCLEIC-CARD™ system. Refer to page 17 for instructions on PCR optimization.

Effect of DNA quantity on results

If excessive 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 problematic for two reasons:

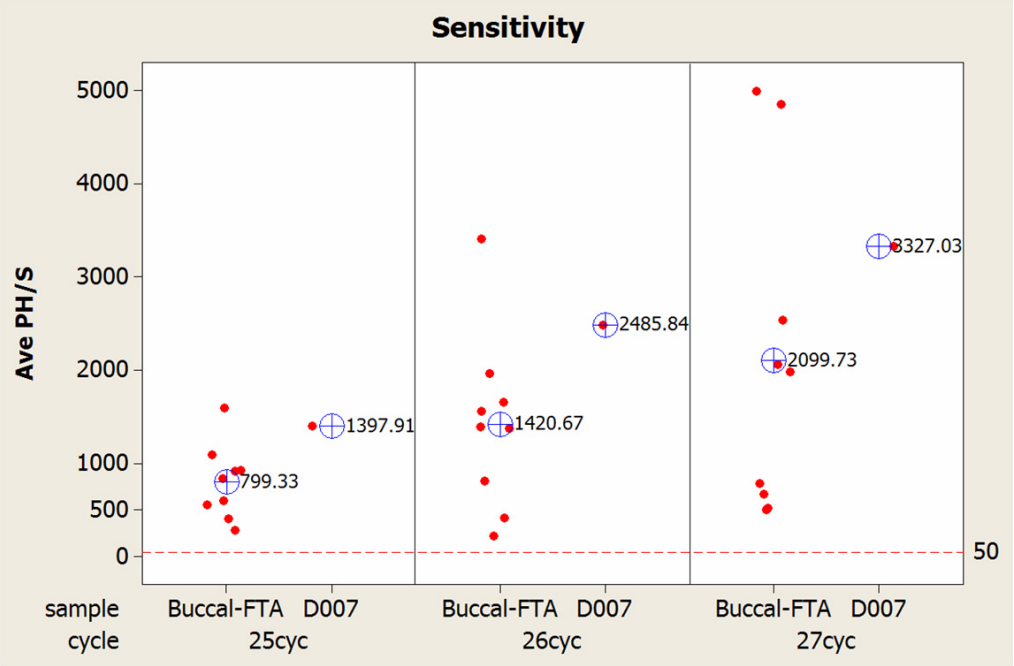
  – Quantitation (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause the corresponding stutter peak to appear higher in relative intensity, thus increasing the calculated percent stutter.

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

- Incomplete A nucleotide addition.

To ensure minimal occurrence of offscale data when using the Yfiler® Direct Kit, optimize PCR cycle number according to instructions on page 17.

Figure 8 Average Yfiler® Direct peak height/sample for buccal samples from 1.2 mm FTA punches and DNA Control 007. 4 ng 007 DNA was amplified for 27 cycles, 6 ng of 007 DNA was amplified for 25 and 26 cycles with Yfiler® Direct.



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 suspect's reference sample, then the suspect is "excluded" as the donor of the biological evidence tested. An exclusion is independent of the frequency of the two genotypes in the population.

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

Population samples used in these studies

The Yfiler® 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.

Population	Number of samples
African-American	333
U.S. Caucasian	254
U.S. Hispanic	175

Gene diversity values

Table 4 shows the Yfiler® Kit gene diversity in three populations, listed as percentages.

Table 4 Yfiler® Kit Gene Diversity values across three different U.S. populations

Locus	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

Gene diversity (D) = $\frac{n(1-\Sigma p_i^2)}{n-1}$ where n = sample size, p_i = allele frequency (Johnson et al., 2003).

Analyzing the population data

In addition to the alleles that were observed and recorded in the Life Technologies 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 5 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 5 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. Hispanic (N=175)	
	DC (%)	UH	DC (%)	UH	DC (%)	UH
Minimal haplotype†	84.6	249	74.8	162	85.1	136
U.S. haplotype‡	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

† The minimal haplotype includes the markers DYS19, DYS385 a/b, DYS389 I/II, DYS390, DYS391, DYS392, DYS393.

‡ The U.S. haplotype includes the minimal haplotype loci plus DYS438 and DYS439.

Mutation rate

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

In previous studies, haplotypes of eight loci amplified by the Yfiler® 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.

A Troubleshooting

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

Table 6 Troubleshooting

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 PCR Master Mix or Yfiler® Direct Primer Set	Repeat amplification.
	No activation of AmpliTaq Gold® DNA Polymerase	Repeat amplification, and hold reactions initially at 95°C for 1 minute.
	Master Mix not vortexed thoroughly before aliquoting	Vortex the Master Mix thoroughly.
	Yfiler® Direct Primer Set exposed to too much light	Store the Primer Set protected from light.
	GeneAmp® PCR System malfunction	Refer to the thermal cycler user's manual and check instrument calibration.
	Use of incorrect thermal cycling parameters	Check the protocol for correct thermal cycling parameters.
	Tubes not seated tightly in the thermal cycler during amplification	Push reaction tubes firmly into contact with block after first cycle. Repeat test.
	Wrong PCR reaction tube	Use MicroAmp® Reaction Tubes with Caps for the GeneAmp® PCR System 9700.
	MicroAmp® Base used with tray/ retainer set and tubes in GeneAmp® 9700	Remove MicroAmp® Base from tray/retainer set and repeat test.
	Insufficient PCR product electrokinetically injected	Prepare PCR product as described in Chapter 3, "Perform Electrophoresis" on page 23.
	Degraded formamide	Check the storage of formamide; do not thaw and refreeze multiple times. Try Hi-Di™ Formamide.
Positive signal from AmpFℓSTR® Control DNA 007 but partial or no signal from DNA test samples	Quantity of test DNA sample is below assay sensitivity	Repeat test with another punch sample.
	Test sample DNA is severely degraded or of insufficient quantity	Repeat test with another punch sample.
More than one allele present at a locus (except for DYS385a/b)	Presence of exogenous DNA	Use appropriate techniques to avoid introducing foreign DNA during laboratory handling.
	Amplification of stutter product	See "Stutter products" on page 67.
	Mixed sample	

Observation	Possible causes	Recommended actions
Some but not all loci visible on electropherogram of DNA test samples	Test-sample DNA is severely degraded or of insufficient quantity	Repeat test with another punch sample.
Poor peak height balance	Incorrect thermal cycler parameters	Check the protocol for correct thermal cycler parameters.
	GeneAmp® PCR System 9700 with Aluminum 96-Well block or third party thermal cyclers	Use GeneAmp® PCR System 9700 with silver or gold-plated silver blocks only, or the Veriti® 96-Well Thermal Cycler.

B

Ordering Information

Equipment and materials not included

Table 7 and Table 8 list required and optional equipment and materials not supplied with the Yfiler® Direct Kit. Unless otherwise noted, many of the items are available from major laboratory suppliers (MLS).

Table 7 Equipment

Equipment	Source
3100/3100-*Avant* Genetic Analyzer	Contact your local Life Technologies sales representative
Applied Biosystems® 3130/3130*xl* Genetic Analyzer	
Applied Biosystems® 3500/3500xL Genetic Analyzer for Human Identification	
Applied Biosystems® 310 Genetic Analyzer	
GeneAmp® PCR System 9700 with the Silver 96-Well Block	N8050001
GeneAmp® PCR System 9700 with the gold-plated silver 96-well block	4314878
Veriti® 96-Well Thermal Cycler	4375786
Silver 96-well sample block	N8050251
Gold-plated silver 96-well sample block	4314443
Tabletop centrifuge with 96-well plate adapters (optional)	MLS
Harris Manual Punch, 1.2 mm	MLS
BSD600-Duet Semi-Automated Dried Sample Punch Instrument with a 1.2 mm punch head	Contact your local Life Technologies support representative for information.
BSD1000-GenePunch Automated Dried Sample Punch Instrument with a 1.2 mm punch head	
Bode Buccal DNA Collector™	4467893 This number is not available for sale in the US.
Copan® NUCLEIC-CARD™	Contact your local Life Technologies support representative for information. This product is not available for sale in the US.

Table 8 User-supplied materials

Item†	Source
AmpFℓSTR® Yfiler® Direct PCR Amplification Kit	4427368
Prep-n-Go™ Buffer	4467079
3100 Analyzer materials	
96-well plate septa	4315933
Reservoir septa	4315932
3100/3130*xl* Genetic Analyzer capillary array, 36-cm	4315931
POP-4® polymer for 3100/3100-*Avant* Genetic Analyzers	4316355
3100/3100-*Avant* Genetic Analyzer Autosampler Plate Kit, 96-well	4316471
GeneScan™ 500 LIZ® Size Standard	4322682
OR	*OR*
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
Running Buffer, 10X	402824
Hi-Di™ Formamide	4311320
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
MicroAmp® Optical 96-well reaction plate	N8010560
250-µL glass syringe (array-fill syringe)	4304470
5.0-mL glass syringe (polymer-reserve syringe)	628-3731
For a complete list of s and accessories for the 3100/3100-*Avant* instrument, refer to Appendix B of the *3100 Genetic Analyzer and 3100-Avant Genetic Analyzer User Reference Guide* (Pub. no. 4335393).	
3130*xl* Analyzer materials	
96-well plate septa	4315933
Reservoir septa	4315932
3100/3130*xl* Genetic Analyzer capillary array, 36-cm	4315931
POP-4® polymer for 3130/3130*xl* Genetic Analyzers	4352755
3100/3100-*Avant* Genetic Analyzer Autosampler Plate Kit, 96-well	4316471
GeneScan™ 500 LIZ® Size Standard	4322682
OR	*OR*
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
Running Buffer, 10X	402824
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
MicroAmp® Optical 96-well reaction plate	N8010560
Hi-Di™ Formamide	4311320
For a complete list of s and accessories for the 3130/3130*xl* instrument, refer to Appendix A of the *Applied Biosystems® 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* (Pub. no. 4352716).	
3500/3500xL Analyzer materials	
Anode buffer container (ABC)	4393927
Cathode buffer container (CBC)	4408256

Item†	Source
POP-4® polymer (960 samples) for 3500/3500xL Genetic Analyzers	4393710
POP-4® polymer (384 samples) for 3500/3500xL Genetic Analyzers	4393715
Conditioning reagent	4393718
8-Capillary array, 36 cm for 3500 Genetic Analyzers	4404683
24-Capillary array, 36 cm for 3500xL Genetic Analyzers	4404687
96-well retainer & base set (Standard) 3500/3500xL Genetic Analyzers	4410228
8-Tube retainer & base set (Standard) for 3500/3500xL Genetic Analyzers	4410231
8-Strip Septa for 3500/3500xL Genetic Analyzers	4410701
96-Well Septa for 3500/3500xL Genetic Analyzers	4412614
Septa Cathode Buffer Container, 3500 series	4410715
GeneScan™ 600 LIZ® Size Standard v2.0	4408399
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
For a complete list of parts and accessories for the 3500/3500xL instrument, refer to the *Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide* (Pub. no. 4401661)	
PCR Amplification	
MicroAmp® 96-well tray	N8010541
MicroAmp® reaction tube with cap, 0.2-mL	N8010540
MicroAmp® 8-tube strip, 0.2-mL	N8010580
MicroAmp® 8-cap strip	N8010535
MicroAmp® 96-well tray/retainer set	403081
MicroAmp® 96-well base	N8010531
MicroAmp® clear adhesive film	4306311
MicroAmp® optical adhesive film	4311971
MicroAmp® optical 96-well reaction plate	N8010560
Other user-supplied materials	
Hi-Di™ Formamide, 25-mL	4311320
Aerosol resistant pipette tips	MLS
Microcentrifuge tubes	MLS
Pipettors	MLS
Tape, labeling	MLS
Tube, 50-mL Falcon	MLS
Tube decapper, autoclavable	MLS
Deionized water, PCR grade	MLS
Tris-HCl, pH 8.0	MLS
EDTA, 0.5 M	MLS
Vortex	MLS

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

C

PCR Work Areas

Work area setup and lab design

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

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

The sensitivity of the DO NOT USE (and other PCR-based tests) enables amplification of minute quantities of DNA, necessitating precautions to avoid contamination of samples yet to be amplified (Kwok and Higuchi, 1989).

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

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

PCR setup work area

IMPORTANT! These items should never leave the PCR setup work area.

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

- Tube decapper, autoclavable
- Vortex

Amplified DNA work area

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

You can use the following systems:

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

  IMPORTANT! The DO NOT USE is not validated for use with the GeneAmp® PCR System 9700 with the Aluminium 96-Well Block. Use of this thermal cycling platform may adversely affect performance of the DO NOT USE.

- Veriti® 96-Well Thermal Cycler

D

Safety

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

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

Chemical safety

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

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

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

Biological hazard safety

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

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

In the U.S.:

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

In the EU:

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

Documentation and Support

Related documentation

Document title	Pub. no.
3100/3100-Avant Data Collection v2.0 User Guide	4347102
3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0 User Bulletin	4350218
3100 Genetic Analyzer User Manual (Data Collection v1.1)	4315834
3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products User Bulletin	4332345
Applied Biosystems® 3130/3100xl Genetic Analyzers Using Data Collection Software v3.0 User Bulletin	4363787
Applied Biosystems® 3130/3130xl Genetic Analyzers Getting Started Guide	4352715
Applied Biosystems® 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide	4352716
Applied Biosystems® 3130/3130xl Genetic Analyzers Quick Reference Card	4362825
Applied Biosystems® 3130/3130xl Genetic Analyzers AB Navigator Software Administrator Guide	4359472
Applied Biosystems® 3130/3100xl DNA Analyzers User Guide	4331468
Applied Biosystems® 3500/3500xL Genetic Analyzer Quick Reference Card	4401662
Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide, Data Collection v1.0	4401661
Applied Biosystems® 3500/3500xL Genetic Analyzer User Bulletin: Solutions to issues related to software, data, hardware, and consumables **Note:** Additional user bulletins may be available at **www.lifetechnologies.com**	4445098
Applied Biosystems® 3730/3730xl Genetic Analyzer Getting Started Guide	4359476
GeneAmp® PCR System 9700 Base Module User's Manual	N805-0200
Veriti® 96-Well Thermal Cycler AmpFℓSTR®Kit Validation User Bulletin	4440754
Quantifiler® Kits: Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit User's Manual	4344790
PrepFiler® Forensic DNA Extraction Kit User Guide	4390932
GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide	4338775
GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial	4335523
Installation Procedures and New Features for GeneMapper® *ID* Software *v3.2 User Bulletin*	4352543
GeneMapper® ID-X Software Version 1.0 Getting Started Guide	4375574
GeneMapper® ID-X Software Version 1.0 Quick Reference Guide	4375670
GeneMapper® ID-X Software Version 1.0 Reference Guide	4375671
GeneMapper® ID-X Software Version 1.1 (Mixture Analysis) Getting Started Guide	4396773
GeneMapper® ID-X Software Version 1.1 (Mixture Analysis) Quick Reference Guide	4402094

Document title	Pub. no.
GeneMapper® ID-X Software Version 1.2 Reference Guide	4426481
GeneMapper® ID-X Software Version 1.2 Quick Reference Guide	4426482

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

Note: To open the user documentation available from the our web site, use the Adobe® Acrobat® Reader® software available from **www.adobe.com**.

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

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