USER GUIDE

applied biosystems
life technologies

AmpFℓSTR® NGM SElect™ PCR Amplification Kit

for use with:
200 reaction kit (Part no. 4457889)
1000 reaction kit (Part no. 4457890)

Publication Number 4458841
Revision F

life technologies

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Preface

Revision history

Revision	Date	Description
A	June 2010	New document.
B	–	–
C	January 2011	Add chapter 5.
D	March 2011	Change “30 cycles” to “29 and 30 cycles” on page 26.
E	March 2012	Update licensing language.
F	February 2015	Add information for Veriti® 96-Well Thermal Cycler and Proflex™ PCR System.

Overview 1

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Product overview

Purpose

The AmpFℓSTR® NGM SElect™ PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that amplifies the 15 STR loci contained in the AmpFℓSTR® NGM™ kit together with the highly polymorphic SE33 (ACTBP2) locus and the gender determination locus Amelogenin. The NGM SElect™ kit delivers a 17-locus multiplex with the highest discrimination power of any AmpFℓSTR® kit and benefits from the same improvements in sensitivity and robustness pioneered with the NGM™ kit. Like the NGM™ kit, the NGM SElect™ kit uses modified PCR cycling conditions for enhanced sensitivity, a new buffer formulation to improve performance with inhibited samples, more loci concentrated in the low molecular-weight region of the profile to improve performance on degraded samples, and an improved process for synthesis and purification of the amplification primers to deliver a much cleaner electrophoretic background.

Product description

The AmpFℓSTR® NGM SElect™ Kit contains all the necessary reagents for the amplification of human genomic DNA.

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

- Applied Biosystems® 3500/3500xL Genetic Analyzer
- ABI Prism® 3100/3100-*Avant* Genetic Analyzer
- Applied Biosystems® 3130/3130*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
- ProFlex™ PCR System

About the primers

The AmpFℓSTR® NGM SElect™ Kit employs the latest improvements in primer synthesis and purification techniques to minimize the presence of dye-labeled artifacts. These improvements result in a much cleaner electropherogram background that enhances the assay signal-to-noise ratio of the assay and simplifies the interpretation of results.

Loci amplified by the kit

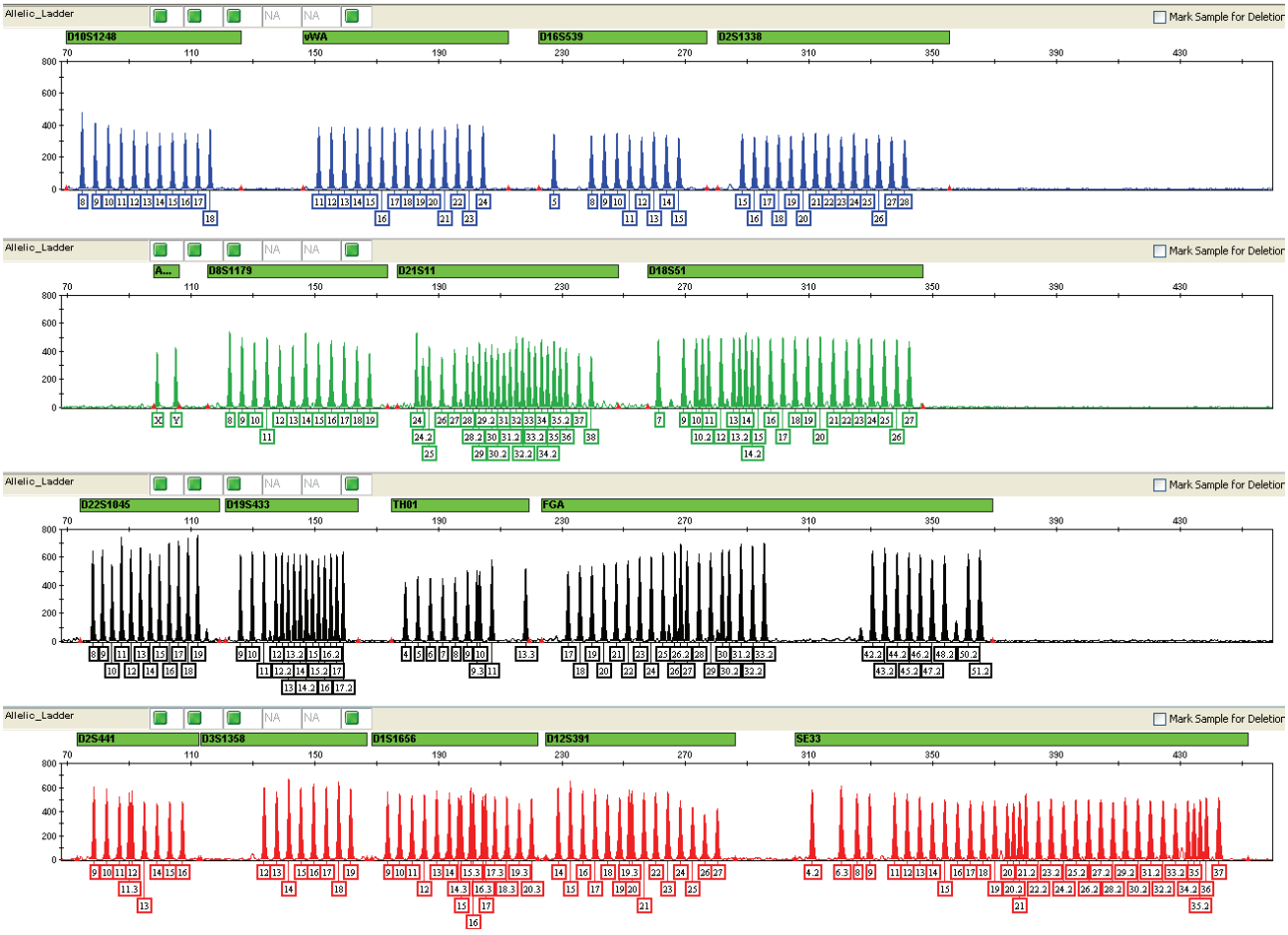
The following table shows the loci amplified, their chromosomal locations, and the corresponding fluorescent marker dyes. The AmpFℓSTR® NGM SElect™ Allelic Ladder is used to genotype the analyzed samples. The alleles contained in the Allelic Ladder and the genotype of the AmpFℓSTR® Control DNA 007 are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

Table 1 AmpFℓSTR® NGM SElect™ Kit loci and alleles

Locus designation	Chromosome location	Alleles included in AmpFℓSTR® NGM SElect™ Allelic Ladder	Dye label	Control DNA 007
D10S1248	10q26.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18	6-FAM™	12, 15
vWA	12p13.31	11,12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24	6-FAM™	14, 16
D16S539	16q24.1	5, 8, 9, 10, 11, 12,13, 14, 15	6-FAM™	9, 10
D2S1338	2q35	15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28	6-FAM™	20, 23
Amelogenin	X: p22.1-22.3 Y: p11.2	X, Y	VIC®	X, Y
D8S1179	8q24.13	8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	VIC®	12, 13
D21S11	21q11.2-q21	24, 24.2, 25, 26, 27, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 37, 38	VIC®	28, 31
D18S51	18q21.33	7, 9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27	VIC®	12, 15
D22S1045	22q12.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	NED™	11, 16
D19S433	19q12	9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2	NED™	14, 15
TH01	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3	NED™	7, 9.3
FGA	4q28	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2	NED™	24, 26
D2S441	2p14	9, 10, 11, 11.3, 12, 13, 14, 15, 16	PET®	14, 15
D3S1358	3p21.31	12, 13, 14, 15, 16, 17, 18, 19	PET®	15, 16
D1S1656	1q42.2	9, 10, 11, 12, 13, 14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18.3, 19.3, 20.3	PET®	13, 16
D12S391	12p13.2	14, 15, 16, 17, 18, 19, 19.3, 20, 21, 22, 23, 24, 25, 26, 27	PET®	18, 19
SE33	6	4.2, 6.3, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20.2, 21, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 27.2, 28.2, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35, 35.2, 36, 37	PET®	17, 25.2

Allelic Ladder profile

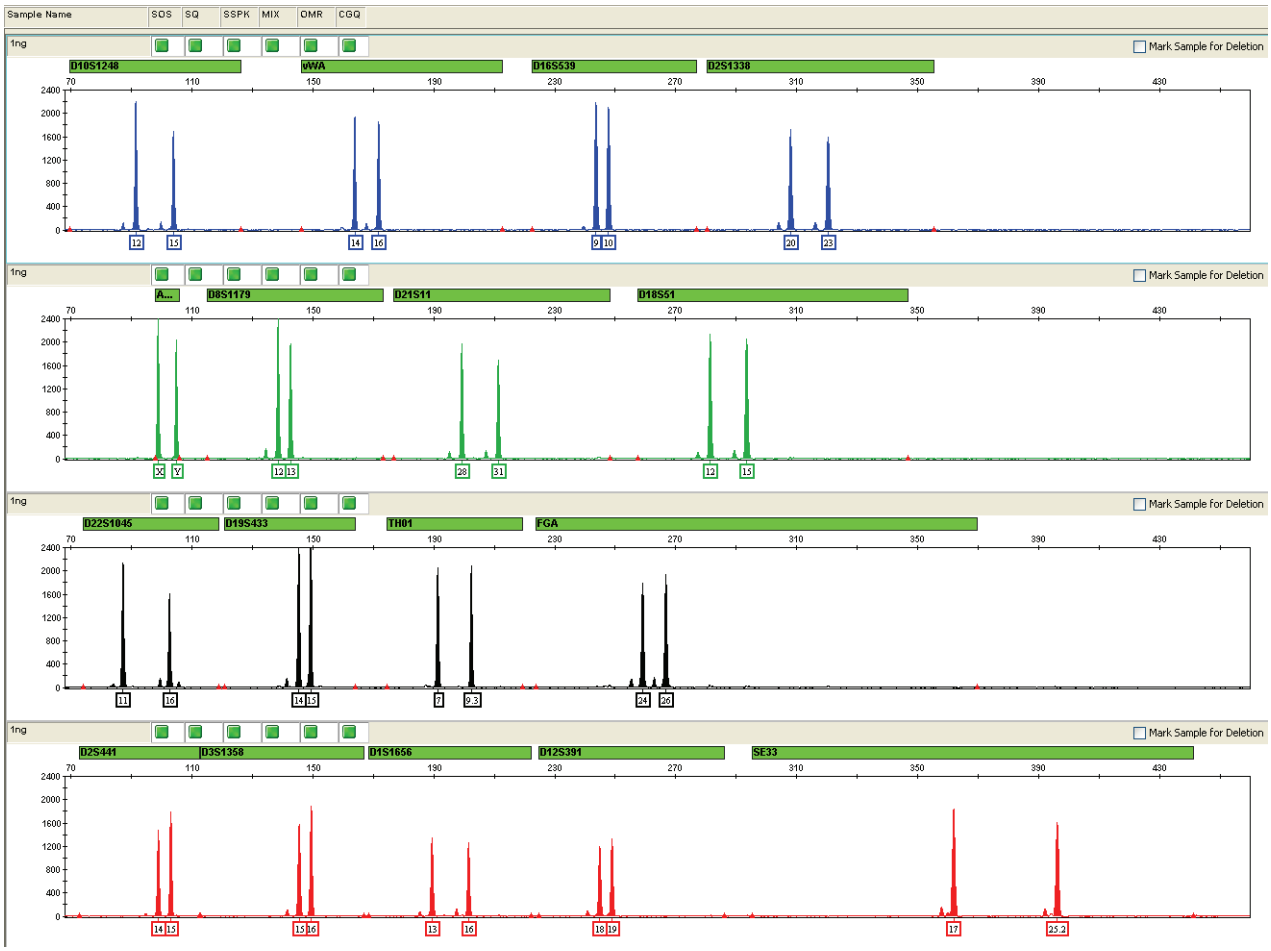
The Allelic Ladder for the AmpFℓSTR® NGM SElect™ Kit is shown below. See “Allelic ladder requirements” on page 34 for information on ensuring accurate genotyping.



GeneMapper® *ID-X* Software plot of the AmpFℓSTR® NGM SElect™ Allelic Ladder

Control DNA 007 profile

Amplification of Control DNA 007 using the AmpFℓSTR® NGM SElect™ Kit is shown below.



1 ng of Control DNA 007 amplified with the AmpFℓSTR® NGM SElect™ Kit and analyzed on the Applied Biosystems® 3130*xl* Genetic Analyzer

Workflow overview

Extract DNA


AutoMate *Express*™ Forensic DNA Extraction System with PrepFiler® Express™ Forensic DNA Extraction Kit

Quantify DNA

Quantifiler® Duo DNA Quantification Kit


Quantifiler® HP and Trio DNA Quantification Kits

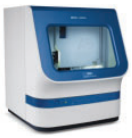
Perform PCR

96-Well GeneAmp® PCR System 9700

ProFlex™ PCR System

Veriti® 96-Well Thermal Cycler

Perform electrophoresis

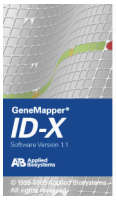


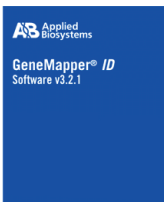
Applied Biosystems® 3500/3500xL Genetic Analyzer for Human Identification

ABI PRISM® 3100/3100-*Avant* Genetic Analyzer

Applied Biosystems® 3130/3130*xl* Genetic Analyzer

Analyze data



GeneMapper® ID-X or GeneMapper® ID Software

Instrument and software overview

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

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

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

Instrument and software compatibility

Table 2 Software specific to each instrument

Instrument	Data Collection Software	Analysis software
3500/3500xL	3500 Series Data Collection Software v1.0 or later	*GeneMapper® ID-X Software* v1.2
3130/3130*xl*‡	3.0 or later	• *GeneMapper® ID Software* v3.2.1 *or* • *GeneMapper® ID-X Software* v1.0.1 or later
3100/3100-*Avant*	1.1 (3100) 1.0 (3100-*Avant*)	
	2.0	

‡ We conducted validation studies for the AmpFℓSTR® NGM SElect™ Kit using this configuration.

About multicomponent analysis

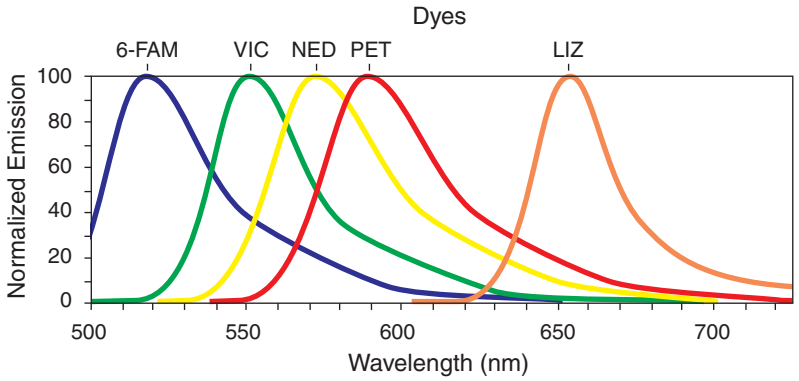
Applied Biosystems fluorescent multi-color dye technology allows the analysis of multiple loci, including loci that have alleles with overlapping size ranges. Alleles for overlapping loci are distinguished by labeling locus-specific primers with different colored dyes.

Multicomponent analysis is the process that separates the five different fluorescent dye colors into distinct spectral components. The four dyes used in the AmpFℓSTR® NGM SElect™ PCR Amplification Kit to label samples are 6-FAM™, VIC®, NED™, and PET® dyes. The fifth dye, LIZ™, 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 Applied Biosystems® and ABI PRISM® 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, as shown in the spectra below. The goal of multicomponent analysis is to correct for spectral overlap.



Emission spectra of the five dyes used in the AmpFℓSTR® NGM SElect™ Kit

Materials and equipment

Kit contents and storage

The AmpFℓSTR® NGM SElect™ PCR Amplification Kit contains materials sufficient to perform 200 (PN 4457889) or 1000 (PN 4457890) amplifications at 25 µL reaction volumes.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set from light when not in use. Amplified DNA, AmpFℓSTR® NGM SElect™ Allelic Ladder, and GeneScan™ 600 LIZ™ Size Standard v2.0 should also be protected from light. Keep freeze-thaw cycles to a minimum.

Table 3 Kit Contents and Storage

Component	Description	200× Volume	1000× Volume	Storage
AmpFℓSTR® NGM SElect™ Primer Set	Contains forward and reverse primers to amplify human DNA targets.	1 tube, 1.0 mL	1 bottle, 5.0 mL	– 15 to – 25 °C on receipt, 2 to 8 °C after initial use
AmpFℓSTR® NGM SElect™ Master Mix	Contains enzyme, salts, dNTPs, carrier protein, and 0.05% sodium azide.	2 tubes, 1.0 mL each	1 bottle, 10.0 mL	
AmpFℓSTR® NGM SElect™ Allelic Ladder	Contains amplified alleles. See Table 1 on page 11 for a list of alleles included in the Allelic Ladder.	1 tube, 50.0 µL	1 tube, 75.0 µL	
AmpFℓSTR® Control DNA 007	Contains 0.10 ng/µL human male 007 DNA in 0.02% sodium azide and buffer‡. See Table 1 on page 11 for profile.	1 tube, 0.3 mL	1 tube, 0.3 mL	2 to 8 °C

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

For information on ordering kits and materials not included, see Appendix B on page 147.

Standards for samples

For the AmpFℓSTR® NGM SElect™ Kit, the panel of standards needed for PCR amplification, PCR product sizing, and genotyping are:

- **Control DNA 007** – A positive control for evaluating the efficiency of the amplification step and STR genotyping using the AmpFℓSTR® NGM SElect™ Allelic Ladder.
- **GeneScan™ 600 LIZ™ Size Standard v2.0** – The following fragments should be defined for use with the AmpFℓSTR® NGM SElect™ Kit: 60, 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. For additional information about size standards, refer to the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (PN 4338775), Appendix D. Order the GeneScan™ 600 LIZ™ Size Standard v2.0 (PN 4408399) separately.

- **AmpFℓSTR® NGM SElect™ Allelic Ladder** – Allelic ladder for accurate characterization of the alleles amplified by the AmpFℓSTR® NGM SElect™ Kit. The AmpFℓSTR® NGM SElect™ Allelic Ladder contains most of the alleles reported for the 16 autosomal loci. See Table 1 on page 11 for a list of the alleles included in the AmpFℓSTR® NGM SElect™ Allelic Ladder.

Materials and equipment not included

For information about required and optional materials and equipment not supplied with the AmpFℓSTR® NGM SElect™ Kit, see Appendix B, "Materials and equipment not included" on page 147.

Chapter 2

PCR Amplification

PCR Amplification 2

This chapter covers:

- PCR work areas 22
- Required user-supplied materials and reagents 23
- DNA quantification 23
- Prepare the amplification kit reactions 25
- Perform PCR 26
- Amplification using bloodstained FTA® cards 27

PCR work areas

Work area setup and lab design

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

- For AmpFℓSTR® NGM SElect™ PCR Amplification Kit forensic DNA testing, refer to: National Institute of Justice Office of Law Enforcement Standards. 1998. *Forensic Laboratories: Handbook for Facility Planning, Design, Construction and Moving*. Washington, DC: National Institute of Justice. 76 pp.
- For AmpFℓSTR® NGM SElect™ Kit parentage DNA testing, refer to: American Association of Blood Banks. 2004. *Guidance for Standards for Parentage Relationship Testing Laboratories*. 7th ed. Bethesda, Md: American Association of Blood Banks. 58 pp.

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

To prevent contamination by human DNA, be careful while handling and processing samples. 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: These laboratory design resources and guidances constitute only a sample of the precautions that need to be observed when using PCR technology. Refer to your laboratory's internal policies and procedures for more information and references.

PCR-setup tools

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

- Calculator, vortex
- 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 tools

The following PCR systems should be placed in the amplified DNA work area.

- 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
- ProFlex™ PCR System

Required user-supplied materials and reagents

Kit contents and storage

The AmpFℓSTR® NGM SElect™ PCR Amplification Kit is available as either a 200 reaction kit or 1000 reaction kit. The number of reactions is based on a 25 µL reaction volume. See "Kit contents and storage" on page 17 for details on kit contents.

User-supplied reagents

In addition to the AmpFℓSTR® NGM SElect™ Kit reagents, the use of low TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) is recommended. You can prepare the buffer as described in the procedure below or order it from Teknova (Cat # T0223).

To prepare low TE buffer:

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

   Note: Adjust the volumes based on your specific needs.

2. Aliquot and autoclave the solutions.

3. Store at room temperature.

DNA quantification

Importance of quantification

Quantifying the amount of DNA in a sample before amplification allows you to determine whether or not sufficient DNA is present to permit amplification and to calculate the optimum amount of DNA to add to the reaction. The optimum amount of DNA for the AmpFℓSTR® NGM SElect™ Kit is 1.0 ng in a maximum input volume of 10 µL at 29 cycles.

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

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

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

Methods of quantifying DNA

We provide several kits for quantifying DNA in samples. See the references cited in the following table for details about these kits.

Product	Description
Quantifiler® Human DNA Quantification Kit (PN 4343895) *and* Quantifiler® Y Human Male DNA Quantification Kit (PN 4343906) For more information, see *Quantifiler® Human DNA Quantification Kits User's Manual* (PN 4344790)	**Properties:** The Quantifiler® Human and Quantifiler® Y Human Male kits are highly specific for human DNA, and they detect total human or male DNA, respectively. The kits detect single-stranded and degraded DNA. **How they work:** The Quantifiler® DNA Quantification kits consist of target-specific and internal control 5' nuclease assays. The Quantifiler® Human and Quantifiler® Y Human Male kits contain different target-specific assays (human DNA or human male DNA, respectively) that each consist of two locus-specific PCR primers and one TaqMan® MGB probe labeled with FAM™ dye for detecting the amplified sequence. The kits each contain a separate internal PCR control (IPC) assay that consists of an IPC template DNA (a synthetic sequence not found in nature), two primers for amplifying the IPC template DNA, and one TaqMan® MGB probe labeled with VIC® dye for detecting the amplified IPC DNA.
Quantifiler® Duo DNA Quantification Kit (PN 4387746) For more information, see *Quantifiler® Duo DNA Quantification Kit User's Manual* (PN 4391294)	**Properties:** The Quantifiler® Duo kit is highly specific for human DNA and combines the detection of both total human and male DNA in one PCR reaction.The kit detects single-stranded and degraded DNA. **How it works:** The Quantifiler® Duo DNA Quantification Kit consists of target-specific and internal control 5' nuclease assays. The Quantifiler® Duo kit combines two human-specific assays in one PCR reaction (for total human DNA and human male DNA). The two human DNA specific assays each consist of two PCR primers and a TaqMan® probe. The TaqMan® probes for the human DNA and human male DNA assays are labeled with VIC® and FAM™ dyes, respectively. In addition, the kit contains an internal PCR control (IPC) assay similar in principle to that used in the other Quantifiler kits, but labeled with NED™ dye.
Quantifiler® HP DNA Quantification Kit (Cat. no. 4482911) Quantifiler® Trio DNA Quantification Kit (Cat. no. 4482910) For more information, see *Quantifiler HP and Trio DNA Quantification Kits User Guide* (Pub no. 4485354)	**Properties:** The Quantifiler® HP Kit is designed to quantify the total amount of amplifiable human DNA in a sample. The Quantifiler® Trio Kit is designed to simultaneously quantify the total amount of amplifiable human DNA and human male DNA in a sample. **How they work:** The Quantifiler® HP and Trio DNA Quantification Kits use multiple-copy target loci for improved detection sensitivity. The human-specific target loci (Small Autosomal, Large Autosomal, and Y-chromosome targets) each consist of multiple copies dispersed on various autosomal chromosomes (Small Autosomal and Large Autosomal). To maximize the consistency of quantification results, genomic targets were selected with conserved primer- and probe-binding sites within individual genomes and also with minimal copy number variability between different individuals and population groups. As a result, the detection sensitivity of the Quantifiler® HP and Trio assays is improved over Quantifiler® Duo, Human, and Y Human Male DNA Quantification Kit assays. The primary quantification targets (Small Autosomal and Y) consist of relatively short amplicons (75 to 80 bases) to improve the detection of degraded DNA samples. In addition, the Quantifiler® HP and Trio Kits each contain a Large Autosomal target with a longer amplicon (>200 bases) to aid in determining if a DNA sample is degraded.

Prepare the amplification kit reactions

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

DNA sample	Volume per reaction
AmpFℓSTR® NGM SElect™ Master Mix	10.0 µL
AmpFℓSTR® NGM SElect™ Primer Set	5.0 µL

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

2. Prepare reagents. Thaw the AmpFℓSTR® NGM SElect™ Master Mix and the AmpFℓSTR® NGM SElect™ Primer Set, then vortex the tubes for 3 seconds and centrifuge them briefly before opening.

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.

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

4. Vortex the reaction mixture for 3 seconds, then centrifuge briefly.

5. Dispense 15 µL of reaction mixture into each reaction well of a MicroAmp® Optical 96-Well Reaction Plate or each MicroAmp® tube.

6. Prepare the DNA samples as shown in the table below. The final reaction volume should be 25 µL.

DNA sample	To prepare...
Negative control	Add 10 µL of low TE buffer (10mM Tris, 0.1mM EDTA, pH 8.0).
Test sample	Dilute a portion of the test DNA sample with low TE buffer so that 1.0 ng of total DNA is in a final volume of 10 µL. Add 10 µL of the diluted sample to the reaction mix.
Positive control	Add 10 µL of 007 control DNA (0.1 ng/µL) to provide 1.0 ng of total DNA in the positive control reaction.

7. Seal the MicroAmp® Optical 96-Well Reaction Plate with MicroAmp® Clear Adhesive Film or MicroAmp® Optical Adhesive Film, or cap the tubes.

8. Centrifuge the tubes or plate at 3000 rpm for about 20 seconds in a tabletop centrifuge (with plate holders, if using 96-well plates) to remove bubbles.

9. Amplify the samples in a GeneAmp® PCR System 9700 with the silver 96-well block, or a GeneAmp® PCR System 9700 with the gold-plated silver 96-well block, a Veriti® 96-Well Thermal Cycler, or a ProFlex™ PCR System.

Note: The AmpFℓSTR® NGM SElect™ Kit is not validated for use with the GeneAmp PCR System 9700 with the aluminium 96-well block. Use of this thermal cycling platform may adversely affect the performance of the AmpFℓSTR® NGM SElect™ Kit.

Perform PCR

1. Program the thermal cycling conditions.
   - When using the GeneAmp PCR System 9700 with either 96-well silver or gold-plated silver block, select the **9600 Emulation Mode**.
   - When using the Veriti® 96-Well Thermal Cycler, refer to the following document for instructions on how to configure the Veriti instrument to run in the 9600 Emulation Mode: *User Bulletin: Veriti® 96-Well Thermal Cycler AmpFℓSTR® Kit Validation* (Part no. 4440754).
   - When using the ProFlex™ PCR System, refer to the *ProFlex™ PCR System Kit Validation User Bulletin* (Pub. no. 100031595) for more information.

Initial incubation step	Cycle (29 or 30 cycles)		Final extension	Final hold
	Denature	Anneal		
HOLD	CYCLE		HOLD	HOLD
95 °C 11 minutes	94 °C 20 seconds	59 °C 3 minutes	60 °C 10 minutes	4 °C indefinite

IMPORTANT! The AmpFℓSTR® NGM SElect™ Kit is validated for use at both 29 and 30 cycles. The optimum conditions for the NGM SElect™ kit are 29 cycles of amplification with a 1 ng input DNA concentration. Laboratories choosing to use the NGM SElect™ kit at 30 cycles should reduce the input DNA concentration to 500 pg. Internal validation studies to evaluate all aspects of kit performance are required for each individual cycle number intended for operational use within the laboratory.

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

IMPORTANT! If using adhesive clear film instead of caps to seal the plate wells, be sure to place a MicroAmp® compression pad (PN 4312639) on top of the plate to prevent evaporation during thermal cycling.

3. Start the run.

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

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

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

Amplification using bloodstained FTA® cards

FTA® cards can be useful for the collection, storage, and processing of biological samples. A small punch disc of the card containing the sample can be placed directly into an amplification tube, purified, and amplified, without transferring the disc. Our studies indicate that a 1.2 mm bloodstained disc contains approximately 5 to 20 ng DNA. An appropriate cycle number for this high quantity of DNA is 24 cycles, determined by our validation studies. However, it is recommended that each laboratory determine the optimum cycle number based on internal validation studies.

In the example shown in Figure 1, a 1.2 mm disc of a bloodstained FTA® card was purified using three washes with FTA® Purification Reagent and two washes with 1✕ low TE buffer. The punch was then amplified directly in the MicroAmp® tube for 24 cycles.

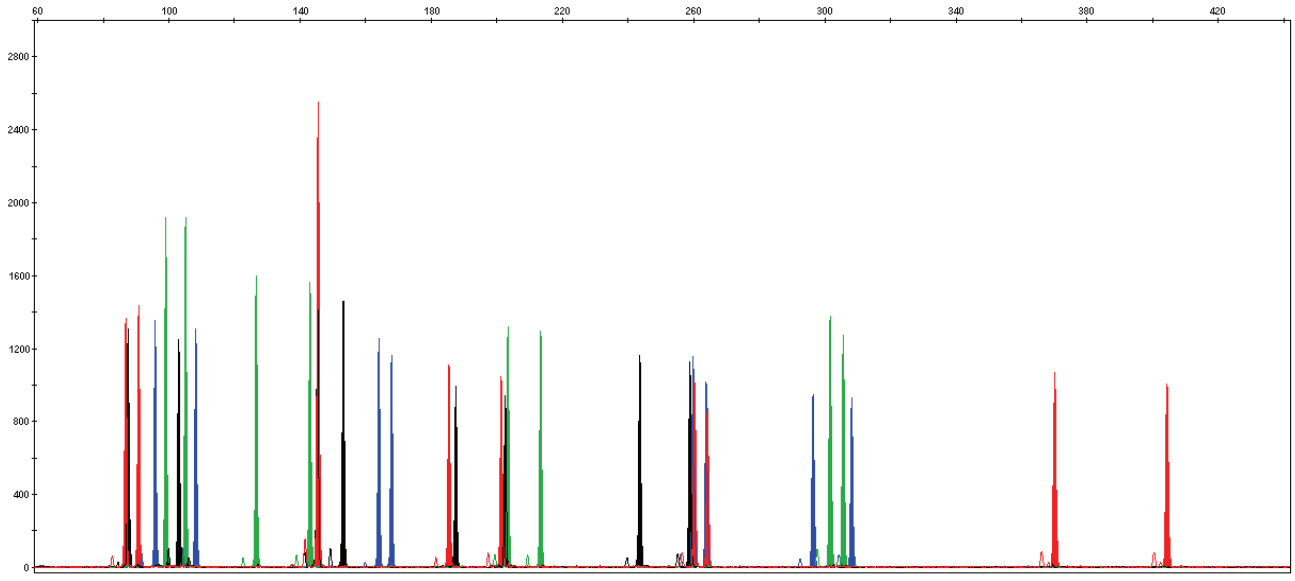


Figure 1 AmpFℓSTR® NGM SElect™ PCR Amplification Kit results from a 1.2 mm FTA® bloodstain disc (24-cycle amplification), analyzed on an Applied Biosystems® 3130*xl* Genetic Analyzer

Chapter 3

Electrophoresis

Electrophoresis 3

This chapter covers:

- Allelic ladder requirements 34

Section 3.1 3100/3100-Avant and 3130/3130xl instruments 35

- Set up the 3100/3100-Avant or 3130/3130xl instrument for electrophoresis . 35
- Prepare samples for electrophoresis on the 3100/3100-Avant or 3130/3130xl instrument 37

Section 3.2 3500/3500xL Series instruments 39

- Set up the 3500/3500xL instrument for electrophoresis 39
- Prepare samples for electrophoresis on the 3500/3500xL instrument. 40

Allelic ladder requirements

To accurately genotype samples, you must run an allelic ladder sample along with the unknown samples. Run the appropriate number of allelic ladder samples per injection and per sample for your instrument:

- Applied Biosystems® 3500 Series Genetic Analyzers:

  Run at least one allelic ladder per every set of 24 samples.

  – Applied Biosystems® 3500xL

    One ladder per injection

    One injection = 24 samples (23 samples + 1 allelic ladder)

  – Applied Biosystems® 3500

    One ladder for every 3 injections

    One injection = 8 samples

- ABI PRISM® 3100 and Applied Biosystems® 3130 Series Genetic Analyzers:

  Run at least one allelic ladder per every set of 16 samples.

  – Applied Biosystems® 3130*xl* or ABI PRISM® 3100 systems

    One ladder per injection

    One injection = 16 samples

  – Applied Biosystems® 3130 or ABI PRISM® 3100-*Avant*

    One ladder for every 4 injections

    One injection = 4 samples

IMPORTANT! Variation in laboratory temperature can affect fragment migration speed and result in sizing variation. We recommend the indicated frequency of allelic ladder injections; this frequency 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.

When genotyping, it is important to use 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.
- Variation in laboratory temperature can affect migration speed (see IMPORTANT above). These variations can result in sizing variations between both single and multiple capillary runs, with a greater size variation between those samples injected in multiple capillary runs, than between those samples injected in a single capillary run.

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

Appendix B, "Materials and equipment not included" on page 147 lists the required materials not supplied with the AmpFℓSTR® NGM SElect™ PCR Amplification Kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the AmpFℓSTR® NGM SElect™ Primer Set from light when not in use. Amplified DNA, AmpFℓSTR® NGM SElect™ Allelic Ladder, and GeneScan™ 600 LIZ™ Size Standard v2.0 should also be protected from light. Keep freeze-thaw cycles to a minimum.

3100/3100-*Avant* or 3130/3130*xl* instrument requirements

The following table lists Data Collection Software and the run modules that can be used to analyze AmpFℓSTR® NGM SElect™ 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* (PN 4363787)
ABI PRISM® 3100	2.0	Windows® 2000	• HIDFragmentAnalysis36_POP4_1 Injection condition: 3kV/10 sec • Dye Set G5	*ABI PRISM® 3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0, Protocols for Processing AmpFℓSTR PCR Amplification Kit PCR Products User Bulletin* (PN 4350218)
ABI PRISM® 3100	1.1	Windows NT®	• GeneScan36vb_DyeSetG5Module Injection condition: 3kV/10 sec • GS600v2.0Analysis.gsp	*ABI PRISM® 3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR PCR Amplification Kit PCR Products User Bulletin* (PN 4332345)

Genetic Analyzer	Data Collection Software	Operating System	Run modules and conditions	References
ABI PRISM® 3100-*Avant*	1.0	Windows NT®	• GeneScan36Avb_DyeSetG5Module Injection condition: 3 kV/5sec • GS600v2.0Analysis.gsp	*ABI PRISM® 3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR PCR Amplification Kit PCR Products User Bulletin* (PN 4332345)

‡ We conducted validation studies for the AmpFℓSTR® NGM SElect™ Kit using this configuration.

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

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

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

Reagent	Volume per reaction
GeneScan™ 600 LIZ™ Size Standard v2.0	0.5 µL
Hi-Di™ Formamide	9.5 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/experiments.

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

   Note: For blank wells, add 11 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 onto 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

Appendix B, "Materials and equipment not included" on page 147 lists the required materials not supplied with the AmpFℓSTR® NGM SElect™ PCR Amplification Kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the AmpFℓSTR® NGM SElect™ Primer Set from light when not in use. Amplified DNA, AmpFℓSTR® NGM SElect™ Allelic Ladder, and GeneScan™ 600 LIZ™ Size Standard v2.0 should also be protected from light. Keep freeze-thaw cycles to a minimum.

3500 instrument requirements

The following table lists Data Collection Software and the run modules that can be used to analyze AmpFℓSTR® NGM SElect™ 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* (PN 4401661) *3500 and 3500xL Genetic Analyzers Quick Reference Card* (PN 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 capillary electrophoresis on the 3500/3500xL instrument immediately before loading.

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

Reagent	Volume per reaction
GeneScan™ 600 LIZ™ Size Standard v2.0	0.5 µL
Hi-Di™ Formamide	9.5 µL

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

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

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

   Note: For blank wells, add 11 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 put it onto the autosampler.
9. Ensure that a plate record is completed and link the plate record to the plate.
10. Start the electrophoresis run.

Chapter 4

Data Analysis

Data Analysis 4

This chapter covers:

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- Before you start 47
- Set up GeneMapper® ID Software for data analysis 48
- Analyze and edit sample files with GeneMapper® ID Software 60
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Section 4.2 GeneMapper® ID-X Software 63

- Before you start 63
- Set up GeneMapper® ID-X Software for data analysis 64
- Analyze and edit sample files with GeneMapper® ID-X Software. 79
- For more information. 81

Section 4.1 GeneMapper® *ID* Software

Before you start

GeneMapper® ID Software is an automated genotyping software for forensic casework, databasing, and paternity data analysis. After electrophoresis, the Data Collection Software stores information for each sample in a .fsa file. Using *GeneMapper® ID Software* v3.2.1 software, you can then analyze and interpret the data from the .fsa files.

Note: Refer to **"Instrument and software overview" on page 15** for a list of compatible instruments.

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 that you 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, verify the sample result according to your laboratory's protocol.

If you are using GeneMapper® *ID-X* Software to perform Human Identification (HID) analysis with AmpFℓSTR® kits, go to "Set up GeneMapper® *ID-X* Software" on page 64 or refer to the *GeneMapper® ID-X Software Version 1.0 Human Identification Analysis Getting Started Guide* (PN 4375574).

Set up GeneMapper® *ID* Software for data analysis

Workflow

To analyze sample (.fsa) files using GeneMapper® *ID* Software v3.2.1 for the first time:

- Import panels and bins into the Panel Manager, as explained in "Import panels and bins" on page 48.
- Create an analysis method, as explained in **"Create a HID analysis method" on page 52**.
- Create a size standard, as explained in **"Create a HID size standard" on page 58**.
- Define custom views of analysis tables.

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

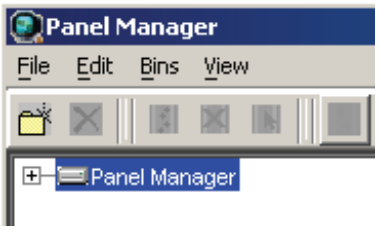
  Refer to Chapter 1 of the GeneMapper® *ID* Software *Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (PN 4335523) for more information.

Import panels and bins

To import the AmpFℓSTR® NGM SElect™ Kit panel and bin set from our website into the GeneMapper® *ID* Software v3.2.1 database:

1. Download and open the file containing panels and bins:
   a. From the Support menu of **www.appliedbiosystems.com**, select **Support ▸ Software Downloads, Patches & Updates ▸** GeneMapper® *ID* Software **v 3.2 ▸ Updates & Patches**, and download the file **NGMSElect Analysis Files GMID**.
   b. Unzip the file.
2. Start the GeneMapper® *ID* Software, then log in with the appropriate user name and password.

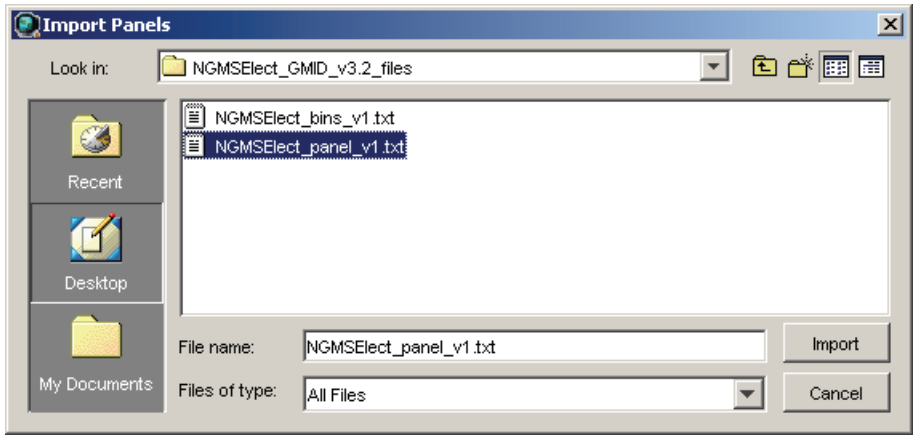
   IMPORTANT! For logon instructions, refer to the GeneMapper® *ID* Software *Version 3.1 Human Identification Analysis User Guide* (PN 4338775).
3. Select **Tools ▸ Panel Manager**.
4. Import NGMSElect_panel_v1:
   a. Select **Panel Manager** in the navigation pane.



b. In the toolbar, select **File ▸ Import Panels** to open the Import Panels dialog box.

c. Locate, then open the **NGMSElect Analysis Files GMID** folder that you unzipped in step 1b on page 48.

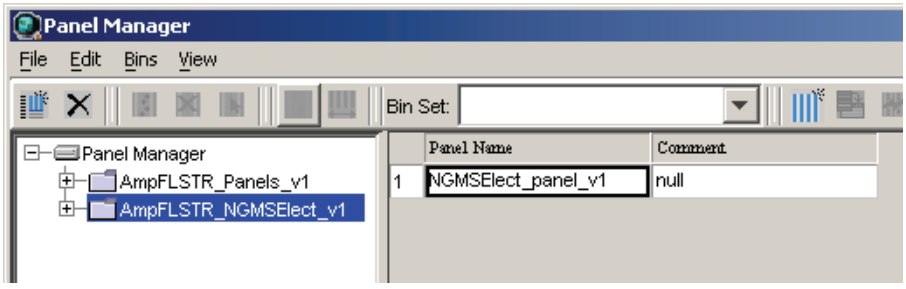
d. Select **NGMSElect_panel_v1.txt**, then click **Import**.



Note: Importing this file creates a new folder in the navigation pane of the Panel Manager, AmpFLSTR_NGMSElect_v1. This folder contains the panel and associated markers.

5. Import **NGMSElect_bins_v1**:

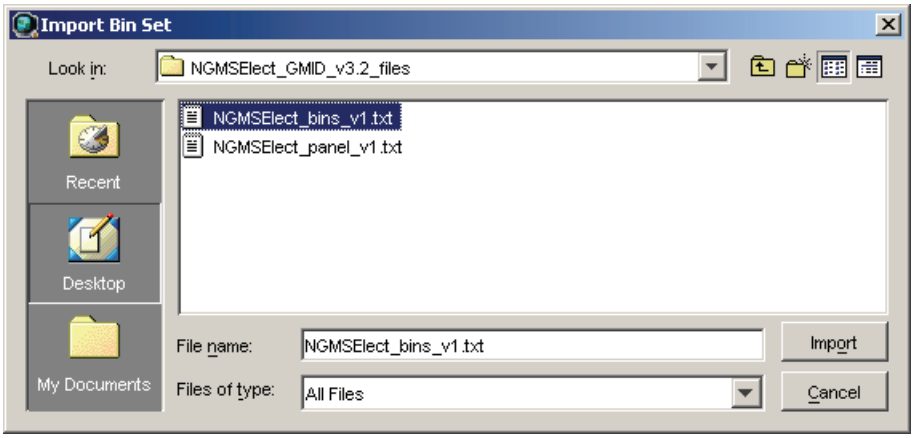
a. Select the **AmpFLSTR_NGMSElect_v1** folder in the left navigation pane.



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

c. Locate, then open the **NGMSElect Analysis Files GMID** folder.

d. Select **NGMSElect_bins_v1**, then click **Import**.

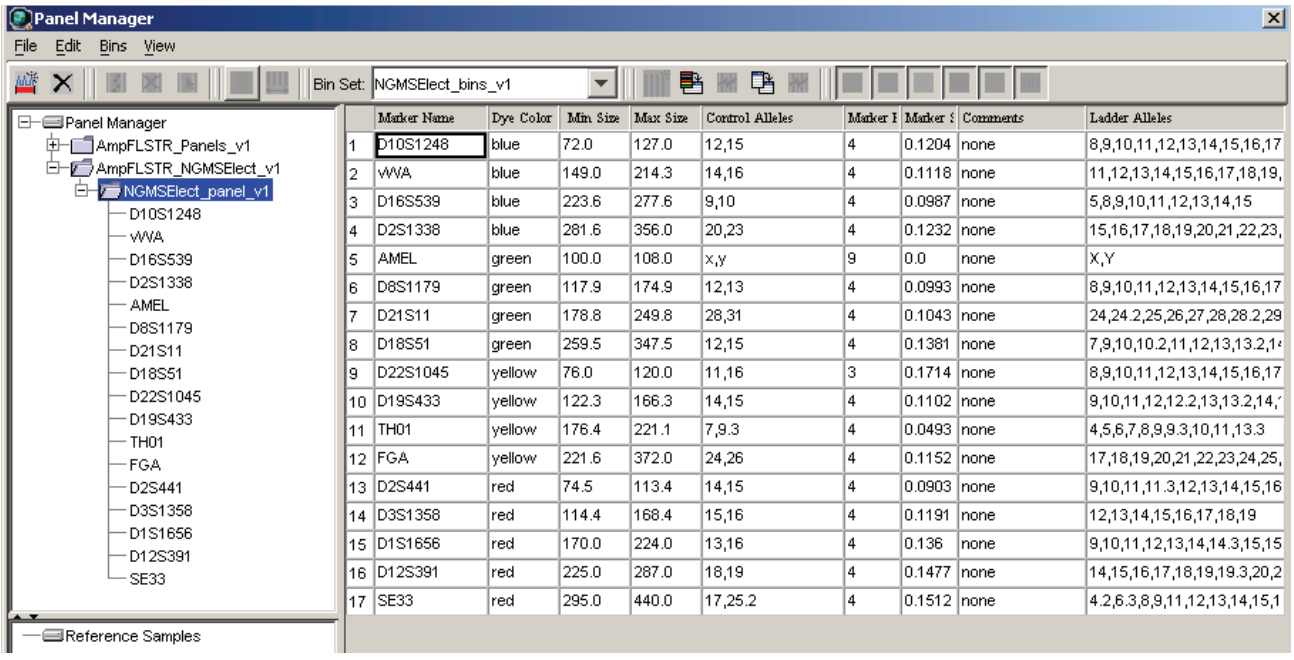


Note: Importing this file associates the bin set with the panels in the NGMSElect_panel_v1 folder.

6. View the imported panels in the navigation pane:

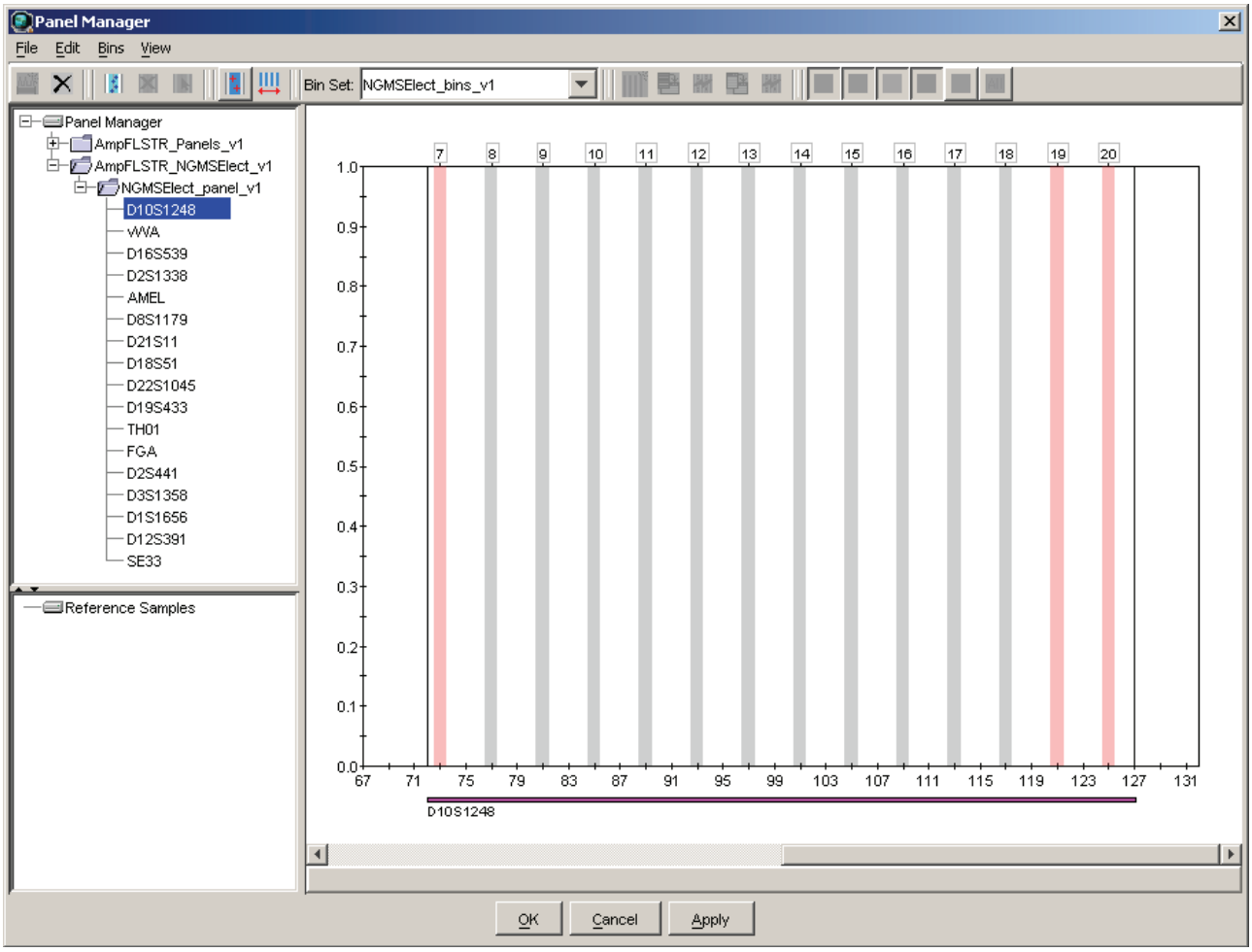
   a. Double-click the **AmpFLSTR_NGMSElect_v1** folder to view the **NGMSElect_panel_v1** folder.

   b. Double-click the **NGMSElect_panel_v1** folder to display the panel information.



	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker I	Marker S	Comments	Ladder Alleles
1	D10S1248	blue	72.0	127.0	12,15	4	0.1204	none	8,9,10,11,12,13,14,15,16,17
2	vWA	blue	149.0	214.3	14,16	4	0.1118	none	11,12,13,14,15,16,17,18,19,
3	D16S539	blue	223.6	277.6	9,10	4	0.0987	none	5,8,9,10,11,12,13,14,15
4	D2S1338	blue	281.6	356.0	20,23	4	0.1232	none	15,16,17,18,19,20,21,22,23,
5	AMEL	green	100.0	108.0	x,y	9	0.0	none	X,Y
6	D8S1179	green	117.9	174.9	12,13	4	0.0993	none	8,9,10,11,12,13,14,15,16,17
7	D21S11	green	178.8	249.8	28,31	4	0.1043	none	24,24.2,25,26,27,28,28.2,29
8	D18S51	green	259.5	347.5	12,15	4	0.1381	none	7,9,10,10.2,11,12,13,13.2,1
9	D22S1045	yellow	76.0	120.0	11,16	3	0.1714	none	8,9,10,11,12,13,14,15,16,17
10	D19S433	yellow	122.3	166.3	14,15	4	0.1102	none	9,10,11,12,12.2,13,13.2,14,
11	TH01	yellow	176.4	221.1	7,9.3	4	0.0493	none	4,5,6,7,8,9,9.3,10,11,13.3
12	FGA	yellow	221.6	372.0	24,26	4	0.1152	none	17,18,19,20,21,22,23,24,25,
13	D2S441	red	74.5	113.4	14,15	4	0.0903	none	9,10,11,11.3,12,13,14,15,16
14	D3S1358	red	114.4	168.4	15,16	4	0.1191	none	12,13,14,15,16,17,18,19
15	D1S1656	red	170.0	224.0	13,16	4	0.136	none	9,10,11,12,13,14,14.3,15,15
16	D12S391	red	225.0	287.0	18,19	4	0.1477	none	14,15,16,17,18,19,19.3,20,2
17	SE33	red	295.0	440.0	17,25.2	4	0.1512	none	4.2,6.3,8,9,11,12,13,14,15,1

c. Select **D10S1248** in the left navigation pane to display the Bin view for the marker.



7. Click **Apply**, then click **OK** to add the AmpFℓSTR® NGM SElect™ Kit panel and bin set to the GeneMapper® *ID* Software database.

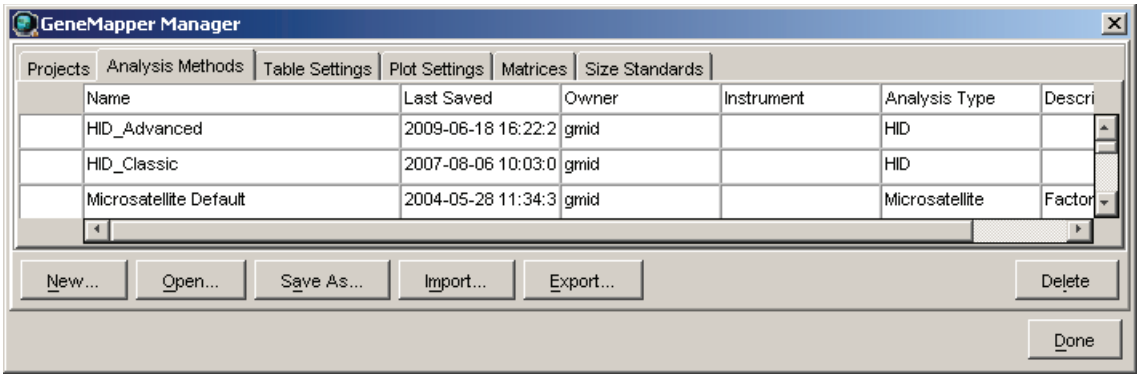
IMPORTANT! If you close the Panel Manager without clicking **Apply** and then **OK**, the panels and bins are not imported into the GeneMapper® *ID* Software database.

Create a HID analysis method

The HID Advanced analysis method for the AmpFℓSTR® NGM SElect™ Kit uses the NGMSElect_bins_v1 file described in step 5 on page 49.

Use the following procedure to create a HID analysis method for the AmpFℓSTR® NGM SElect™ Kit.

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



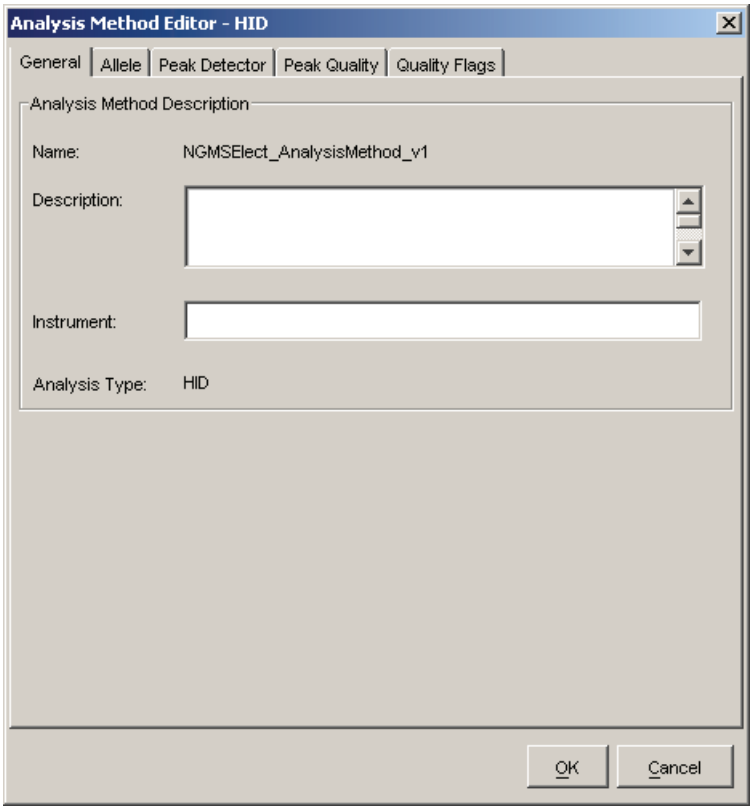
2. Select the **Analysis Methods** tab, then click **New** to open the New Analysis Method dialog box.

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.

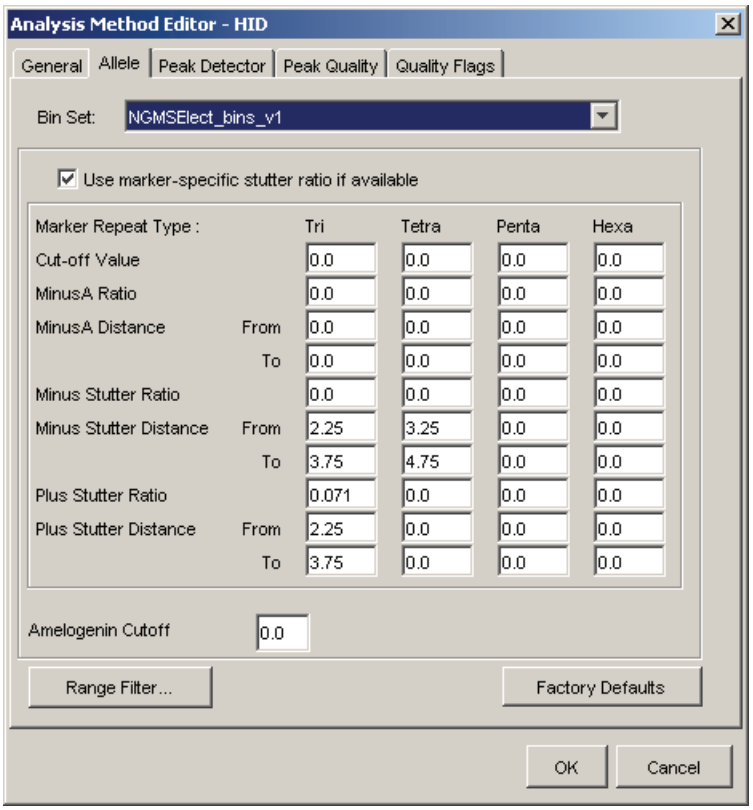
Note: The Analysis Method Editor closes when you save your settings (See step 5 on page 57). To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.

General tab settings



In the Name field, either type the name as shown for consistency with files supplied with other AmpFℓSTR® kits, 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.

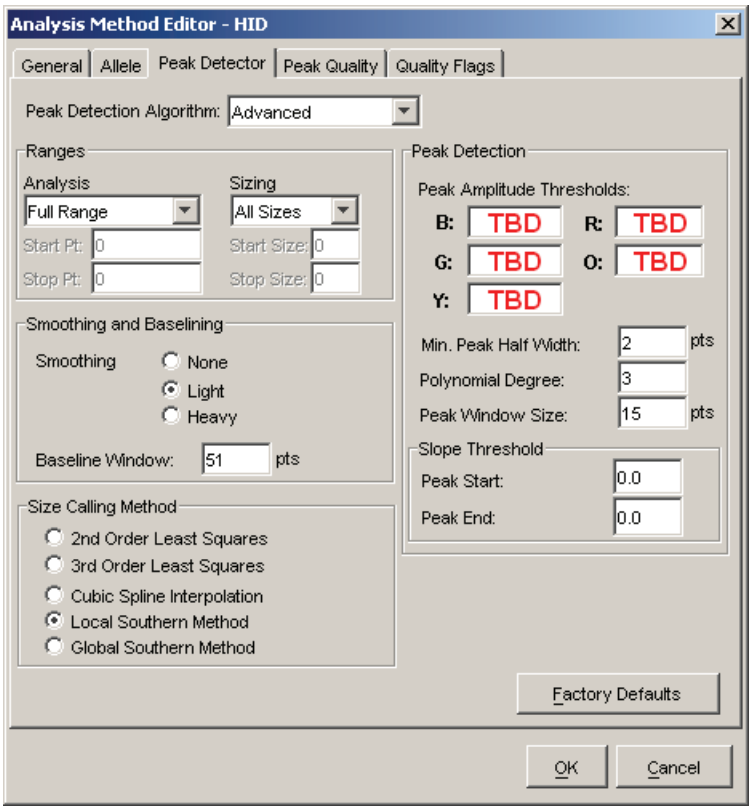
Allele tab settings



- In the Bin Set field, select the **NGMSElect_bins_v1** 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.
- The "Use marker-specific stutter ratio if available" check box is selected by default. Consequently, the software applies the stutter ratio filters supplied in the NGMSElect_panel_v1 file. GeneMapper ID Software v3.2.1 specifies locus-specific filter ratios for minus stutters, but not for plus stutters, in the panel file. However, validation studies with the NGM SElect™ kit show that the trinucleotide repeat D22S1045 locus produces a relatively large amount of plus stutter compared to tetranucleotide repeat loci. The relatively large amount of stutter may cause the stutter peak to be labeled during routine analysis.

  The plus stutter at the D22S1045 locus can be filtered by assigning a global plus stutter filter for trinucleotide repeat loci in the Analysis Parameter file. Because D22S1045 is the only trinucleotide repeat locus in the NGM Select™ kit, this stutter filter setting is applied only to plus stutter peaks at the D22S1045 locus. The settings shown above resulted in little or no labeling of D22S1045 plus stutter peaks during validation studies. However, we recommend that users determine the settings appropriate for use in their laboratory during internal validation studies.

Peak Detector tab settings

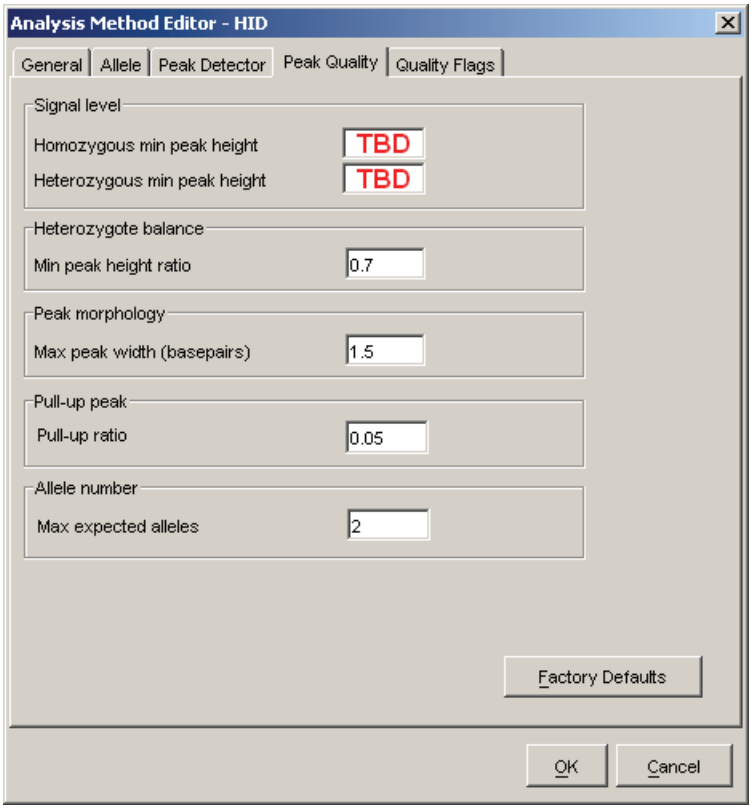


IMPORTANT! TBD indicates values to be determined in your laboratory. Laboratories must perform the appropriate internal studies to determine the peak amplitude thresholds for interpretation of NGM SElect™ kit data.

Fields include:

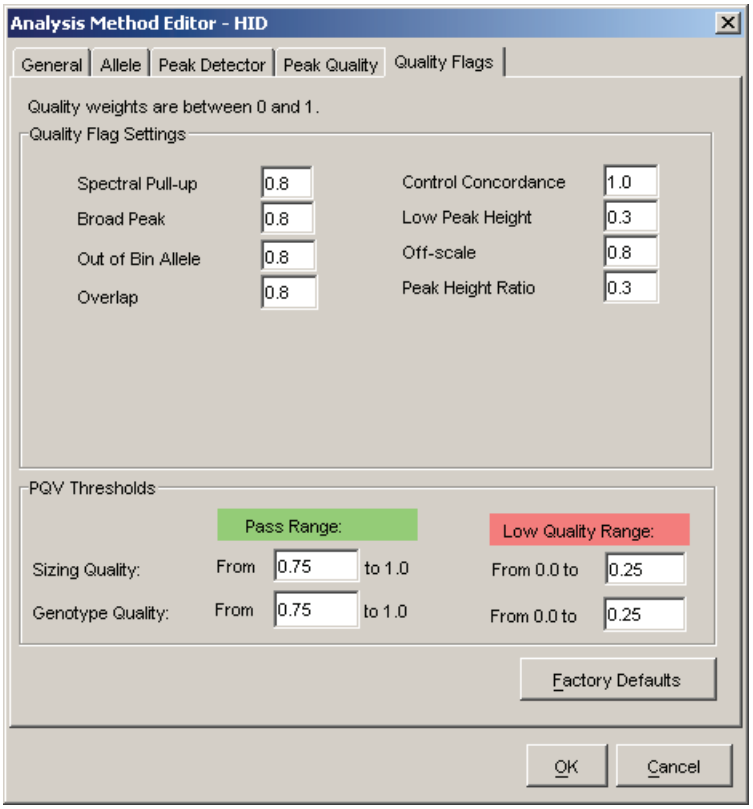
- **Peak amplitude thresholds** – The software uses these parameters to specify the minimum peak height, in order to limit the number of detected peaks. Although GeneMapper® *ID* Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the genotype of these peaks.
- **Size calling method** – The NGM SElect™ kit has been validated using the Local Southern sizing method. Alternative sizing methods should be selected only after extensive evaluation as part of an internal validation study in the user's laboratory.

Peak Quality tab settings



IMPORTANT! TBD indicates values to be determined in your laboratory. Laboratories need to perform the appropriate internal validation studies to determine the minimum heterozygous and homozygous minimum peak height thresholds and the minimum peak height ratio threshold that allow for reliable interpretation of AmpFℓSTR® NGM SElect™ Kit data.

Quality Flags tab settings



IMPORTANT! The values shown are the software defaults and are the values used by us during developmental validation. Laboratories must perform appropriate internal validation studies to determine the appropriate values to use.

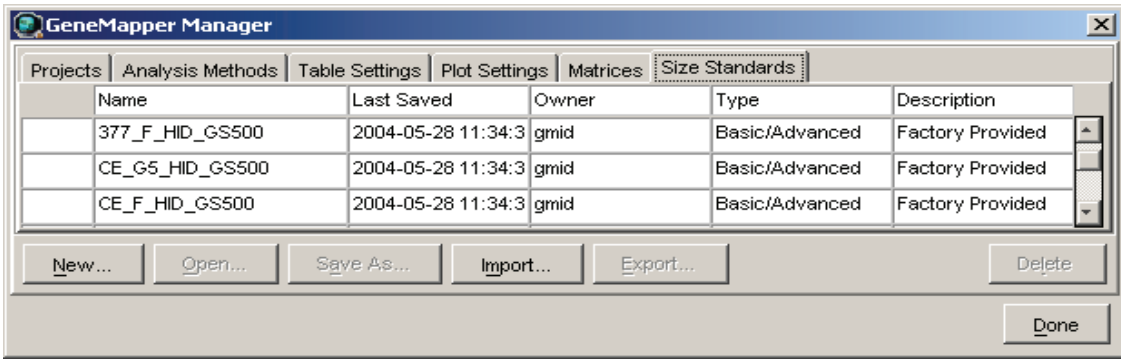
5. Click **Save**.

Create a HID size standard

The size standard for the AmpFℓSTR® NGM SElect™ PCR Amplification Kit uses the following GeneScan™ 600 LIZ™ Size Standard v2.0 peaks in its sizing algorithm: 60, 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.

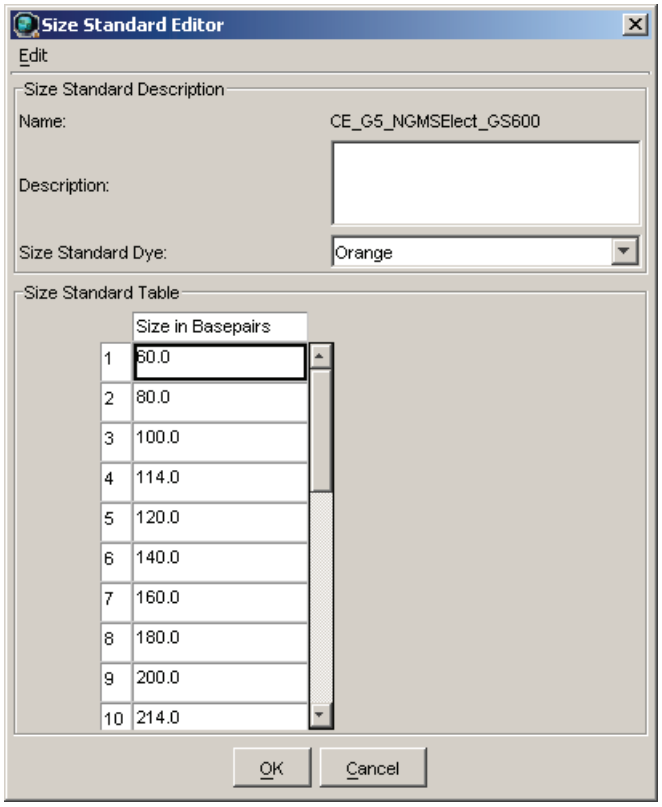
Use the following procedure to create the size standard for the AmpFℓSTR® NGM SElect™ Kit.

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



2. Select the **Size Standards** tab, then click **New**.

3. Complete the Name field as shown below or with a name of your choosing. In the Size Standard Dye field, select **Orange**. In the Size Standard Table, enter the sizes specified in "Create a HID size standard" on page 58.



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

Analyze a project

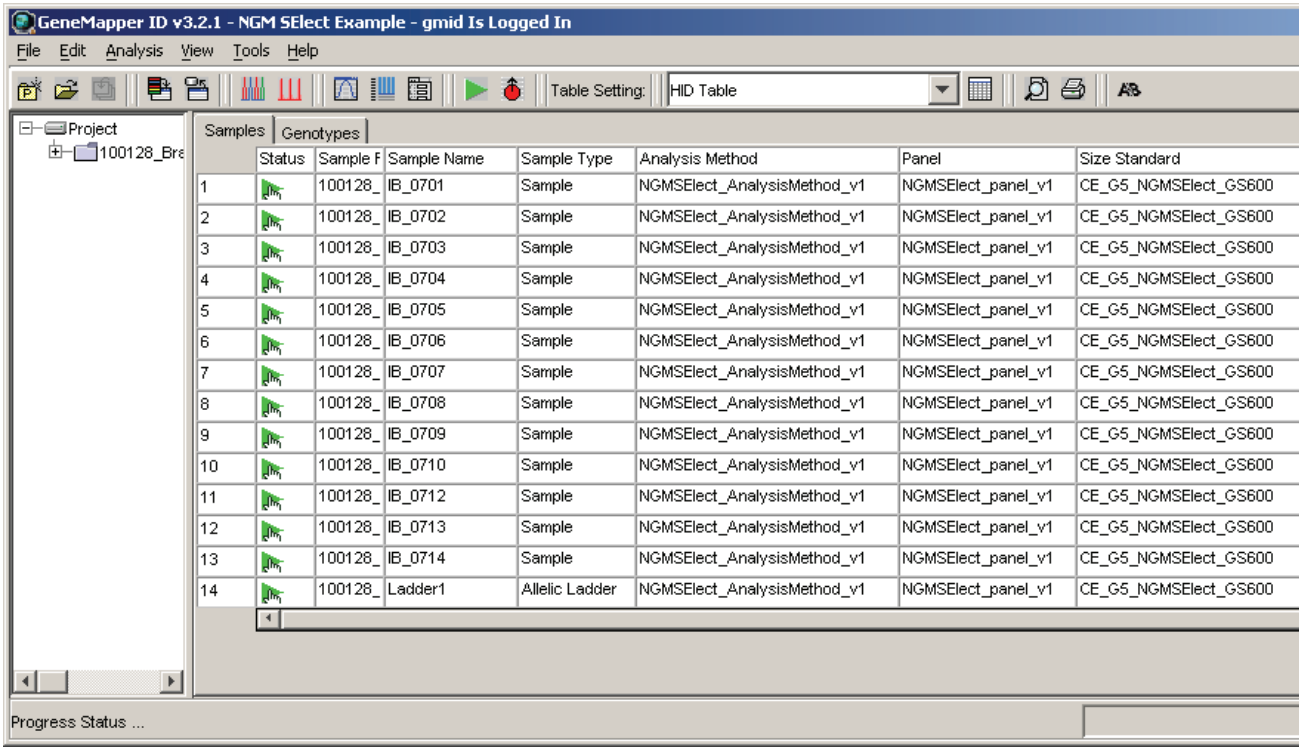
1. In the Project window, select **File ▸ Add Samples to Project**, then locate the disk or directory containing the sample files.

2. Apply analysis settings to the samples in the project. The names of the settings shown are the names suggested in the sections above. If you use names of your own choosing, select those names instead.

Parameter	Settings
Sample Type	Select the sample type
Analysis Method	**NGMSElect_AnalysisMethod_v1**
Panel	**NGMSElect_panel_v1**
Size Standard	**CE_G5_NGMSElect_GS600**

   - Size Standard: For more information about how the Size Caller works, refer to the ABI Prism® GeneScan® Analysis Software for the *Windows NT® Operating System Overview of the Analysis Parameters and Size Caller User Bulletin* (PN 4335617).
   - CE_G5_NGMSElect_GS600: 60, 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 (size standard fragments defined in the AmpFℓSTR® NGM SElect™ Kit). For additional information about size standards, refer to the GeneMapper® *ID* Software *Version 3.1 Human Identification Analysis User Guide* (PN 4338775), Appendix D.

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 completed 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 screenshot below is an example. Some columns may have different names from the names shown.
   - The Genotypes tab becomes available 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 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* (PN 4335523)
- GeneMapper® *ID* Software *Version 3.1 Human Identification Analysis User Guide* (PN 4338775)
- *Installation Procedures and New Features for* GeneMapper® *ID* Software *Version v3.2 User Bulletin* (PN 4352543)

Section 4.2 GeneMapper® *ID-X* Software

Before you start

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. Using GeneMapper® *ID-X* Software v1.0.1 or higher, you can then analyze and interpret the data from the .fsa files.

Note: Refer to **"Instrument and software overview" on page 15** for a list of compatible instruments.

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 that you 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, verify the sample result according to your laboratory's protocol.

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

Workflow

To analyze sample (.fsa) files using GeneMapper® *ID-X* Software v1.0.1 or higher for the first time:

- Import panels, bins, and marker stutter into the Panel Manager, as explained in **"Import panels, bins, and marker stutter" on page 64**.
- Create an analysis method, as explained in **"Create an analysis method" on page 70**.
- Create a size standard, as explained in **"Create a size standard" on page 77**.
- Define custom views of analysis tables.

  Refer to Chapter 1 of the GeneMapper® *ID-X* Software *Version 1.0 Getting Started Guide* (PN 4375574) for more information.
- Define custom views of plots.

  Refer to Chapter 1 of the GeneMapper® *ID-X* Software *Version 1.0 Getting Started Guide* (PN 4375574) for more information.

Import panels, bins, and marker stutter

To import the AmpFℓSTR® NGM SElect™ Kit panels, bin sets, and marker stutter from our website into the GeneMapper® *ID-X* Software v1.0.1 or higher database:

1. Download and open the file containing panels, bins, and marker stutter:

   a. From the Support menu of **www.appliedbiosystems.com**, select **Support ▸ Software Downloads, Patches & Updates ▸** GeneMapper® *ID-X* Software **▸ Updates & Patches**, and download the file **NGMSElect Analysis Files GMIDX**.

   b. Unzip the file.

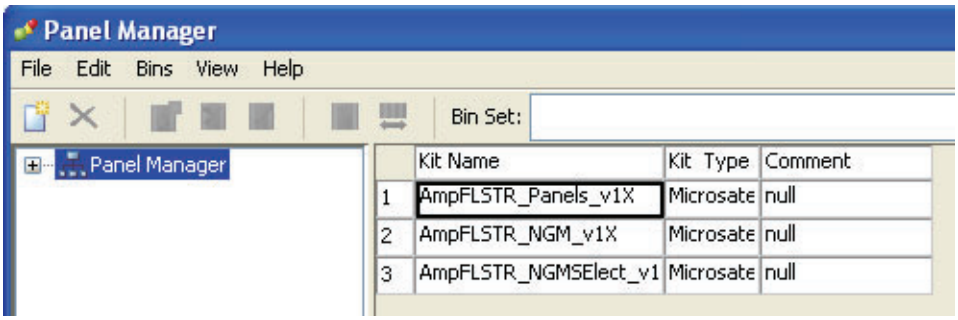
2. Start the GeneMapper® *ID-X* Software, then log in with the appropriate user name and password.

   IMPORTANT! For logon instructions, refer to the GeneMapper® *ID-X* Software *Version 1.0 Getting Started Guide* (PN 4375574).

3. Select **Tools ▸ Panel Manager**.

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

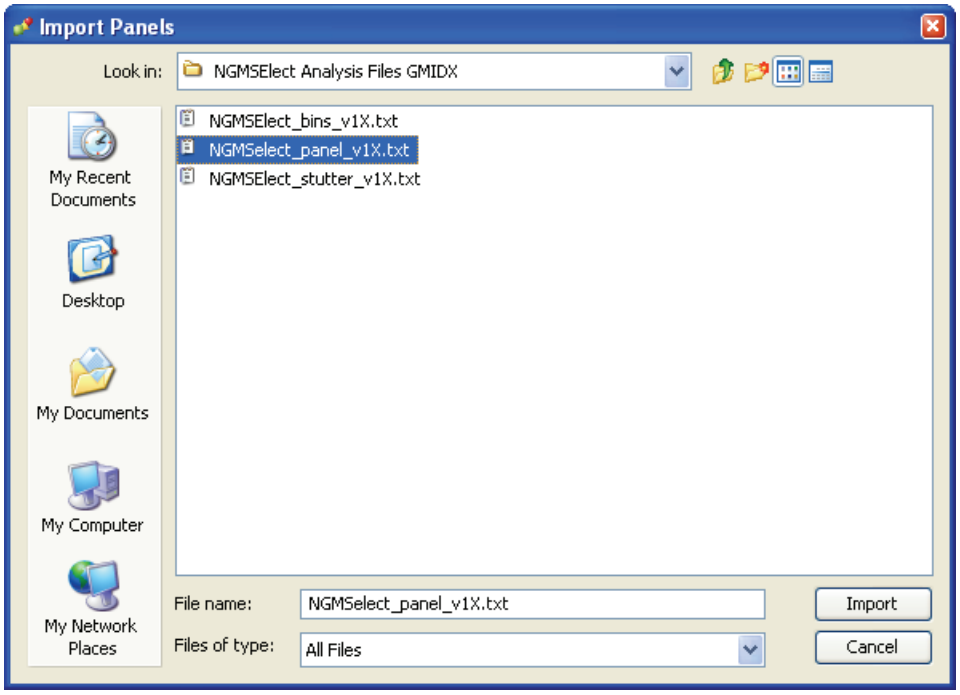
   a. Select **Panel Manager** in the navigation pane.



b. In the toolbar, select **File** ▸ **Import Panels** to open the Import Panels dialog box.

c. Locate, then open the **NGMSElect Analysis Files GMIDX** folder that you unzipped in step 1 on page 64.

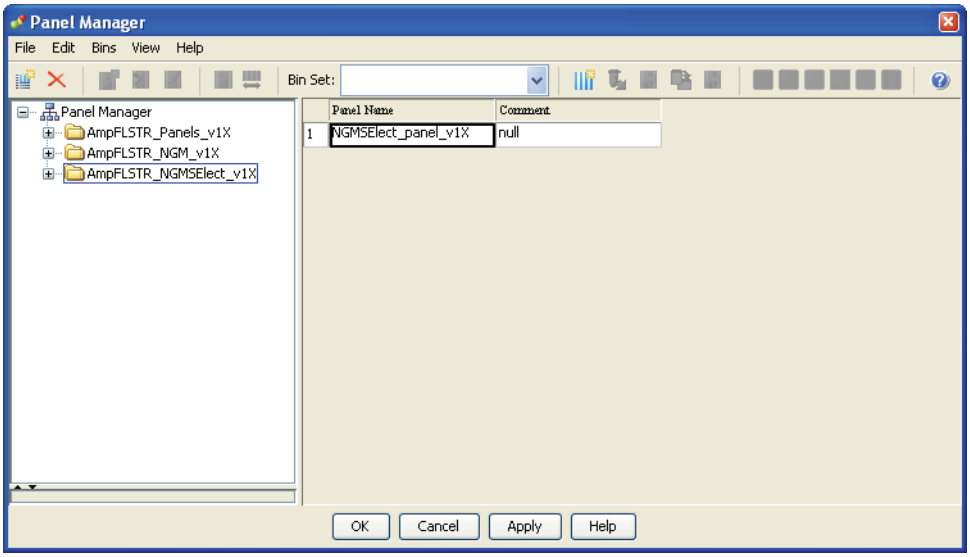
d. Select **NGMSElect_panel_v1X**.txt, then click **Import**.



Note: Importing this file creates a new folder in the navigation pane of the Panel Manager "AmpFLSTR_NGMSElect_v1X". This folder contains the panel and associated markers.

5. Import NGMSElect_bins_v1X:

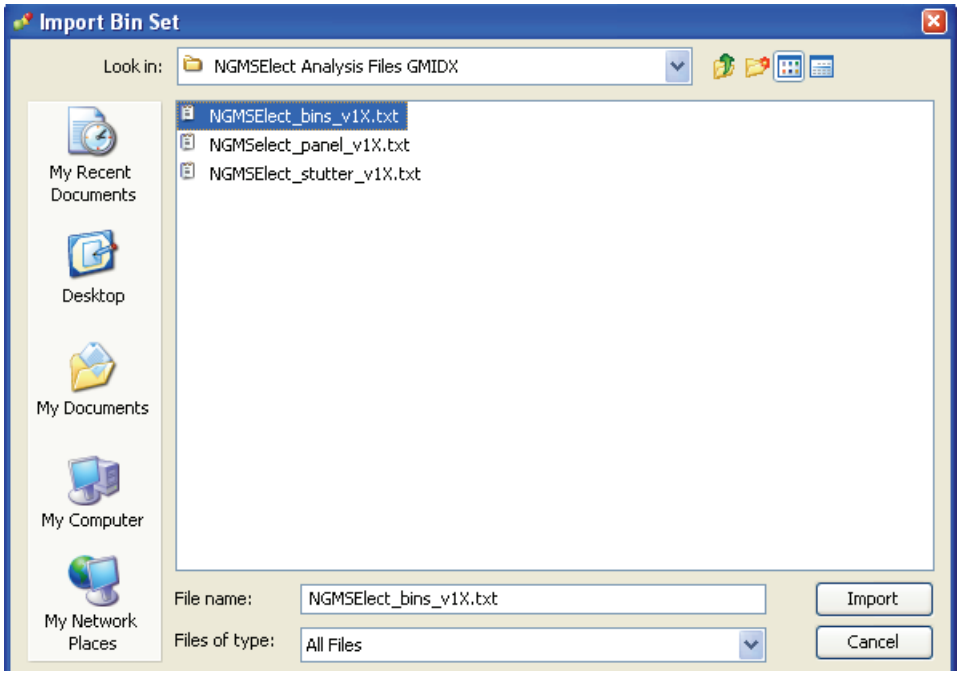
   a. Select the **AmpFLSTR_NGMSElect_v1X** folder in the left navigation pane.



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

   c. Locate, then open the **NGMSElect Analysis Files GMIDX** folder.

   d. Select **NGMSElect_bins_v1X**, then click **Import**.

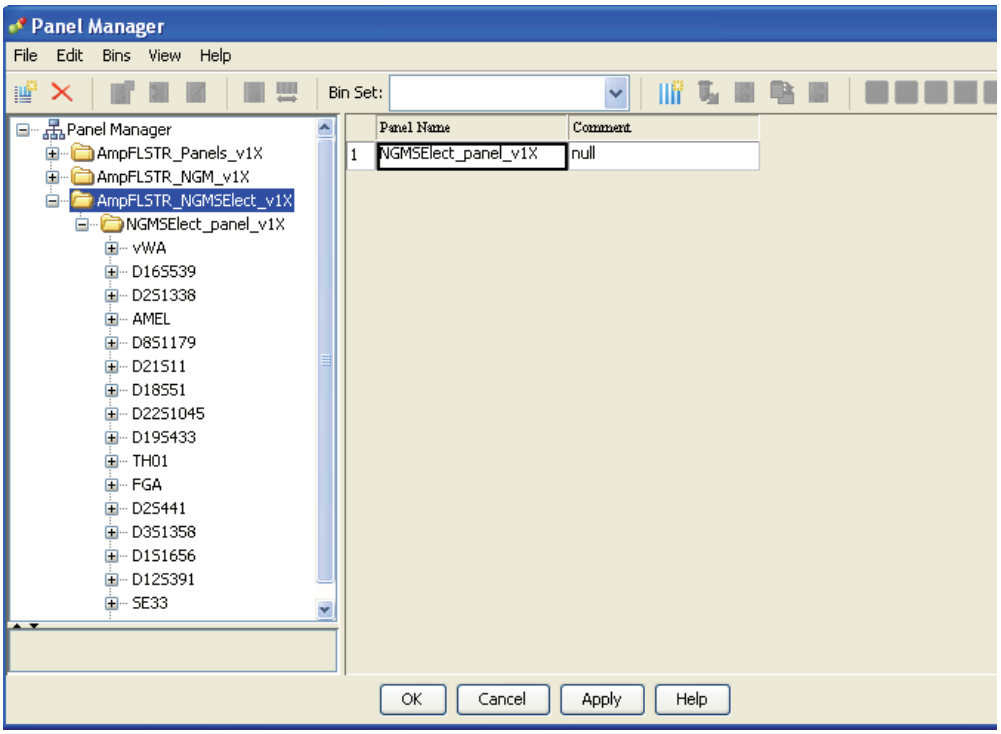


Note: Importing this file associates the bin set with the panels in the NGMSElect_panel_v1X folder.

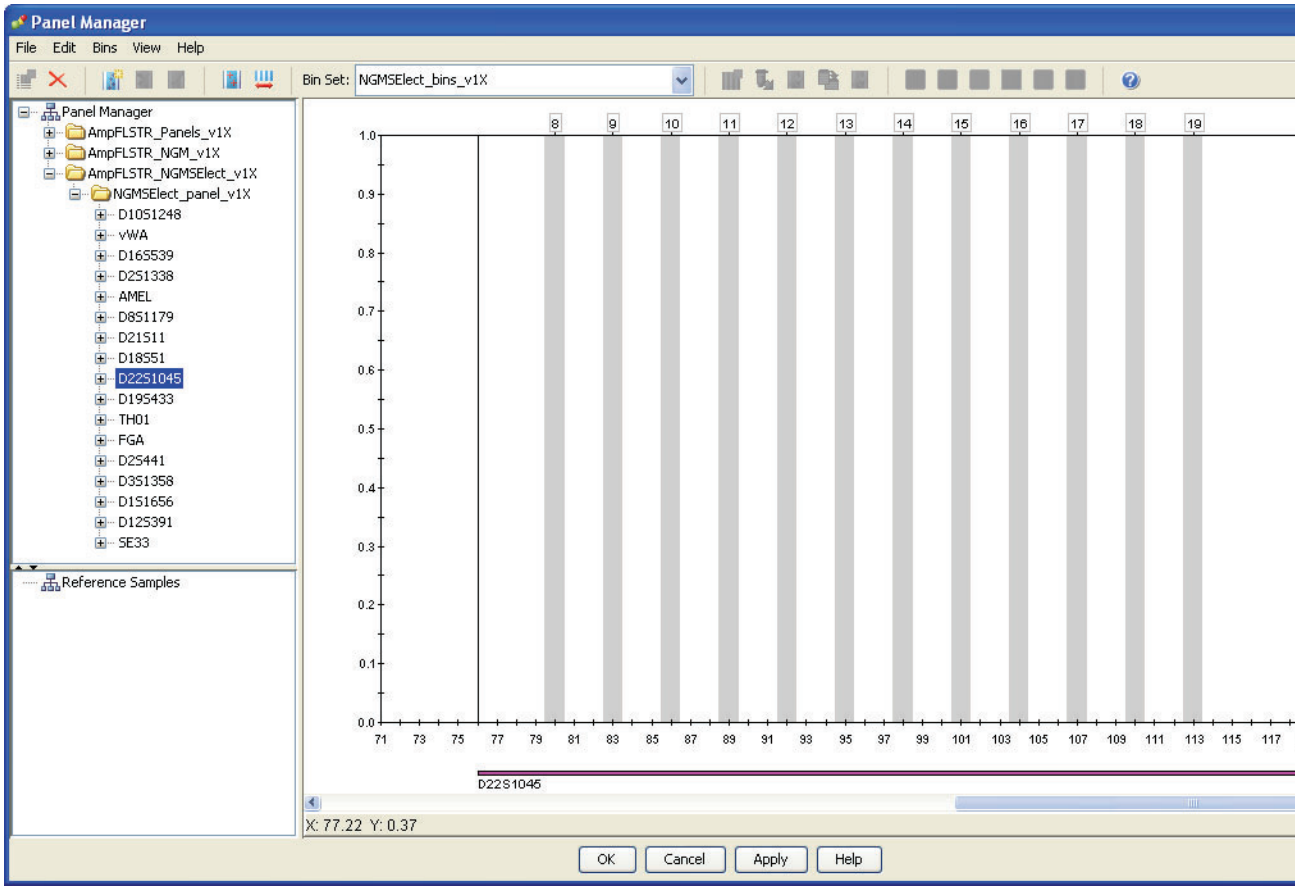
6. View the imported panels in the navigation pane:

   a. Double-click the **AmpFLSTR_NGMSElect_v1X** folder to view the NGMSElect_panel_v1X folder.

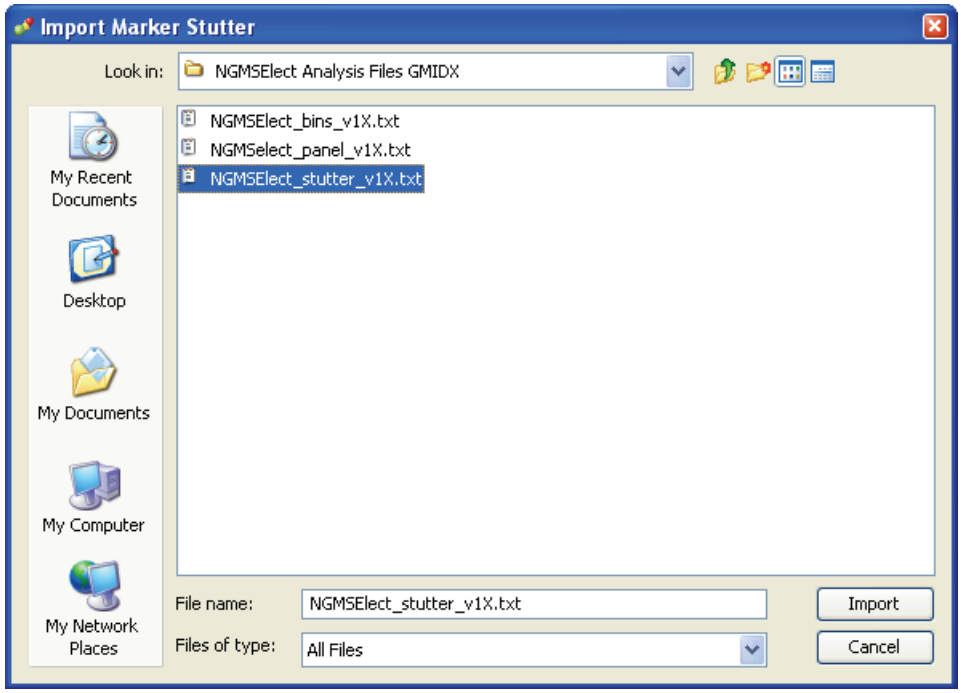
   b. Double-click the **NGMSElect_panel_v1X** folder to display the panel information and the markers below it.



c. Select **D22S1045** to display the Bin view for the marker.



7. Import NGMSElect_stutter_v1X:
   a. Select the **AmpFLSTR_NGMSElect_v1X** folder in the navigation pane.
   b. Select File ▸ **Import Marker Stutter** to open the Import Marker Stutter dialog box.
   c. Locate, then open the **NGMSElect Analysis Files GMIDX** folder.
   d. Select **NGMSElect_stutter_v1X**, then click **Import**.

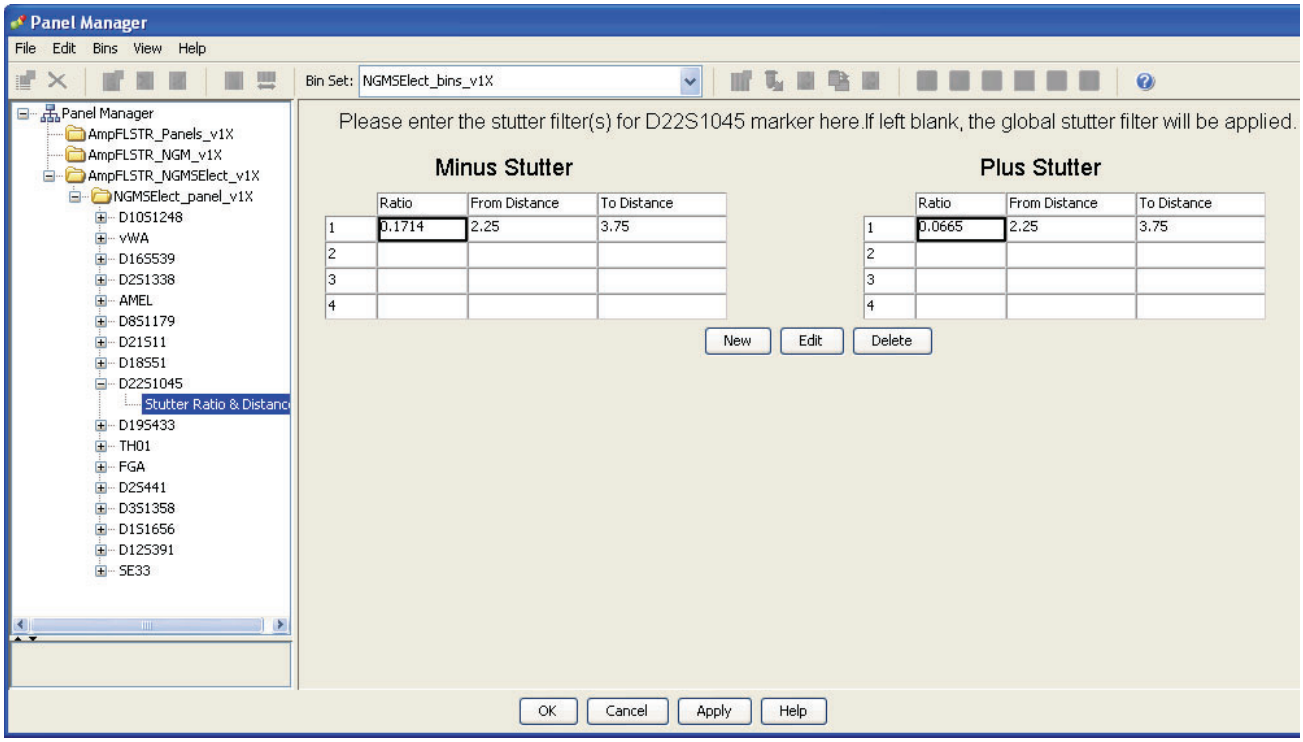


Note: Importing this file associates the marker stutter ratio with the bin set in the NGMSElect_bins_v1X folder.

8. View the imported marker stutters in the navigation pane:
   a. Select the **NGMSElect_panel_v1X** folder to display its list of markers.
   b. Double-click the **NGMSElect_panel_v1X** folder to display its list of markers below it.

c. Double-click **D22S1045** and select **Stutter Ratio & Distance** to display the Stutter Ratio & Distance view for the marker.

Because D22S1045 has a trinucleotide repeat unit, it produces a higher level of plus stutter than tetranucleotide markers, and so requires the use of a plus stutter filter. The settings for the D22S1045 plus stutter filter can be seen in the table in the right pane. Other markers may not require a plus stutter filter, in which case the settings for plus stutter are left blank.



9. Click **Apply**, then **OK** to add the AmpFℓSTR® NGM SElect™ Kit panels, bin sets, and marker stutter to the GeneMapper® *ID-X* Software database.

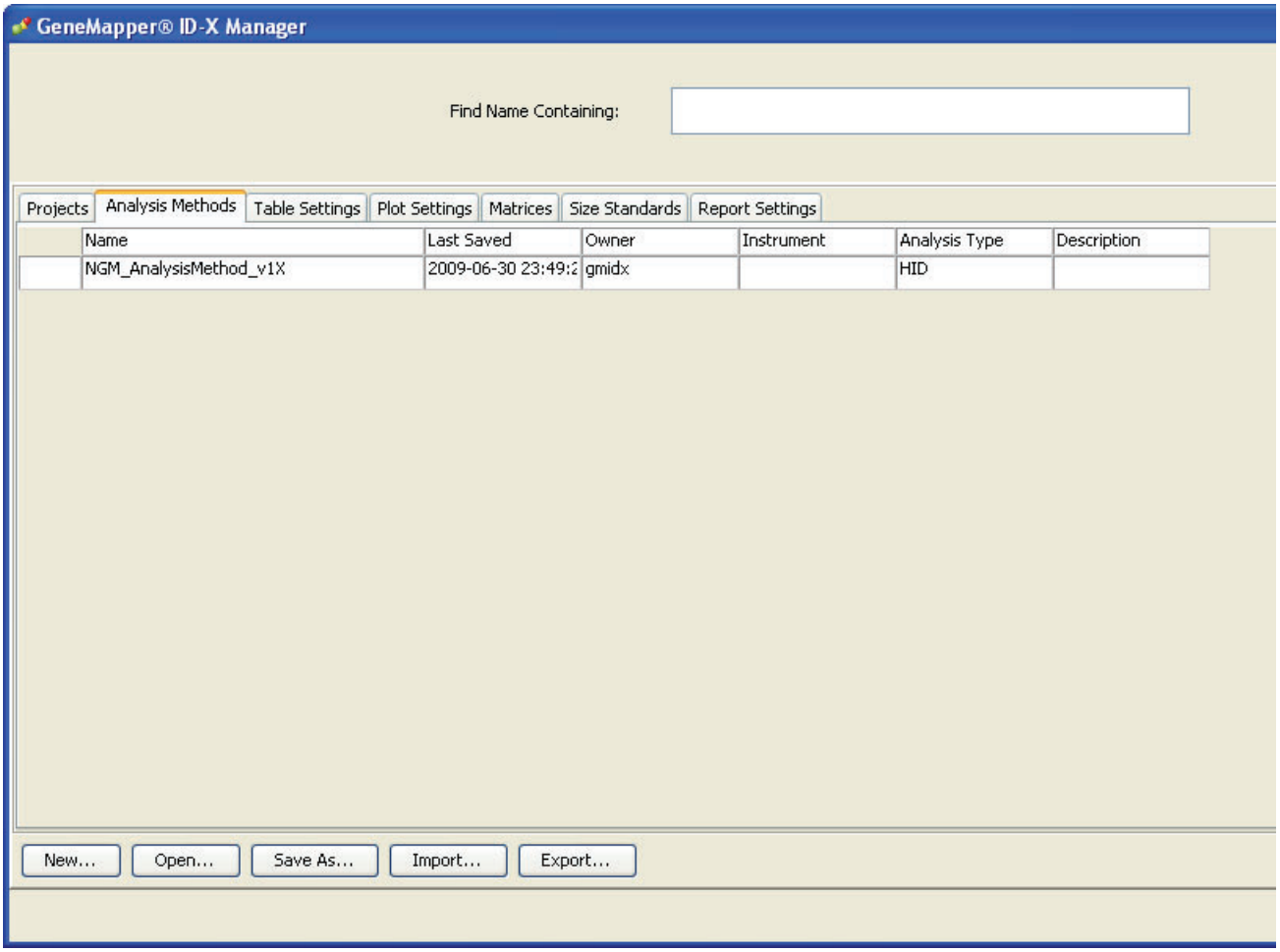
IMPORTANT! If you close the Panel Manager without clicking **Apply**, the panels, bin sets, and marker stutter will not be imported into the GeneMapper® *ID-X* Software database.

Create an analysis method

Use the following procedure to create an analysis method for the NGM SElect™ 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 GeneMapper® *ID-X* Software v1.1.1 or with GeneMapper® *ID* Software version 3.2.1.

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

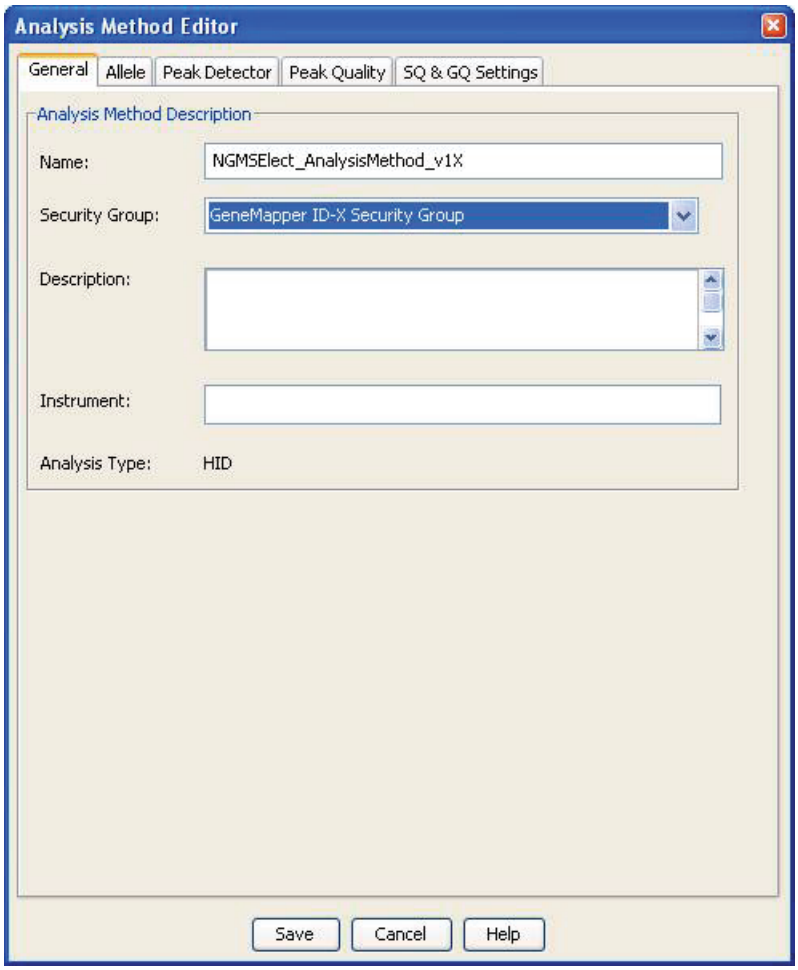


2. Select the **Analysis Methods** tab, then click **New** to open the Analysis Method Editor with the **General** tab selected.

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.

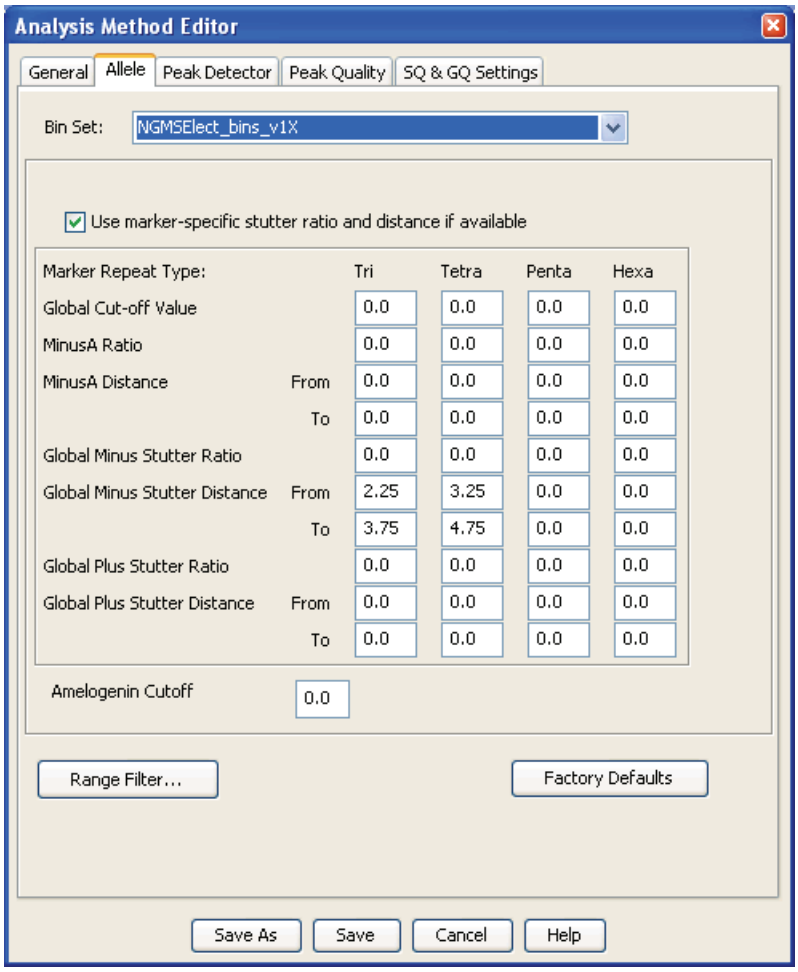
   Note: The Analysis Method Editor closes when you save your settings (See step 4 on page 76). To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.

General tab settings



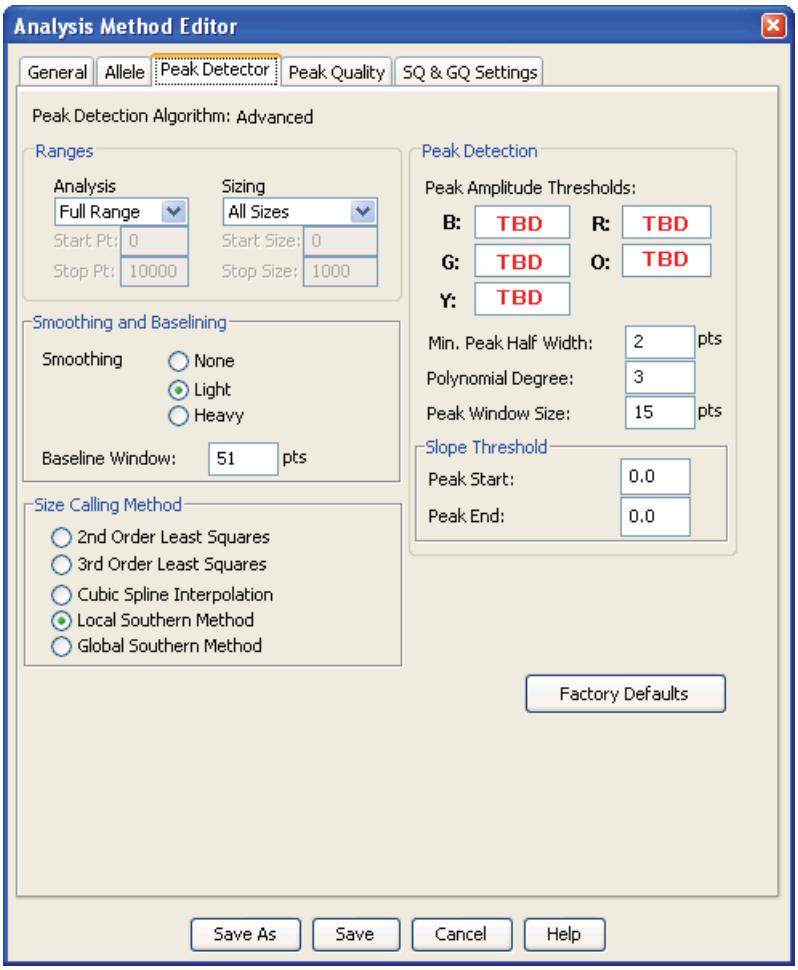
In the Name field, either type the name as shown for consistency with files supplied with other AmpFℓSTR® kits or enter a name of your choosing. In the Security Group field, select the Security Group appropriate to your software configuration from the dropdown list. The Description and Instrument fields are optional.

Allele tab settings



- In the Bin Set field, select the **NGMSElect_bins_v1X** bin set imported previously and configure the stutter distance parameters as shown.
- GeneMapper® *ID-X* Software v1.0.1 or higher allows you to specify 4 types of marker repeat motifs: tri, tetra, penta and hexa. You can enter parameter values for each type of repeat in the appropriate column.
- The "Use marker-specific stutter ratio if applicable" check box is selected by default. When this box is checked, the software applies the stutter ratio filters in the NGMSElect_stutter_v1X file.

Peak Detector tab settings



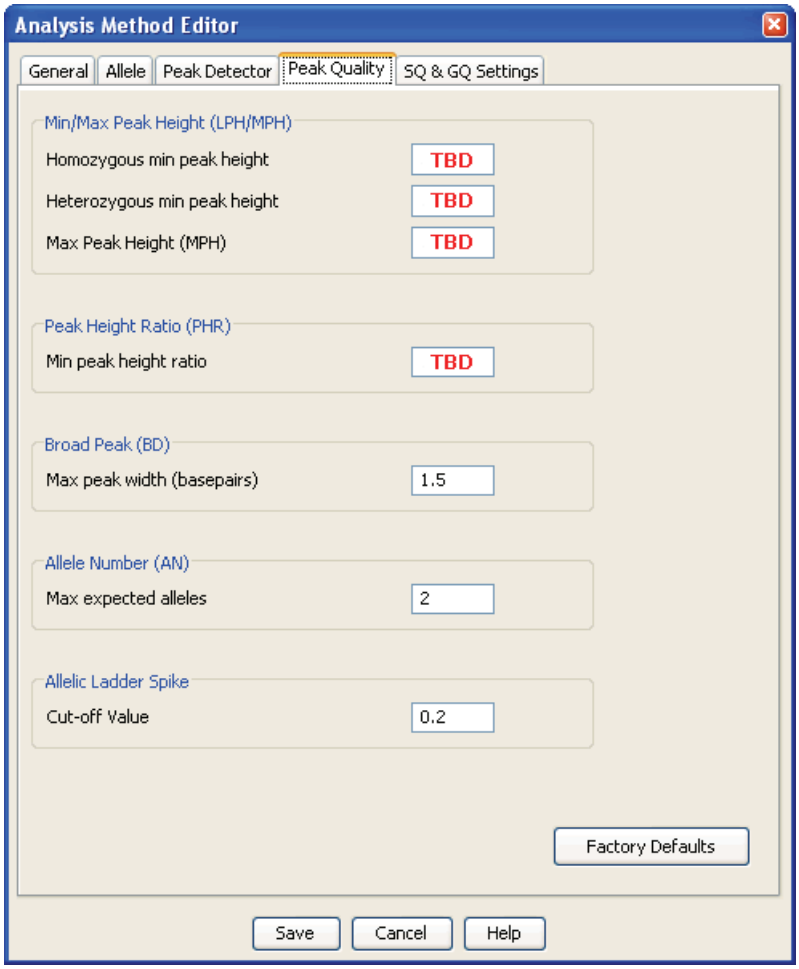
IMPORTANT! TBD indicates values to be determined in your laboratory. Laboratories must perform the appropriate internal studies to determine the appropriate peak amplitude thresholds for interpretation of AmpFℓSTR® NGM SElect™ Kit data.

Fields include:

- **Peak amplitude thresholds** – The software uses these parameters to specify the minimum peak height, in order to limit the number of detected peaks. Although GeneMapper® *ID-X* Software displays peaks that fall below the specified amplitude in electropherograms, the software does not label or determine the genotype of these peaks.
- **Size calling method** – The NGM SElect™ kit has been validated using the Local Southern sizing method. Alternative sizing methods should be selected only after extensive evaluation as part of an internal validation study in the user's laboratory.

- **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 NGM SElect™ kit data.

Peak Quality tab settings



IMPORTANT! TBD indicates values to be determined in your laboratory. Laboratories must perform the appropriate internal validation studies to determine the minimum heterozygous and homozygous minimum peak height thresholds, maximum peak height threshold and the minimum peak height ratio threshold for reliable interpretation of AmpFℓSTR® NGM SElect™ Kit data.

SQ & GQ tab settings

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

4. Click **Save**.

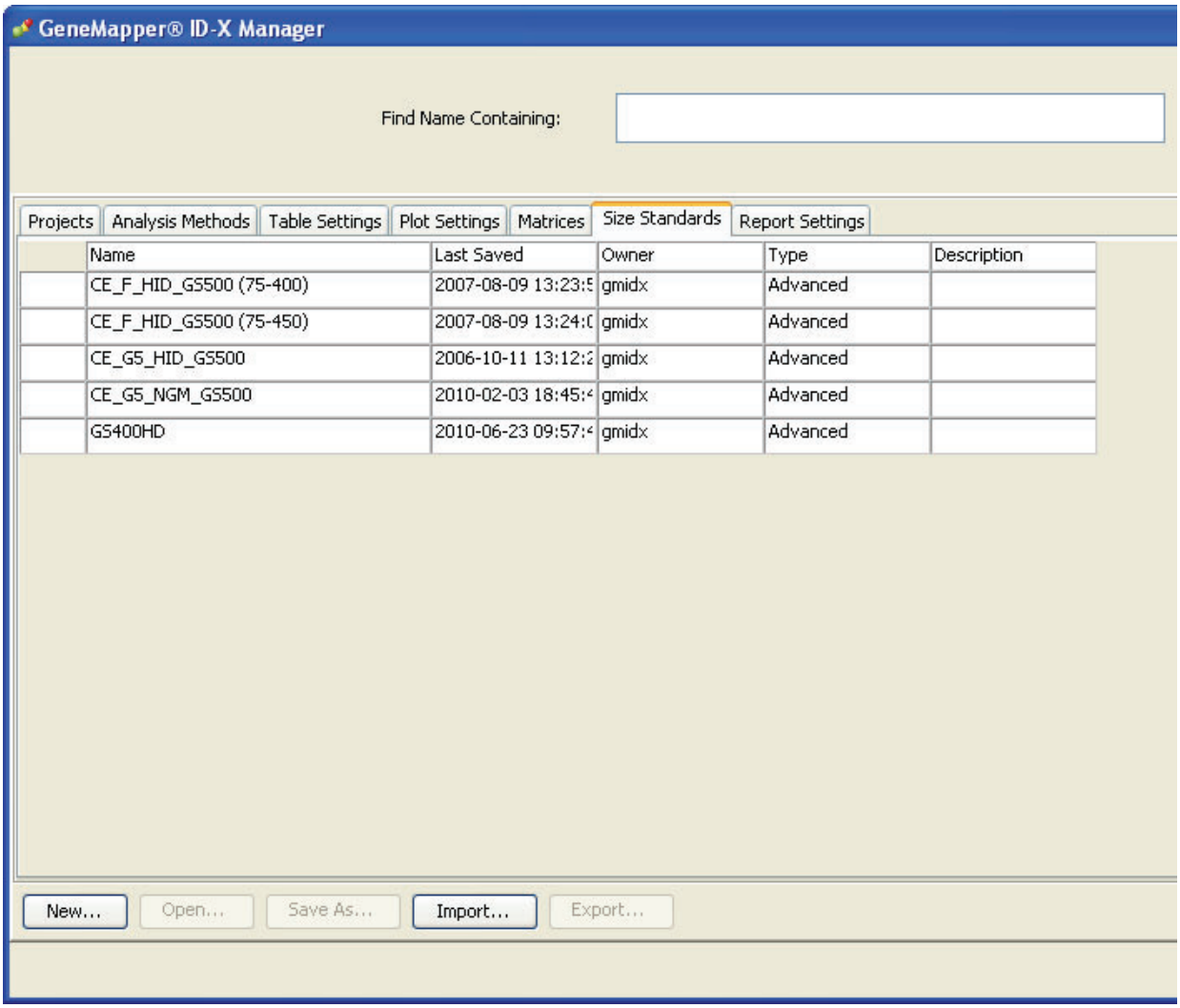
Create a size standard

The size standard for the AmpFℓSTR® NGM SElect™ PCR Amplification Kit uses the following GeneScan™ 600 LIZ™ Size Standard v2.0 peaks in its sizing algorithm: 60, 80, 100, 114, 120, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440 and 460.

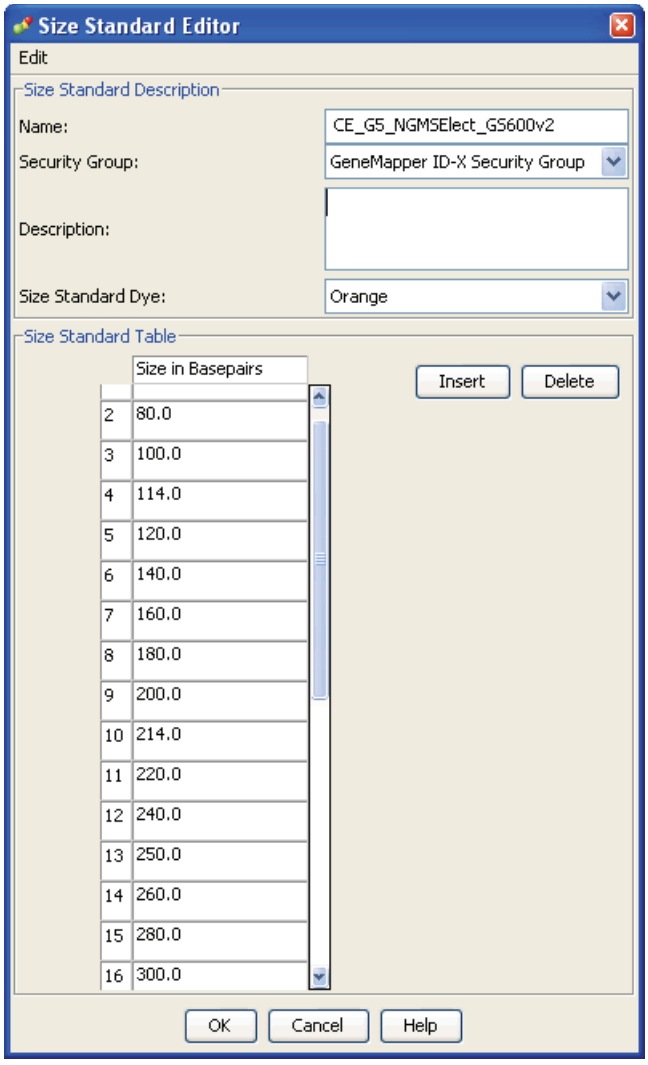
Use the following procedure to create the size standard for the NGM SElect™ kit.

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

2. Select the **Size Standards** tab, then click **New**.



3. Complete the Name field as shown below or with a name of your choosing. In the Security Group field, select the Security Group appropriate to your software configuration from the dropdown list. In the Size Standard Dye field, select **Orange**. In the Size Standard Table, enter the sizes specified in "Create a size standard" on page 77.



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

Analyze a project

1. In the Project window, select **File ▸ Add Samples to Project**, then go to the disk or directory containing the sample files.

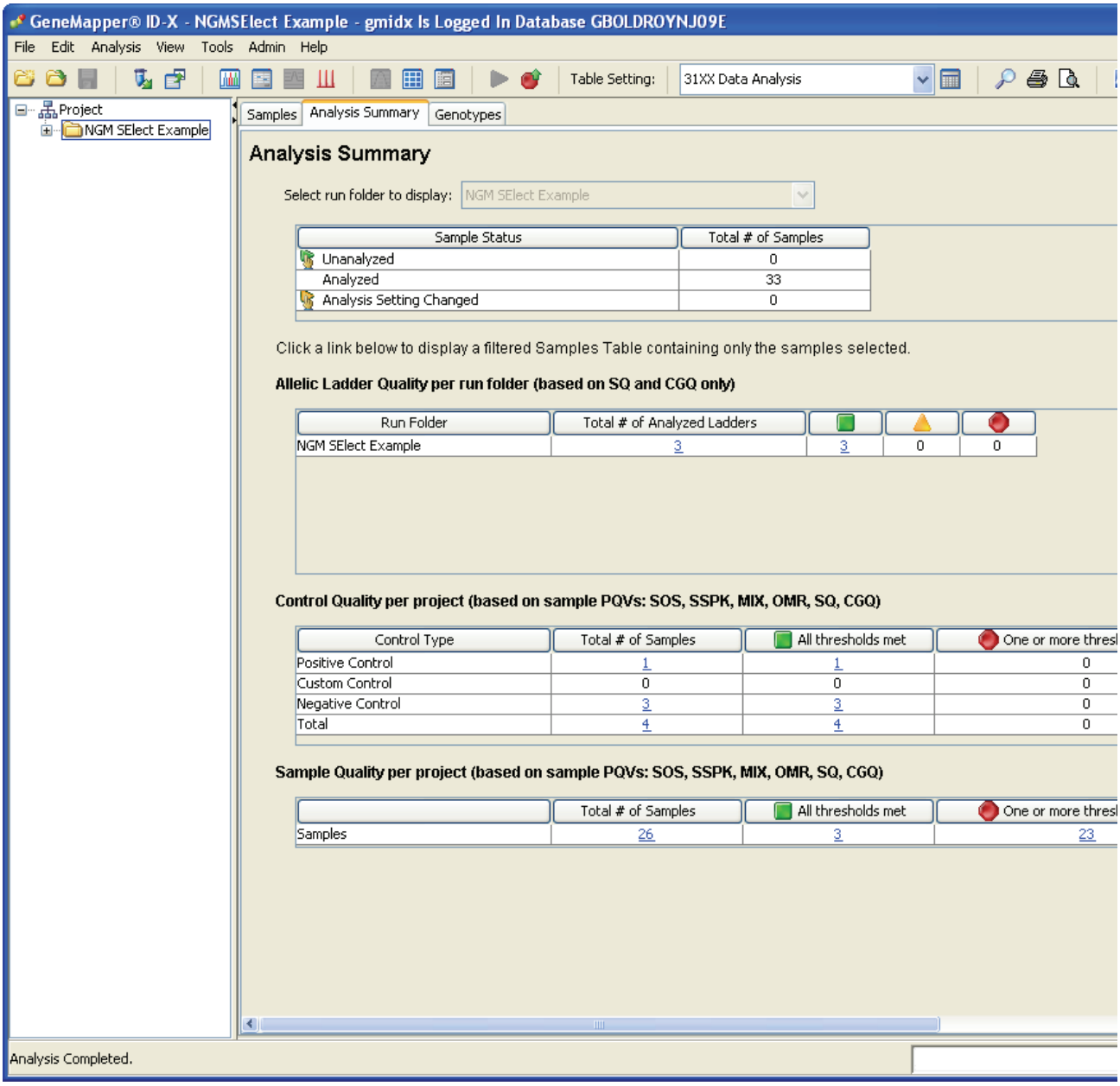
2. Apply analysis settings to the samples in the project. The names of the settings shown are the names suggested in the sections above. If you use names of your own choosing, select those names.

Parameter	Settings
Sample Type	Select the sample type
Analysis Method	**NGMSElect_AnalysisMethod_v1X**
Panel	**NGMSElect_panel_v1X**
Size Standard	**CE_G5_NGMSElect_GS600v2**

   - Size Standard: For more information about how the Size Caller works, refer to the ABI PRISM® GeneScan® Analysis Software for the *Windows NT® Operating System Overview of the Analysis Parameters and Size Caller User Bulletin* (PN 4335617).
   - CE_G5_NGMSElect_GS600v2 (size standard fragments defined in the NGM SElect™ kit): 60, 80, 100, 114, 120, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440 and 460. For additional information about size standards, refer to the GeneMapper® *ID* Software *Version 3.1 Human Identification Analysis User Guide* (PN 4338775), Appendix D.

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 completed 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 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 quick set-up instructions, refer to the GeneMapper® *ID-X* Software *Version 1.0 Getting Started Guide* (PN 4375574).
- For details about GeneMapper® *ID-X* Software features, allele filters, peak detection algorithms, and project editing, refer to:
  - GeneMapper® *ID-X* Software *Version 1.0 Getting Started Guide* (PN 4375574)
  - GeneMapper® *ID-X* Software *Version 1.0 Quick Reference Guide* (PN 4375670)
  - GeneMapper® *ID-X* Software *Version 1.0 Reference Guide* (PN 4375671)
  - GeneMapper® *ID-X* Software *Version 1.1 (Mixture Analysis Tool) Getting Started Guide* (PN 4396773)
  - GeneMapper® *ID-X* Software *1.1 (Mixture Analysis Tool) Quick Reference Guide* (PN 4402094)

Chapter 5

Experiments and Results

Experiments and Results 5

This chapter covers:

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- Sensitivity 117
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- Population data 128
- Mutation rate 129
- Probability of identity 130
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Overview

Experiments using the AmpFℓSTR® NGM SElect™ Kit

This chapter provides results of the developmental validation experiments performed using the AmpFℓSTR® NGM SElect™ PCR Amplification Kit.

Importance of validation

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

Experiment conditions

Experiments to evaluate the performance of the AmpFℓSTR® NGM SElect™ PCR Amplification Kit were performed. The experiments were performed according to the revised guidelines from the Scientific Working Group on DNA Analysis Methods (SWGDAM, July 10, 2003). Based on these guidelines, we conducted experiments that comply with guidelines 1.0 and 2.0 and its associated subsections. This DNA methodology is not novel. (Moretti et al., 2001; Frank et al., 2001; Wallin et al., 2002; and Holt et al., 2000).

This chapter discusses many of the experiments performed and provides examples of results obtained. We chose conditions that produced optimum PCR product yield and that met reproducible performance standards. It is our opinion that while these experiments are not exhaustive, they are appropriate for a manufacturer of STR kits intended for forensic and/or parentage testing use.

IMPORTANT! Each laboratory using the AmpFℓSTR® NGM SElect™ PCR Amplification Kit must perform internal validation studies.

Developmental validation

SWGDAM guideline 1.2.1

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

SWGDAM guideline 2.10.1

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

PCR components

We examined the concentration of each component of the AmpFℓSTR® NGM SElect™ Kit and established that the concentration of each component was within the range where data indicated that the amplification met the required performance criteria for specificity, sensitivity, and reproducibility. For example, 1ng of Control DNA 007 was amplified in the presence of varying concentrations of magnesium chloride, and the results were analyzed on an Applied Biosystems® 3130*xl* Genetic Analyzer. Results are shown in Figure 2. The performance of the multiplex is most robust within ±20% of the optimal magnesium chloride concentration.

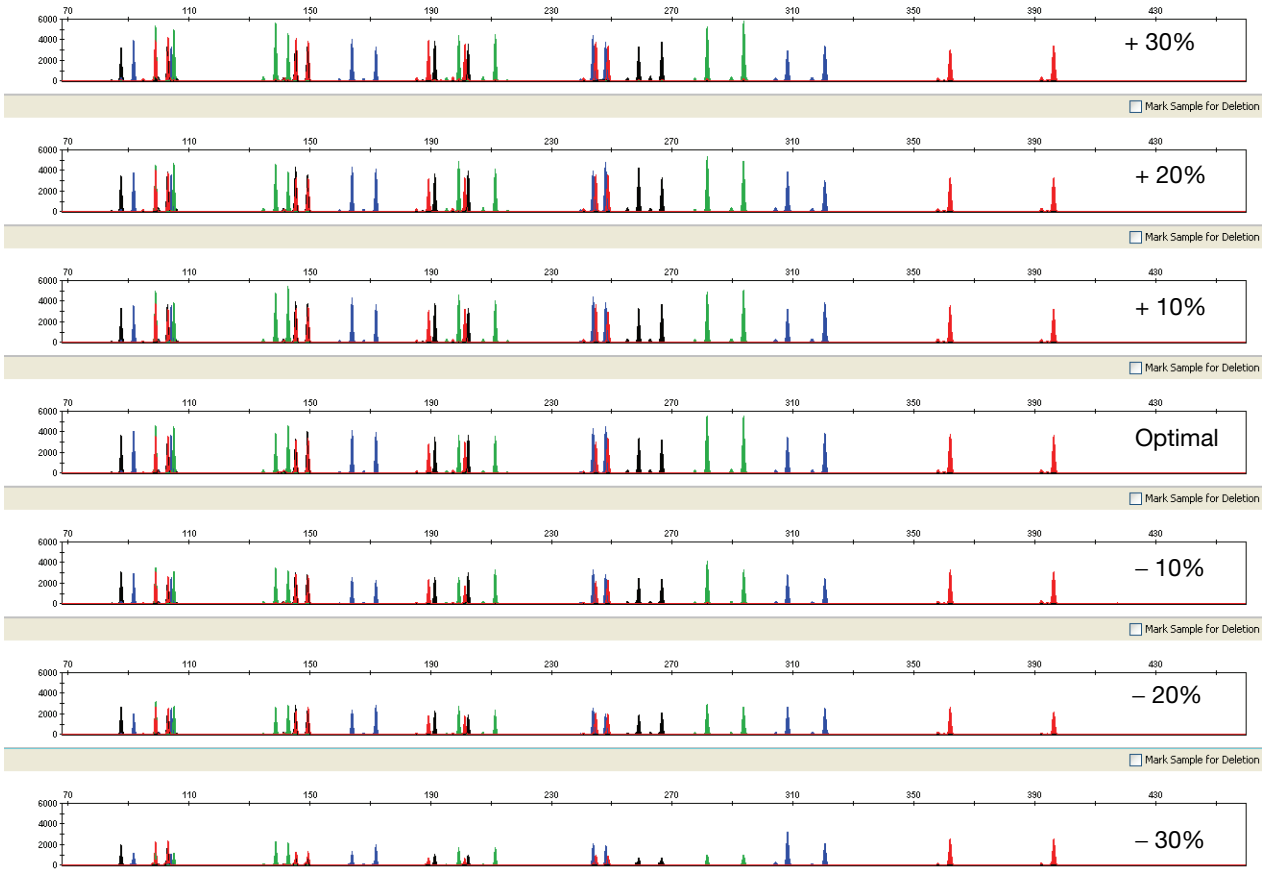

Figure 2 1.0 ng of control DNA 007 amplified with the AmpFℓSTR® NGM SElect™ Kit in the presence of varying concentrations of magnesium chloride and analyzed on an Applied Biosystems® 3130*xl* Genetic Analyzer.

Thermal cycler parameters

Thermal cycling parameters were optimized using a Design of Experiments (DOE) approach that attempts to identify the combination of temperatures and hold times that produce the best assay performance. Optimal assay performance was determined through evaluation of assay sensitivity, peak-height balance and resistance to PCR inhibitors.

For example, annealing temperatures of 55, 57, 59, 61, and 63 °C were tested using a Silver 96-Well GeneAmp® PCR System 9700 (Figure 3). The PCR products were analyzed using an Applied Biosystems® 3130*xl* Genetic Analyzer.

Of the tested annealing temperatures, 55 to 61 °C produced robust profiles. At 63 °C the yield of the majority of loci was significantly reduced. The optimal combination of specificity, sensitivity, and resistance to PCR inhibition was observed at 59 °C. Thermal cycler temperature is critical to assay performance; therefore, routine, regularly scheduled thermal cycler calibration is strongly recommended.

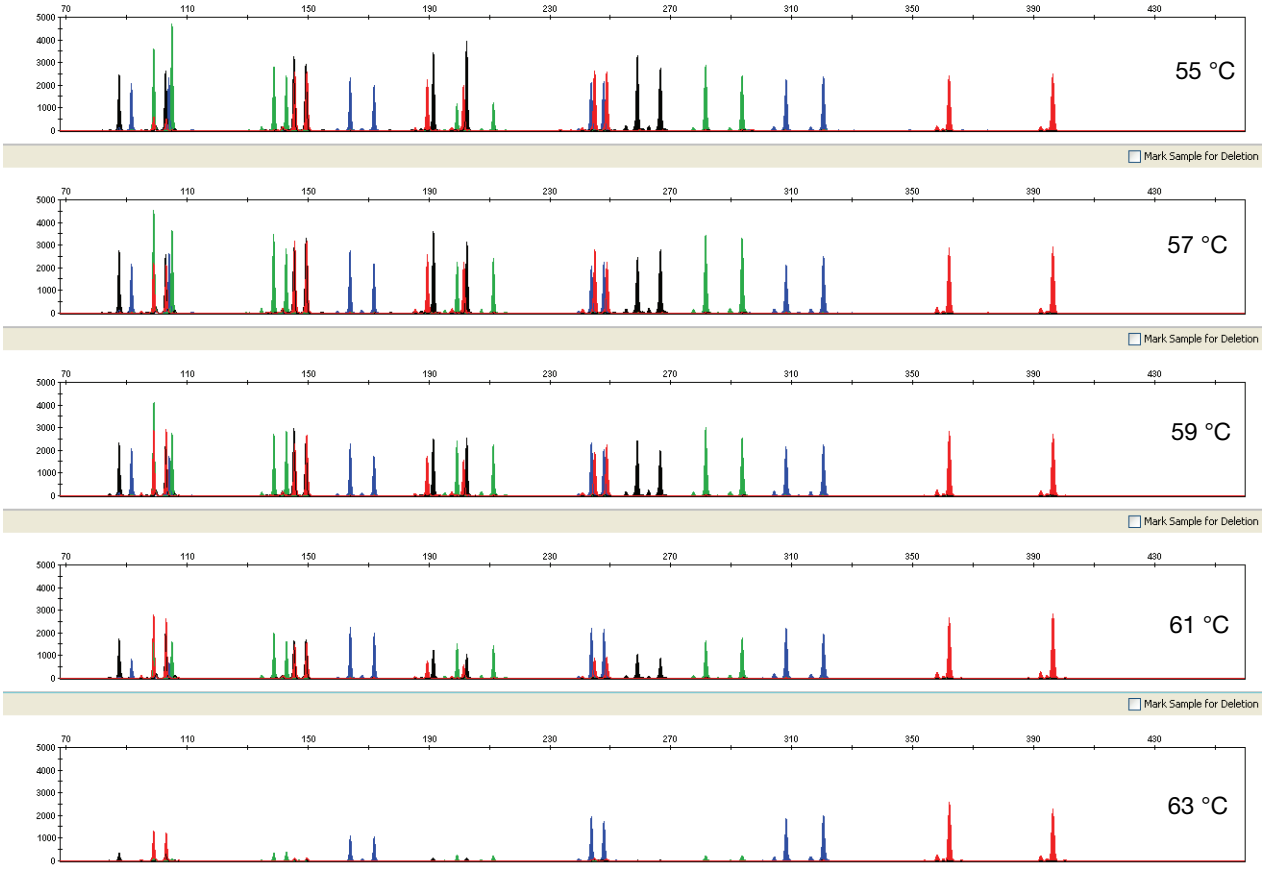

Figure 3 Electropherograms obtained from amplification of 1.0 ng of control DNA 007 at annealing temperatures of 55 °C, 57 °C, 59 °C, 61 °C, and 63 °C, analyzed on an Applied Biosystems® 3130*xl* Genetic Analyzer, (Y-axis scale 0 to 3,000 RFU).

PCR cycle number

AmpFℓSTR® NGM SElect™ Kit reactions were amplified for 27, 28, 29, 30, and 31 cycles on the Silver 96-Well GeneAmp® PCR System 9700 using 1.0 ng of each of three DNA samples. As expected, the amount of PCR product increased with the number of cycles. A full profile was generated for all numbers of thermal cycles (27–31) and off-scale data were collected for several allele peaks at 30 and 31 cycles (Figure 4).

Optimal sensitivity was produced by 29 cycles when the amplified products were analyzed on Applied Biosystems® 3130*xl* Genetic Analyzers. None of the cycle numbers tested produced nonspecific peaks.

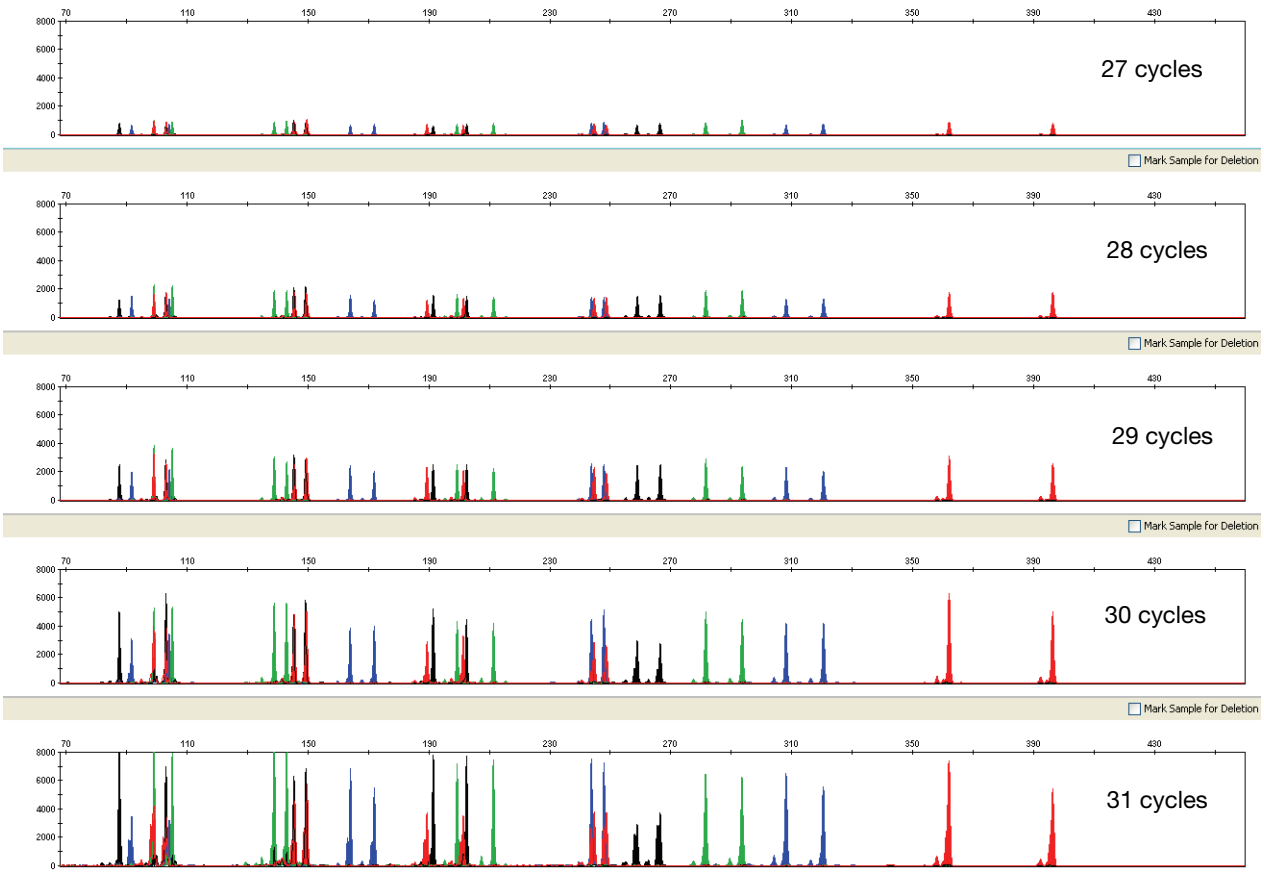

Figure 4 Representative AmpFℓSTR® NGM SElect™ Kit profiles obtained from amplification of 1.0 ng DNA template using 27, 28, 29, 30, and 31 cycles, analyzed on an Applied Biosystems® 3130*xl* Genetic Analyzer, (Y-axis scale 0 to 8,000 RFU)

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)

Accuracy

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

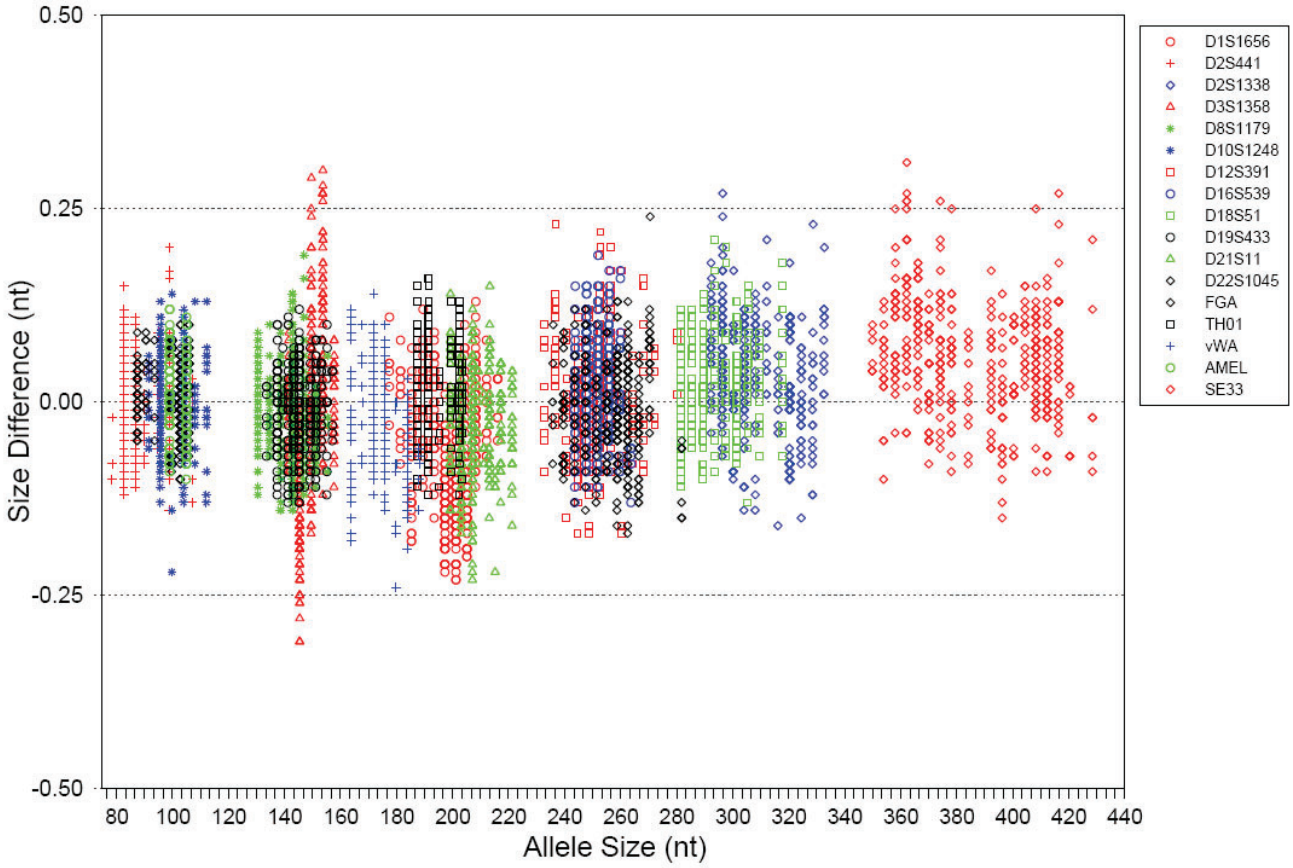

Figure 5 Allele Size vs. Allelic Ladder Sizing for 42 samples analyzed on an Applied Biosystems® 3130*xl* Genetic Analyzer. Size and ladder sizing for the AmpFℓSTR® NGM SElect™ Kit were calculated using the GeneScan™ 600 LIZ™ v2.0 Size Standard.

Precision and size windows

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

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

or

- An allele that does correspond to an Allelic Ladder allele, but whose size is just outside a window because of measurement error

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

Table 4 on page 95 shows typical precision results obtained from five runs (16 capillaries/run) of the AmpFℓSTR® NGM SElect™ Allelic Ladder on an Applied Biosystems® 3130*xl* Genetic Analyzer (36-cm capillary and POP-4™ polymer), using the GeneScan™ 600 LIZ™ v2.0 Size Standard. The results were obtained within a set of injections on a single capillary array.

Sample alleles may occasionally size outside of the ±0.5-nt window for a respective Allelic Ladder allele because of measurement error. The frequency of such an occurrence is lowest in detection systems having the smallest standard deviations in sizing. Figure 5 on page 93 illustrates the tight clustering of allele sizes obtained on the Applied Biosystems® 3130*xl* Genetic Analyzer, where the standard deviation in sizing is typically less than 0.15 nt. The instance of a sample allele sizing outside the ±0.5-nt window because of measurement error is relatively rare when the standard deviation in sizing is approximately 0.15 nt or less (Smith, 1995).

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

GeneMapper® *ID* Software and GeneMapper® *ID-X* Software automatically flag sample alleles that do not size within the prescribed window around an allelic ladder allele by labelling the allele as OL (off-ladder).

Maximum sizing precision is obtained within the same set of capillary injections. Cross-platform sizing differences occur due to a number of factors including type and concentration of polymer, run temperature, and electrophoresis conditions. Variations in sizing can also occur between runs on the same instrument and between runs on different instruments of the same platform type because of these factors.

We strongly recommend that the allele sizes be compared to the sizes obtained for known alleles in the AmpFℓSTR® NGM SElect™ Allelic Ladder from the same run and then be converted to genotypes as described in "Before you start" on pages 47 (GeneMapper® *ID* Software) and 63 (GeneMapper® *ID-X* Software). See Table 4 for the results of five runs of the AmpFℓSTR® NGM SElect™ Allelic Ladder on an Applied Biosystems® 3130*xl* Genetic Analyzer. For more information on precision and genotyping, see Lazaruk et al., 1998 and Mansfield et al., 1998.

In Table 4, the mean sizes for all the alleles in each run (16 capillaries) were calculated. The mean range shown in the table represents the lowest and highest mean size values obtained across all five runs. Similarly, the standard deviation for the allele sizing was calculated for all the alleles in each run. The standard deviation range shown in Table 4 represents the lowest and highest standard deviation values obtained across all five runs.

Table 4 Precision results of five runs (16 capillaries/run) of the AmpFℓSTR® NGM SElect™ Allelic Ladder

Allele	Mean	Standard Deviation
AMEL		
X	98.96–98.98	0.024–0.038
Y	104.99–105.02	0.027–0.039
D10S1248		
8	74.74–74.83	0.030–0.044
9	78.97–79.06	0.026–0.043
10	83.17–83.26	0.028–0.044
11	87.34–87.45	0.031–0.053
12	91.49–91.60	0.028–0.042
13	95.64–95.76	0.037–0.045
14	99.76–99.86	0.024–0.043
15	103.93–104.05	0.032–0.052
16	108.06–108.18	0.028–0.044
17	112.14–112.26	0.033–0.054
18	116.14–116.25	0.026–0.046
D12S391		
14	228.77–228.81	0.034–0.051
15	232.73–232.75	0.036–0.049
16	236.74–236.76	0.033–0.044
17	240.68–240.71	0.035–0.041
18	244.72–244.76	0.031–0.045
19	248.71–248.75	0.037–0.046
19.3	251.71–251.75	0.038–0.041
20	252.66–252.70	0.035–0.047
21	256.51–256.54	0.025–0.039

Allele	Mean	Standard Deviation
22	260.26–260.31	0.036–0.044
23	264.22–264.27	0.034–0.043
24	268.24–268.30	0.034–0.045
25	272.22–272.27	0.030–0.047
26	276.16–276.21	0.031–0.049
27	280.22–280.26	0.022–0.040
D16S539		
5	227.39–227.51	0.050–0.060
8	239.52–239.63	0.040–0.055
9	243.66–243.78	0.040–0.051
10	247.78–247.89	0.042–0.055
11	251.82–251.92	0.037–0.055
12	255.79–255.89	0.045–0.049
13	259.73–259.83	0.040–0.049
14	263.74–263.85	0.035–0.059
15	267.76–267.89	0.034–0.049
D18S51		
7	261.17–261.26	0.027–0.051
9	269.29–269.40	0.036–0.051
10	273.36–273.48	0.032–0.054
10.2	275.38–275.46	0.028–0.049
11	277.44–277.53	0.037–0.043
12	281.46–281.57	0.030–0.043
13	285.48–285.59	0.031–0.050
13.2	287.45–287.57	0.031–0.046
14	289.52–289.62	0.034–0.038
14.2	291.48–291.59	0.033–0.051
15	293.53–293.62	0.032–0.050
16	297.53–297.63	0.031–0.051
17	301.51–301.60	0.028–0.047
18	305.47–305.55	0.031–0.041
19	309.49–309.56	0.034–0.050
20	313.55–313.62	0.033–0.042
21	317.81–317.89	0.031–0.038
22	322.07–322.18	0.033–0.051
23	326.12–326.21	0.037–0.050
24	330.22–330.30	0.039–0.047
25	334.30–334.38	0.029–0.045
26	338.33–338.40	0.033–0.049

Allele	Mean	Standard Deviation
27	342.41–342.48	0.028–0.042
D19S433		
9	125.98–126.02	0.033–0.040
10	129.78–129.82	0.031–0.038
11	133.60–133.65	0.027–0.044
12	137.45–137.50	0.026–0.038
12.2	139.41–139.47	0.024–0.043
13	141.35–141.38	0.032–0.037
13.2	143.34–143.38	0.031–0.040
14	145.29–145.32	0.029–0.038
14.2	147.27–147.31	0.023–0.039
15	149.22–149.25	0.030–0.040
15.2	151.21–151.26	0.026–0.043
16	153.18–153.22	0.027–0.035
16.2	155.16–155.21	0.027–0.037
17	157.12–157.18	0.031–0.042
17.2	159.12–159.18	0.024–0.036
D1S1656		
9	173.48–173.54	0.027–0.047
10	177.41–177.48	0.033–0.048
11	181.37–181.43	0.031–0.044
12	185.36–185.41	0.027–0.036
13	189.38–189.43	0.024–0.042
14	193.45–193.49	0.024–0.036
14.3	196.44–196.50	0.030–0.047
15	197.33–197.36	0.026–0.042
15.3	200.40–200.46	0.024–0.043
16	201.25–201.30	0.022–0.045
16.3	204.31–204.37	0.030–0.039
17	205.16–205.21	0.027–0.041
17.3	208.21–208.28	0.025–0.044
18.3	212.13–212.20	0.030–0.034
19.3	216.11–216.16	0.029–0.039
20.3	220.12–220.16	0.030–0.042
D21S11		
24	182.86–182.97	0.030–0.054
24.2	184.92–185.04	0.032–0.055
25	186.95–187.07	0.026–0.051
26	191.05–191.17	0.031–0.051

Allele	Mean	Standard Deviation
27	195.18–195.28	0.030–0.044
28	199.22–199.34	0.032–0.047
28.2	201.20–201.30	0.035–0.037
29	203.17–203.29	0.024–0.043
29.2	205.23–205.33	0.032–0.046
30	207.19–207.29	0.033–0.040
30.2	209.18–209.28	0.032–0.046
31	211.18–211.29	0.031–0.045
31.2	213.17–213.29	0.033–0.047
32	215.21–215.32	0.031–0.048
32.2	217.24–217.36	0.035–0.048
33	219.31–219.44	0.036–0.055
33.2	221.27–221.41	0.039–0.050
34	223.41–223.55	0.043–0.053
34.2	225.34–225.47	0.035–0.050
35	227.42–227.55	0.035–0.052
35.2	229.37–229.51	0.039–0.053
36	231.38–231.51	0.033–0.049
37	235.46–235.61	0.038–0.058
38	239.44–239.56	0.032–0.055
D22S1045		
5	78.18–78.21	0.031–0.043
6	81.29–81.32	0.032–0.040
7	84.36–84.39	0.031–0.047
8	87.45–87.48	0.027–0.043
9	90.52–90.56	0.028–0.042
10	93.59–93.63	0.021–0.038
11	96.66–96.70	0.028–0.036
12	99.74–99.77	0.022–0.038
13	102.83–102.87	0.025–0.039
14	105.91–105.97	0.024–0.045
15	108.98–109.04	0.030–0.046
16	112.04–112.09	0.034–0.041
D2S1338		
15	288.31–288.45	0.034–0.051
16	292.31–292.45	0.042–0.056
17	296.27–296.42	0.041–0.045
18	300.19–300.32	0.036–0.050
19	304.11–304.25	0.035–0.059

Allele	Mean	Standard Deviation
20	308.06–308.20	0.045–0.051
21	312.06–312.20	0.034–0.051
22	316.20–316.34	0.038–0.054
23	320.41–320.54	0.027–0.056
24	324.52–324.64	0.040–0.050
25	328.58–328.71	0.036–0.049
26	332.62–332.74	0.037–0.053
27	336.67–336.77	0.035–0.054
28	340.93–341.03	0.033–0.049
D2S441		
9	78.49–78.50	0.030–0.037
10	82.63–82.66	0.027–0.037
11	86.76–86.79	0.023–0.038
12	89.94–89.97	0.024–0.039
13	90.87–90.88	0.025–0.044
14	94.82–94.84	0.027–0.036
15	98.92–98.94	0.025–0.034
16	103.05–103.08	0.026–0.038
D3S1358		
12	133.60–133.70	0.026–0.039
13	137.62–137.71	0.027–0.050
14	141.52–141.62	0.031–0.048
15	145.40–145.49	0.025–0.050
16	149.51–149.61	0.030–0.041
17	153.62–153.72	0.027–0.040
18	157.61–157.71	0.036–0.044
19	161.51–161.61	0.031–0.053
D8S1179		
8	122.53–122.56	0.033–0.040
9	126.53–126.56	0.027–0.039
10	130.55–130.57	0.028–0.042
11	134.60–134.63	0.023–0.037
12	138.67–138.71	0.026–0.040
13	142.90–142.92	0.023–0.041
14	147.03–147.06	0.027–0.036
15	151.18–151.20	0.028–0.040
16	155.33–155.37	0.023–0.040
17	159.49–159.52	0.008–0.040
18	163.59–163.62	0.024–0.039

Allele	Mean	Standard Deviation
19	167.67–167.70	0.027–0.038
FGA		
17	232.00–232.06	0.026–0.047
18	235.84–235.90	0.030–0.047
19	239.67–239.72	0.036–0.047
20	243.58–243.64	0.032–0.045
21	247.49–247.55	0.036–0.049
22	251.34–251.40	0.023–0.053
23	255.12–255.17	0.030–0.047
24	258.85–258.92	0.034–0.047
25	262.64–262.73	0.036–0.050
26	266.48–266.58	0.034–0.057
26.2	268.35–268.46	0.029–0.051
27	270.39–270.50	0.036–0.057
28	274.24–274.35	0.034–0.053
29	278.05–278.17	0.031–0.048
30	281.80–281.92	0.032–0.051
30.2	284.05–284.19	0.033–0.046
31.2	287.85–287.98	0.035–0.047
32.2	291.64–291.77	0.035–0.046
33.2	295.44–295.57	0.034–0.053
42.2	330.53–330.68	0.040–0.055
43.2	334.39–334.54	0.037–0.055
44.2	338.38–338.53	0.041–0.061
45.2	342.30–342.45	0.044–0.067
46.2	345.90–346.07	0.045–0.057
47.2	349.74–349.92	0.046–0.073
48.2	353.77–353.93	0.046–0.063
50.2	361.42–361.59	0.053–0.074
51.2	365.26–365.44	0.037–0.070
SE33		
4.2	311.01–311.05	0.030–0.039
6.3	320.41–320.46	0.031–0.062
8	325.55–325.58	0.031–0.061
9	329.62–329.66	0.035–0.057
11	337.65–337.71	0.034–0.047
12	341.73–341.79	0.039–0.054
13	345.82–345.87	0.038–0.046
14	349.89–349.93	0.028–0.050

Allele	Mean	Standard Deviation
15	353.93–353.96	0.032–0.041
16	358.04–358.09	0.032–0.057
17	362.12–362.18	0.029–0.055
18	366.17–366.23	0.031–0.048
19	370.10–370.16	0.032–0.049
20	374.14–374.20	0.021–0.041
20.2	376.13–376.18	0.026–0.047
21	378.13–378.19	0.025–0.043
21.2	380.11–380.18	0.024–0.043
22.2	384.19–384.25	0.035–0.048
23.2	388.29–388.34	0.027–0.043
24.2	392.33–392.38	0.031–0.045
25.2	396.31–396.36	0.032–0.050
26.2	400.39–400.44	0.029–0.048
27.2	404.38–404.41	0.028–0.056
28.2	408.32–408.37	0.033–0.061
29.2	412.30–412.35	0.035–0.050
30.2	416.38–416.44	0.033–0.040
31.2	420.40–420.45	0.020–0.048
32.2	424.52–424.59	0.035–0.057
33.2	428.55–428.60	0.032–0.053
34.2	432.53–432.58	0.030–0.047
35	434.53–434.61	0.029–0.050
35.2	436.53–436.62	0.032–0.055
36	438.56–438.61	0.027–0.052
37	442.58–442.65	0.039–0.059
TH01		
4	179.32–179.35	0.032–0.050
5	183.33–183.39	0.024–0.041
6	187.37–187.42	0.034–0.050
7	191.40–191.45	0.029–0.042
8	195.42–195.46	0.031–0.043
9	199.45–199.49	0.031–0.052
9.3	202.45–202.49	0.030–0.043
10	203.36–203.41	0.028–0.045
11	207.31–207.34	0.021–0.043
13.3	218.29–218.33	0.027–0.049
vWA		
11	151.26–151.34	0.031–0.042

Allele	Mean	Standard Deviation
12	155.35–155.44	0.026–0.040
13	159.51–159.57	0.034–0.037
14	163.78–163.84	0.028–0.042
15	167.71–167.77	0.031–0.043
16	171.76–171.84	0.033–0.049
17	175.80–175.89	0.028–0.039
18	179.79–179.87	0.027–0.051
19	183.90–184.00	0.025–0.050
20	187.99–188.08	0.031–0.050
21	192.05–192.13	0.031–0.048
22	196.11–196.19	0.035–0.049
23	200.08–200.15	0.027–0.041
24	204.40–204.47	0.030–0.043

Extra peaks in the electropherogram

Causes of extra peaks

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

Stutter products

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

The proportion of the stutter product relative to the main allele (percent stutter) is measured by dividing the height of the stutter peak by the height of the main allele peak. Peak heights were measured for amplified samples (N = 1,080) at the loci used in the AmpFℓSTR® NGM SElect™ Kit. All data were generated on the Applied Biosystems® 3500xL Genetic Analyzer.

Some conclusions from these measurements and observations are:

- For each AmpFℓSTR® NGM SElect™ Kit locus, the percent stutter generally increases with allele length, as shown in Figures 6 to 11 on pages 103 to 106.
- Smaller alleles display a lower level of stutter relative to the longer alleles within each locus.
- Each allele within a locus displays a percent stutter that is consistent.

- Stutter filter sets in GeneMapper® *ID* and GeneMapper® *ID-X* Software, calculated as the mean stutter for the locus plus three standard deviations (N = 1,080), are shown in Table 5 on page 107. Peaks in the stutter position that are above the stutter filter percentage specified in the software are not filtered. Peaks in the stutter position that have not been filtered and remain labeled can be further evaluated. For evaluation of mixed samples, see Figure 22 on page 126.
- The measurement of percent stutter for allele peaks that are off-scale may be unusually high due to artificial truncation of the main allele peak.

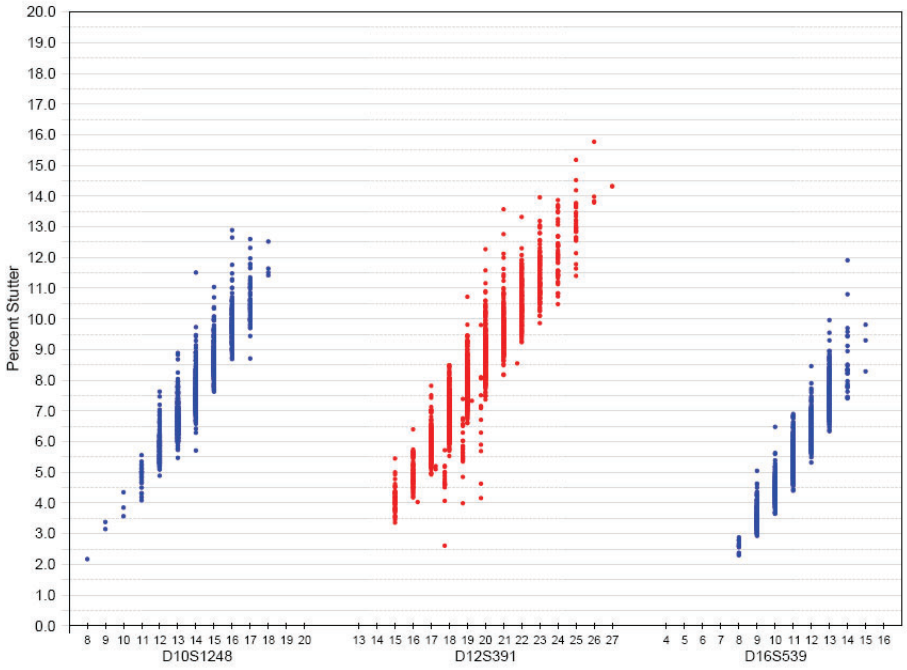

Figure 6 Stutter percentages for D10S1248, D12S391 and D16S539 loci. (Blue and red colors indicate loci labeled with FAM™ and PET® dyes, respectively.)

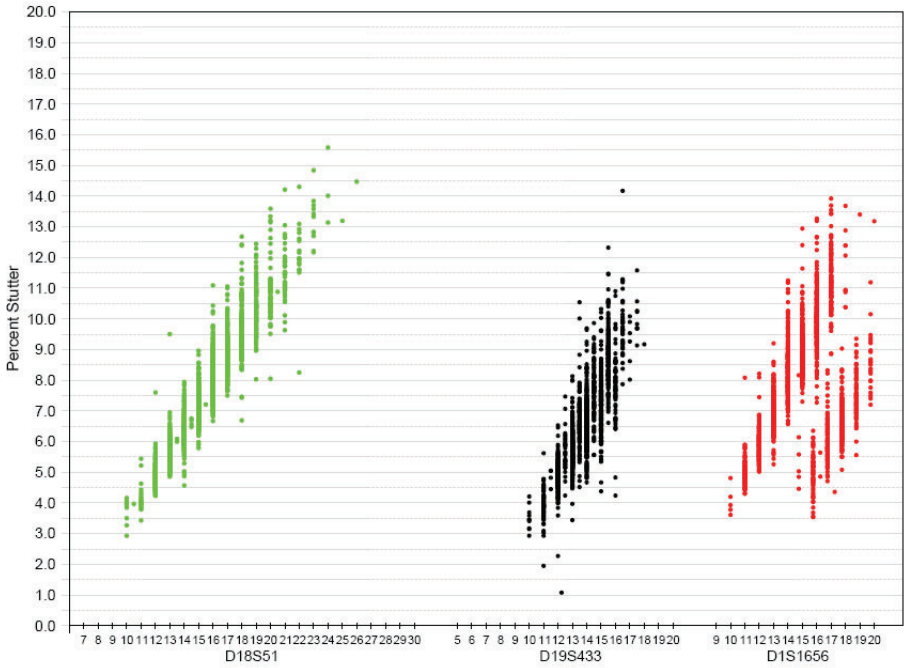

Figure 7 Stutter percentages for the D18S51, D19S433 and D1S1656 loci. (Green, black and red colors indicate loci labeled with VIC®, NED™ and PET® dyes, respectively.)

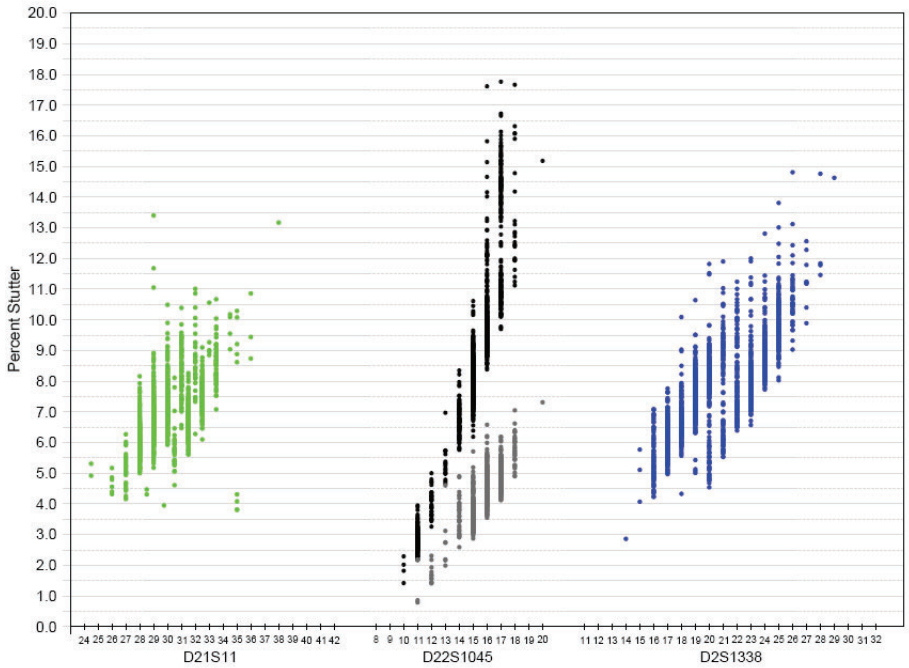

Figure 8 Stutter percentages for the D21S11, D22S1045 and D2S1338 loci. (Green, black/gray, and blue colors indicate loci labeled with VIC®, NED™ and FAM™ dyes, respectively. Black and gray data points associated with the D22S1045 locus indicate minus and plus stutter, respectively.)

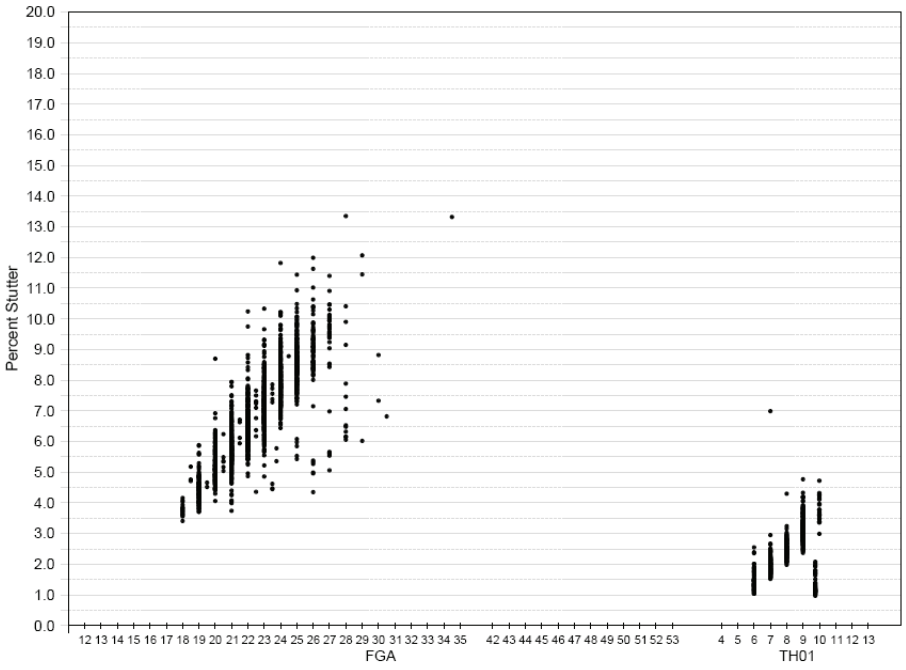

Figure 9 Stutter percentages for the FGA and TH01 loci. (Black data points indicate loci labeled with NED™ dye.)

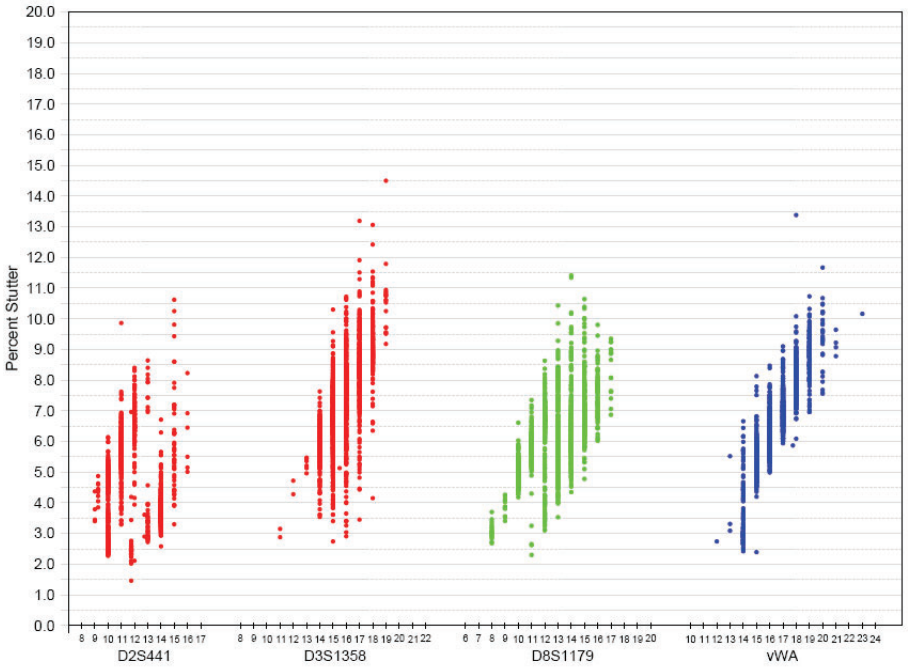

Figure 10 Stutter percentages for the D2S441, D3S1358, D8S1179, and vWA loci. (Red, green, and blue colors indicate loci labeled with and PET®, VIC®, and FAM™ dyes, respectively.)

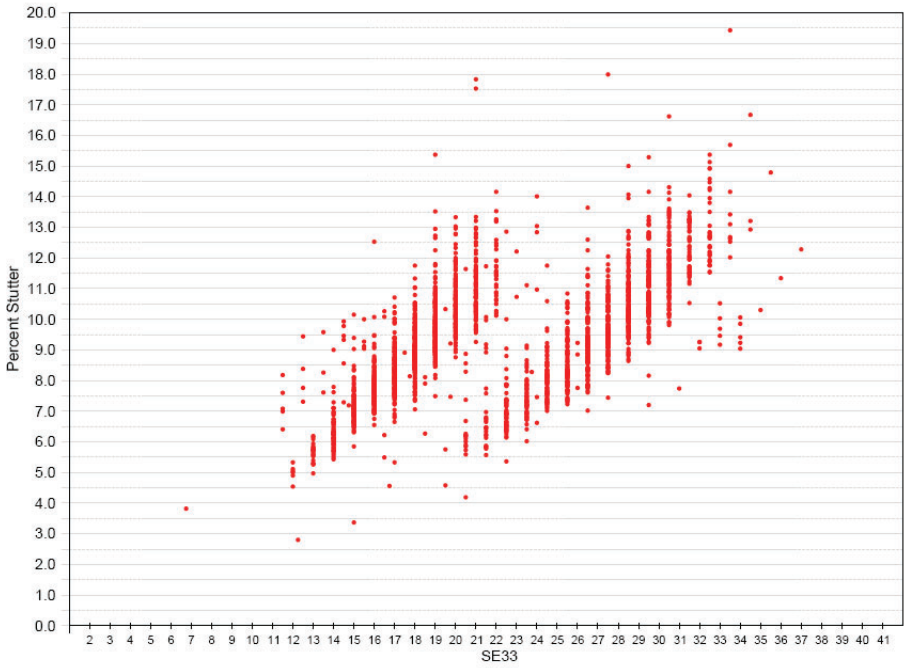

Figure 11 Stutter percentages for the SE33 locus. (Red data points indicate loci labeled with PET® dye.)

Table 5 Marker-specific stutter filter percentages for AmpFℓSTR® NGM SElect™ Kit loci

Locus‡	% Stutter
D10S1248	12.04
D12S391	14.77
D16S539	9.87
D18S51	13.81
D19S433	11.02
D1S1656	13.60
D1S1656 (– 2 nt)§	7.51
D21S11	10.43
D22S1045	17.14
D22S1045 (+3 nt)	6.65
D2S1338	12.32
D2S441	9.03
D3S1358	11.91
D8S1179	9.93
FGA	11.52
SE33	15.12
SE33 (– 2 nt)§	5.95
TH01	4.93
vWA	11.18

‡ These percentages are used as stutter filters in GeneMapper® *ID* v3.2.1 NGMSElect_panel_v1 and GeneMapper® *ID-X* NGMSElect_stutter_v1X.

§ The - 2nt stutter filters are not included in GeneMapper® *ID* NGMSElect_panel v1 due to functional limitations of the software.

IMPORTANT! The values shown are the values determined during developmental validation studies. We recommend that laboratories perform internal validation studies to determine the appropriate values to use.

Addition of 3′ A nucleotide

Many DNA polymerases can catalyze the addition of a single nucleotide (predominantly adenosine) to the 3′ ends of double-stranded PCR products (Clark, 1988; Magnuson et al., 1996). This nontemplate addition results in a PCR product that is one nucleotide longer than the actual target sequence. The PCR product with the extra nucleotide is referred to as the "+A" form.

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

- The primer sequences have been optimized to encourage +A addition.

- The new, highly robust PCR chemistry allows complete +A addition with a short final incubation at 60 °C for 10 minutes.

This final extension step gives the DNA polymerase additional time to complete +A addition to all double-stranded PCR products. See Figure 12 on page 108 for examples of incomplete and normal +A addition. Final extension incubation for longer than the recommended 10 minutes may result in double +A addition, in which *two* non-template adenosine residues are added to the PCR product. Double +A addition can cause "shoulders" on the right side of main allele peaks, and is therefore to be avoided.

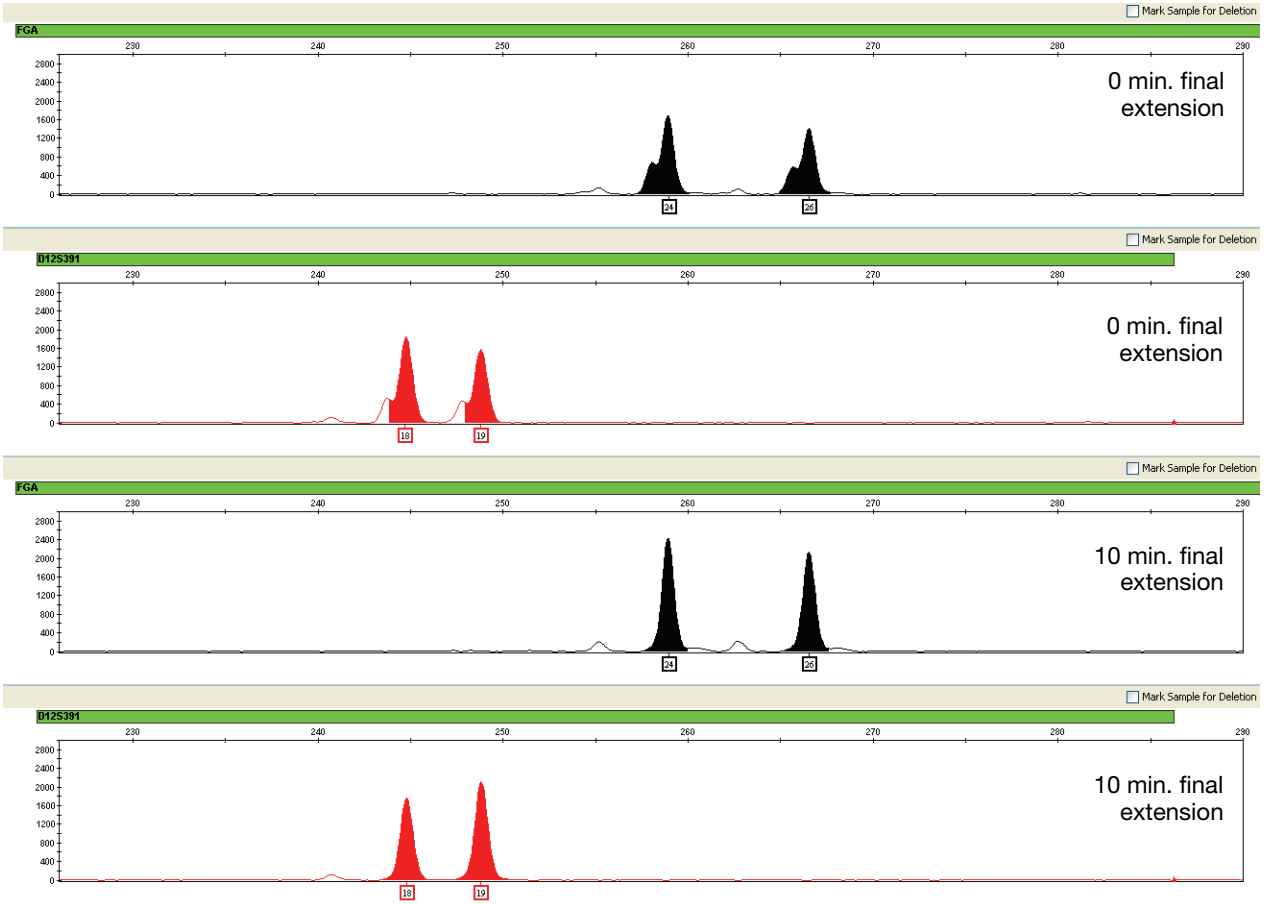

Figure 12 Omitting the final extension step results in shoulders on main allele peaks due to incomplete A nucleotide addition. Data are from an Applied Biosystems® 3130*xl* Genetic Analyzer using the AmpFℓSTR® NGM SElect™ Kit.

Due to improved PCR buffer chemistry, the lack of +A addition is generally less of an issue with the AmpFℓSTR® NGM SElect™ Kit than with earlier generation kits. However, "shouldering" of allele peaks may still be observed if the amount of input DNA is greater than that recommended by the NGM SElect™ kit protocol. Amplification of excess input DNA may also result in offscale data.

Artifacts

Artifacts and anomalies are seen in all molecular biological systems. Artifacts are typically reproducible while anomalies are non-reproducible, intermittent occurrences that are not observed consistently in a system (for example, spikes and baseline noise). Due to improvements in PCR primer manufacturing processes, the incidence of artifacts has been greatly reduced in the AmpFℓSTR® NGM SElect™ Kit. Kit electropherograms are essentially free of reproducible dye artifacts within the kit's read region of 68 – 45 nt. Figure 13 on page 110 shows the very low baseline level fluorescence of a typical negative control PCR using the AmpFℓSTR® NGM SElect™ Kit.

Most STR loci produce minus-stutter peaks as a byproduct of PCR amplification. A process of "slippage" has been proposed as a molecular mechanism for stutter, where the Taq DNA polymerase enzyme "slips" on the template DNA during replication and produces a minority PCR product that is shorter than the template strand, usually by one repeat unit. The stutter process may also occur in the opposite direction to produce amplicon DNA that is usually one repeat unit longer than the template strand, termed plus-stutter. While plus-stutter is normally much less significant than minus-stutter in STR loci with tetranucleotide repeats, the incidence of plus-stutter may be more significant in trinucleotide repeat-containing loci. The D22S1045 locus in the NGM SElect™ kit is a trinucleotide repeat locus, and shows an elevated level of plus-stutter. For example, Figure 15 on page 112 is an electropherogram of the D22S1045 locus showing plus stutter. GeneMapper® *ID* and GeneMapper® *ID-X* analysis files supplied for use with the NGM SElect™ kit contain a plus-stutter filter to prevent these peaks from being called in normal profiles.

Figure 14 on page 111 shows an example of a non-standard (minus 2-nt) stutter that may be observed in certain STR loci such as SE33 and D1S1656 that include more complex nucleotide sequences including regions of dinucleotide repeats. In cases where these stutter peaks exceed the peak amplitude threshold (e.g., 50 RFU), they may be detected by analysis software as additional alleles in the profile.

It is important to consider possible noise and artifacts when interpreting data from the AmpFℓSTR® NGM SElect™ Kit on the Applied Biosystems® 3500/3500xL, Applied Biosystems® 3130/3130*xl*, and ABI PRISM® 3100/3100-*Avant* Genetic Analyzers. Note that a high degree of magnification is used in the sample electropherograms shown in Figures 13 to 15 on pages 110 to 112.

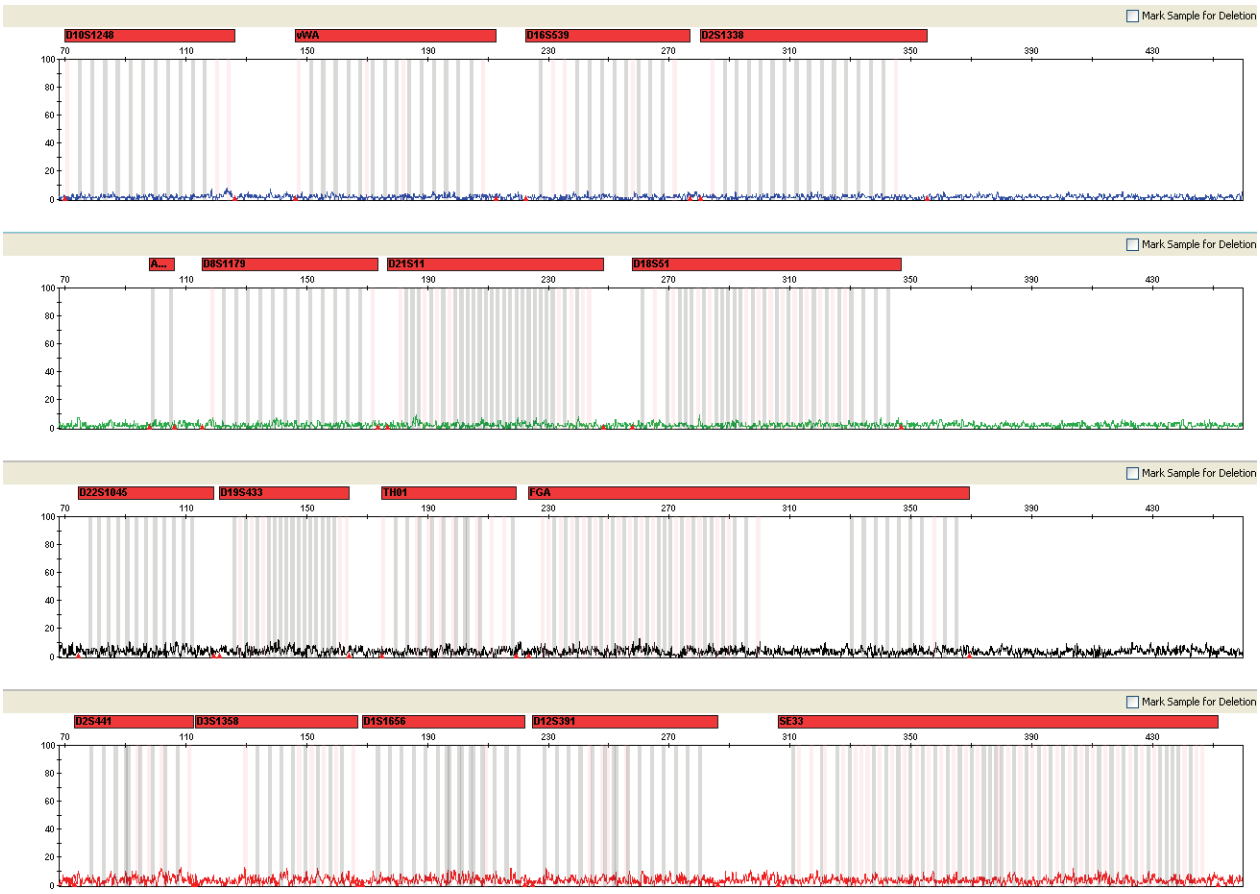

Figure 13 Examples of fluorescence background in data produced on an Applied Biosystems® 3130*xl* Genetic Analyzer, (Y-axis scale 0–100 RFU).

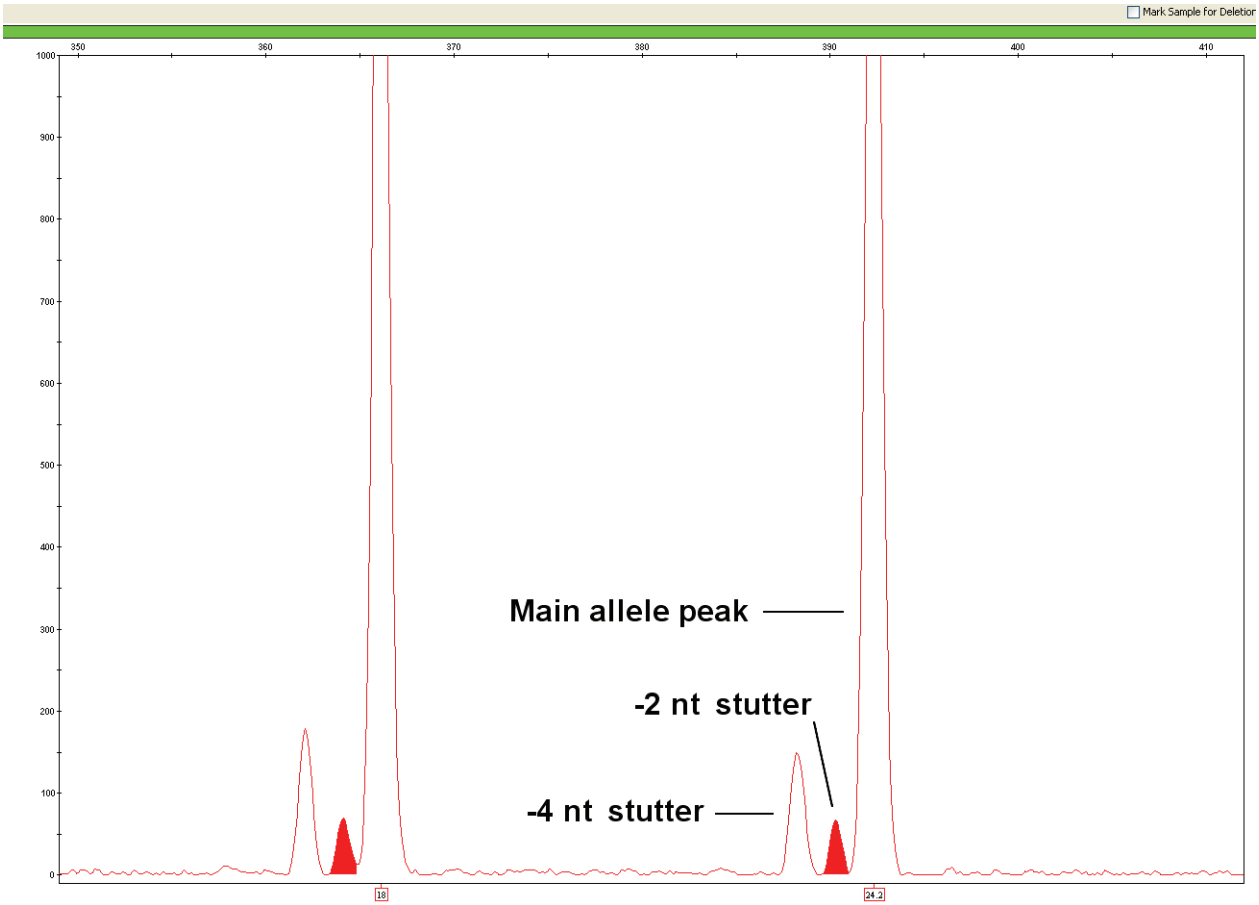

Figure 14 Example of a – 2 nt reproducible artifact at the SE33 locus. Data produced on an Applied Biosystems® 3130*xl* Genetic Analyzer.

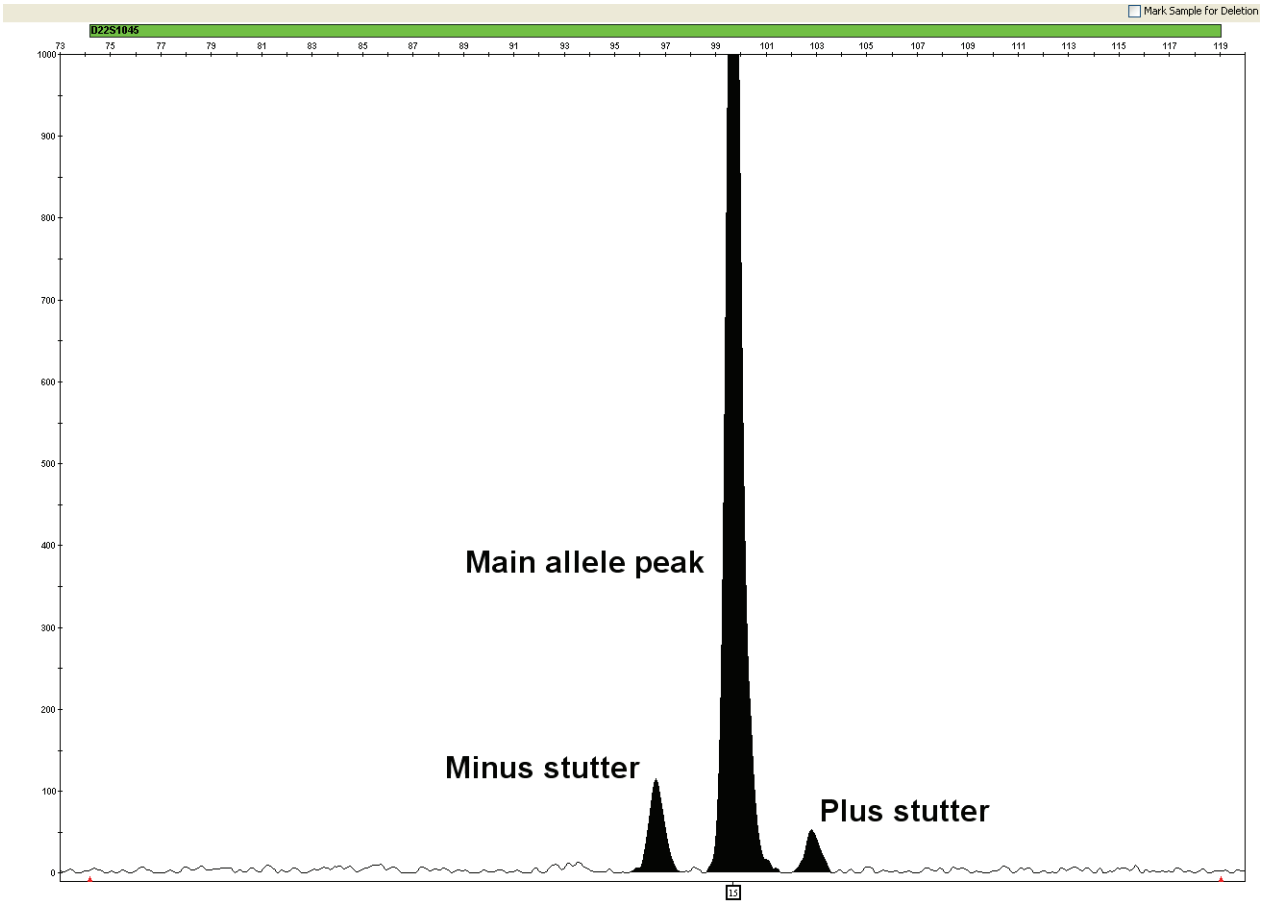

Figure 15 NGM SElect™ kit electropherogram showing plus stutter associated with the D22S1045 STR locus. Data produced on an Applied Biosystems® 3130*xl* Genetic Analyzer.

Characterization of loci

SWGDAM guideline 2.1

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

This section describes basic characteristics of the 15 loci and the sex-determining marker, Amelogenin, which are amplified with the AmpFℓSTR® NGM SElect™ Kit. Most of these loci have been extensively characterized by other laboratories.

Nature of the polymorphisms

The primers for the Amelogenin locus flank a 6-nucleotide deletion within intron 1 of the X homolog. Amplification results in 104-nt and 110-nt products from the X and Y chromosomes, respectively. (Sizes are the actual nucleotide size according to sequencing results, including 3′ A nucleotide addition, and size may not correspond exactly to allele mobility observed on capillary electrophoresis platforms.) With the sole exception of D22S1045, a trinucleotide STR, the remaining AmpFℓSTR® NGM SElect™ Kit loci are tetranucleotide short tandem repeat (STR) loci. The length differences among alleles of a particular locus result from differences in the number of repeat units.

All the alleles in the AmpFℓSTR® NGM SElect™ Kit Allelic Ladder, including microvariants, have been subjected to sequencing. In addition, other groups in the scientific community have sequenced alleles at some of these loci (Nakahori et al., 1991; Puers et al., 1993; Möller et al., 1994; Barber et al., 1995; Möller and Brinkmann, 1995; Barber et al., 1996; Barber and Parkin, 1996; Brinkmann et al., 1998; Momhinweg et al., 1998; Watson et al., 1998). Among the various sources of sequence data on the AmpFℓSTR® NGM SElect™ Kit loci, there is consensus on the repeat patterns and structure of the STRs.

Inheritance

The Centre d'Etude du Polymorphisme Humain (CEPH) has collected DNA from families of Utah Mormon, French Venezuelan, and Amish descent. These DNA sets have been extensively studied all over the world and are routinely used to characterize the mode of inheritance of various DNA loci. Each family set contains three generations, generally including four grandparents, two parents, and several offspring. Consequently, the CEPH family DNA sets are ideal for studying inheritance patterns (Begovich et al., 1992). Observations from the analysis of three CEPH family genomic DNA sets are noted in "Estimating germ-line mutations" on page 129.

Mapping

The AmpFℓSTR® NGM SElect™ Kit loci have been mapped, and the chromosomal locations have been published (Nakahori et al., 1991; Edwards et al., 1992; Kimpton et al., 1992; Mills et al., 1992; Sharma and Litt, 1992; Li et al., 1993; Straub et al., 1993; Barber and Parkin, 1996).

Genetic linkage

Two sets of STR loci in the AmpFℓSTR® NGM SElect™ Kit are located on the same chromosomes. vWA and D12S391 are located approximately 6.3 million bp apart on the p arm of chromosome 12, while D2S1338 and D2S441 are located approximately 150 million bp apart on opposite arms of chromosome 2. Linkage disequilibrium analysis was conducted on the genotype results from 1,034 individuals of three

ethnic groups (350 African-American, 349 Caucasian, and 335 Hispanic). STR locus genotype results from the population study were analyzed using the Linkage Disequilibrium module of GenePop software version 4.0.10 (Raymond and Rousset, 1995; Rousset, 2008). See Table 6 for results.

The relatively high probability values indicate that there is no statistically significant linkage disequilibrium found between the pairs of loci located on the same chromosome.

An independent analysis of data from the same collection of population samples (Budowle, et al., 2010) also concluded that the 15 STR loci shared between the NGM™ and NGM SElect™ kits were independent at the population level (note that the SE33 locus was not part of this analysis). Therefore, to calculate the rarity of a profile for comparison to single-source and mixture samples, the frequencies of all loci including vWA and D12S391 could be multiplied. However, the analysis of the CEPH pedigree families demonstrated a degree of linkage between vWA and D12S391 that does not support the assumption of independence for the purpose of kindship analysis.

Table 6 GenePop LD Result (p value for pairwise analysis of loci)

Locus	Chromosome Map Position‡	Chromosome Nuclear Coordinates‡ (million bp)	African-American (N = 350)	Caucasian (N = 350)	Hispanic (N = 293)
vWA	p13.31	5.9	0.86	0.29	0.27
D12S391	p13.2	12.2			
D2S441	p14	68	0.11	0.32	0.19
D2S1338	q35	218			

‡ STR locus mapping data was obtained from the NCBI Map Viewer **http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=9606** or the UCSC Genome Browser (**http://genome.ucsc.edu/**). GenePop LD analysis probability results (p values) greater than 0.05 were considered to indicate that linkage disequilibrium between the loci within the population tested was not statistically significant.

Species specificity

SWGDAM Guideline 2.2

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

The AmpFℓSTR® NGM SElect™ Kit provides the required specificity for detecting human alleles.

Nonhuman studies

Nonhuman DNA may be present in forensic casework samples. The following species were tested (in the specified amounts) using standard PCR and capillary electrophoresis conditions for the AmpFℓSTR® NGM SElect™ Kit.

- Primates: gorilla, chimpanzee, and macaque (1.0 ng each)
- Non-primates: mouse, dog, sheep, pig, rabbit, cat, horse, hamster, rat, chicken, and cow (10.0 ng each)
- Microorganisms: Candida albicans, Staphylococcus aureus, Escherichia coli, Neisseria gonorrhoeae, Bacillus subtilis, and Lactobacillus rhamnosus (equivalent to 105 copies)

Results were assessed for the presence of any amplified peaks that would indicate cross reactivity of the NGM SElect™ kit with any of these non-human species.

Figure 16 on page 116 shows example electropherogram results from the species specificity tests. The chimpanzee and gorilla DNA samples produced partial profiles within the 70 to 350 nucleotide region (gorilla data not shown). Macaque DNA produced a strong Amelogenin X peak and two small PET® dye peaks at 315 and 339 bp.

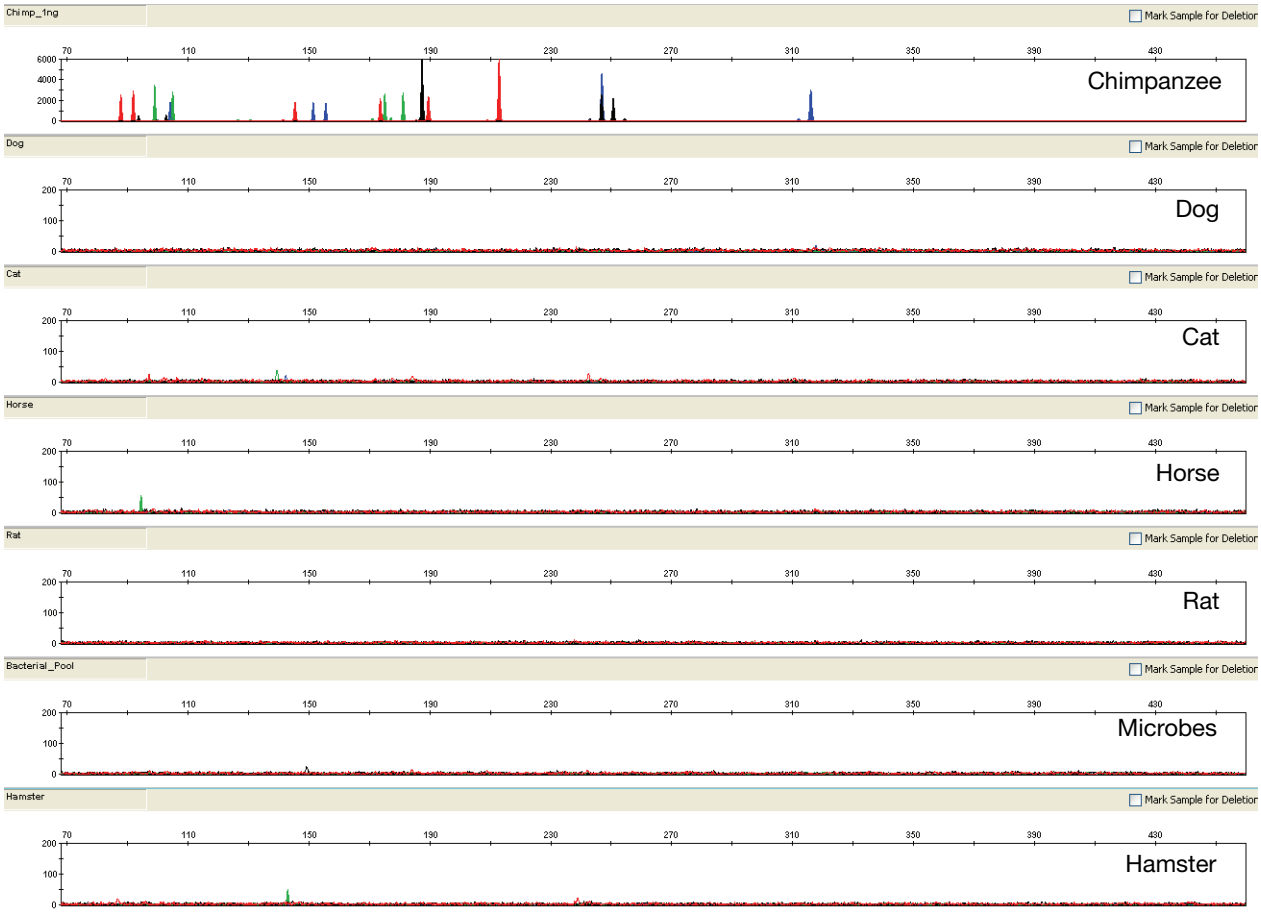

Figure 16 Representative electropherograms for some species tested in a species specificity study including positive and non-template controls (NTC)

Among the non-primate species, most produced no peaks over the 50 RFU threshold. Horse yielded reproducible VIC® dye peaks at 94 bp (less than 100 RFU) and individual replicate PCRs of cat, hamster and pig yielded single, small (less than 100 RFU), non-reproducible peaks.

Sensitivity

SWGDAM guideline 2.3

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

Importance of quantification

The recommended amount of input DNA for the AmpFℓSTR® NGM SElect™ Kit is 1.0 ng for 29-cycle PCR and 0.5 ng for 30-cycle PCR based on quantification using either the Quantifiler® Human or Quantifiler® Duo Quantification kit; individual laboratories should determine the optimum input DNA amount according to the quantification method in use in the laboratory. If the sample contains degraded or inhibited DNA, amplification of a higher concentration of DNA may be beneficial. In Figure 17 on page 118, the control DNA 007 was serially diluted from 1.0 ng to 0.031 ng. Full profiles (34 PCR products) were consistently obtained at 0.125 ng, but occasional partial profiles resulted at lower concentrations. Sensitivity at 30 PCR cycles was greater than at 29 cycles.

Effect of DNA quantity on results

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

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

To address these issues, reamplify the sample using less DNA.

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

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

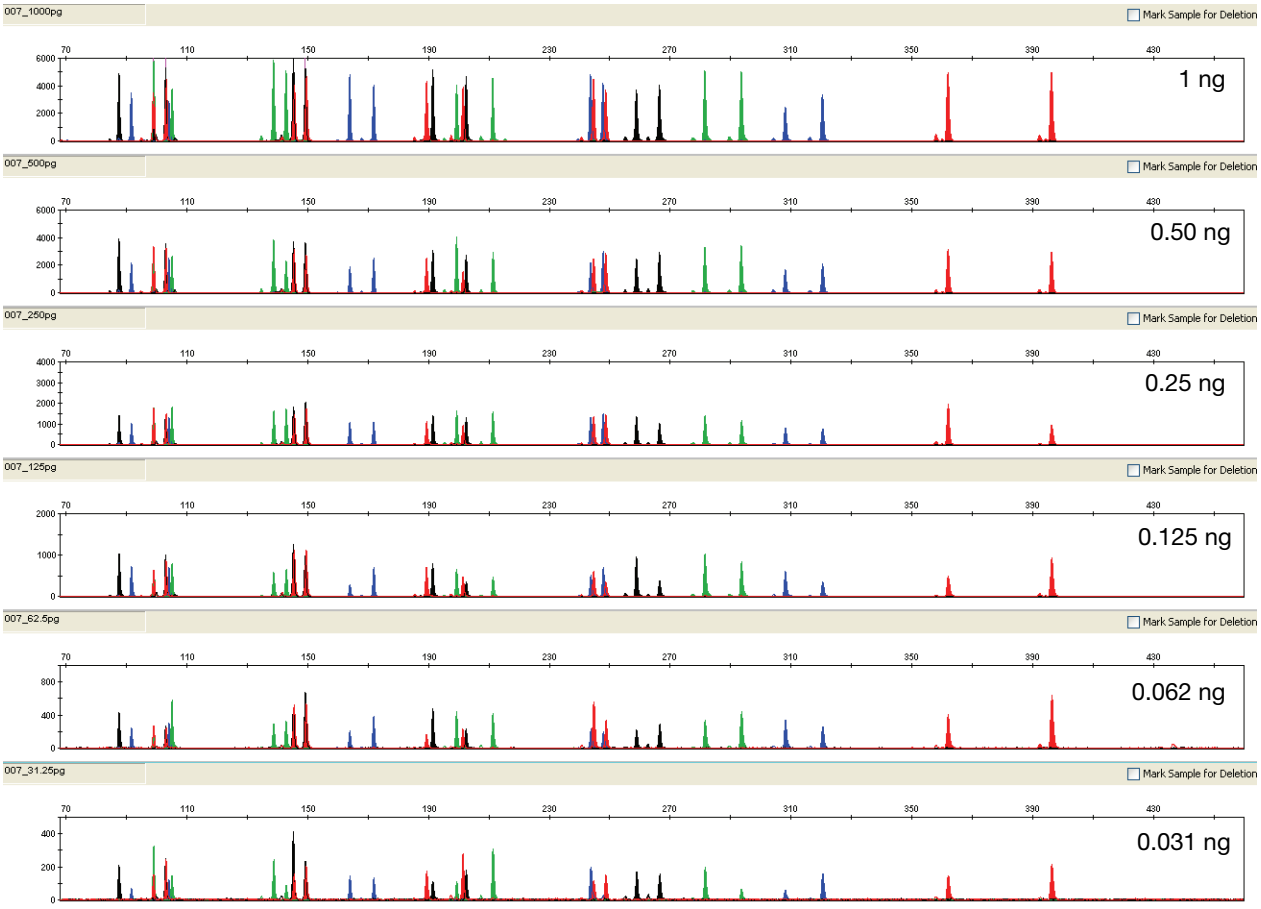

Figure 17 Electropherograms for amplifications using 1 ng, 0.50 ng, 0.25 ng, 0.125 ng, 0.062 ng, and 0.031 ng of control DNA 007. Electrophoresis was performed on an Applied Biosystems® 3130*xl* Genetic Analyzer. Note that the Y-axis scale is magnified for the smaller input amounts of DNA.

Stability

SWGDAM guideline 2.4

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

Degraded DNA

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

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

Amplification of 0.5 ng of degraded DNA using the AmpFℓSTR® NGM SElect™ Kit was performed. As the DNA became progressively degraded, the loci failed to amplify robustly in order of decreasing size. Preferential amplification was not observed.

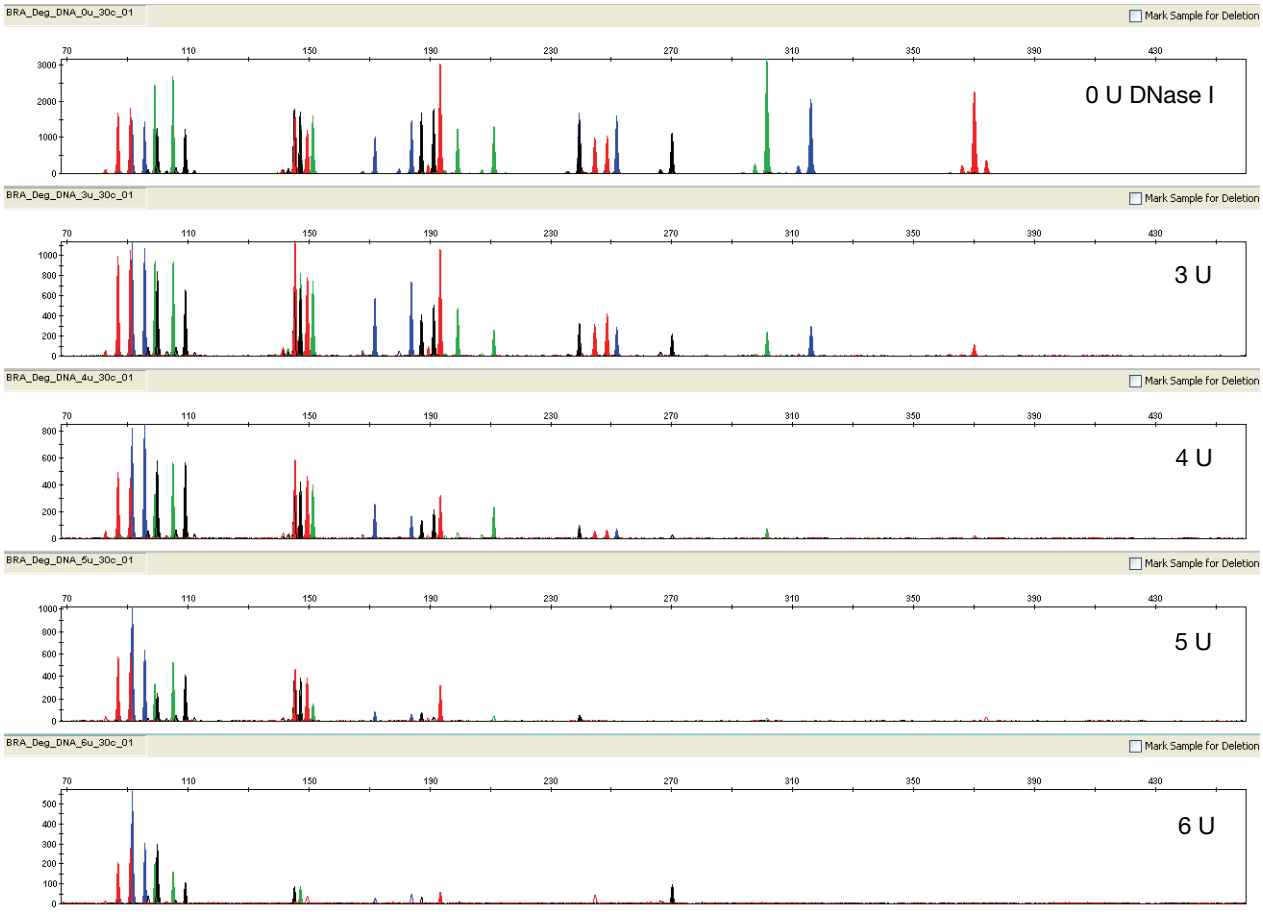

Figure 18 Amplification of Raji DNA samples sonicated and incubated with increasing doses of DNase I. Panels 1, 2, 3, 4, and 5 correspond to 0, 3, 4, 5, and 6 units of DNase I. The same set of samples were analyzed with both 29 and 30 PCR cycles (the results shown are from the 30-cycle amplification). Note that the Y-axis scale is magnified for more degraded samples, which generate lower peak heights.

Effect of inhibitors — hematin

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

To examine the effects of hematin on the performance of the AmpFℓSTR® NGM SElect™ Kit, 0.5 ng of control DNA 007 was amplified in the presence of increasing concentrations of hematin for 29 and 30 cycles of amplification (Figure 19 on page 121).The concentrations of hematin used were 0 µM, 50 µM, 150 µM, and 300 µM (see Table 7 on page 121).

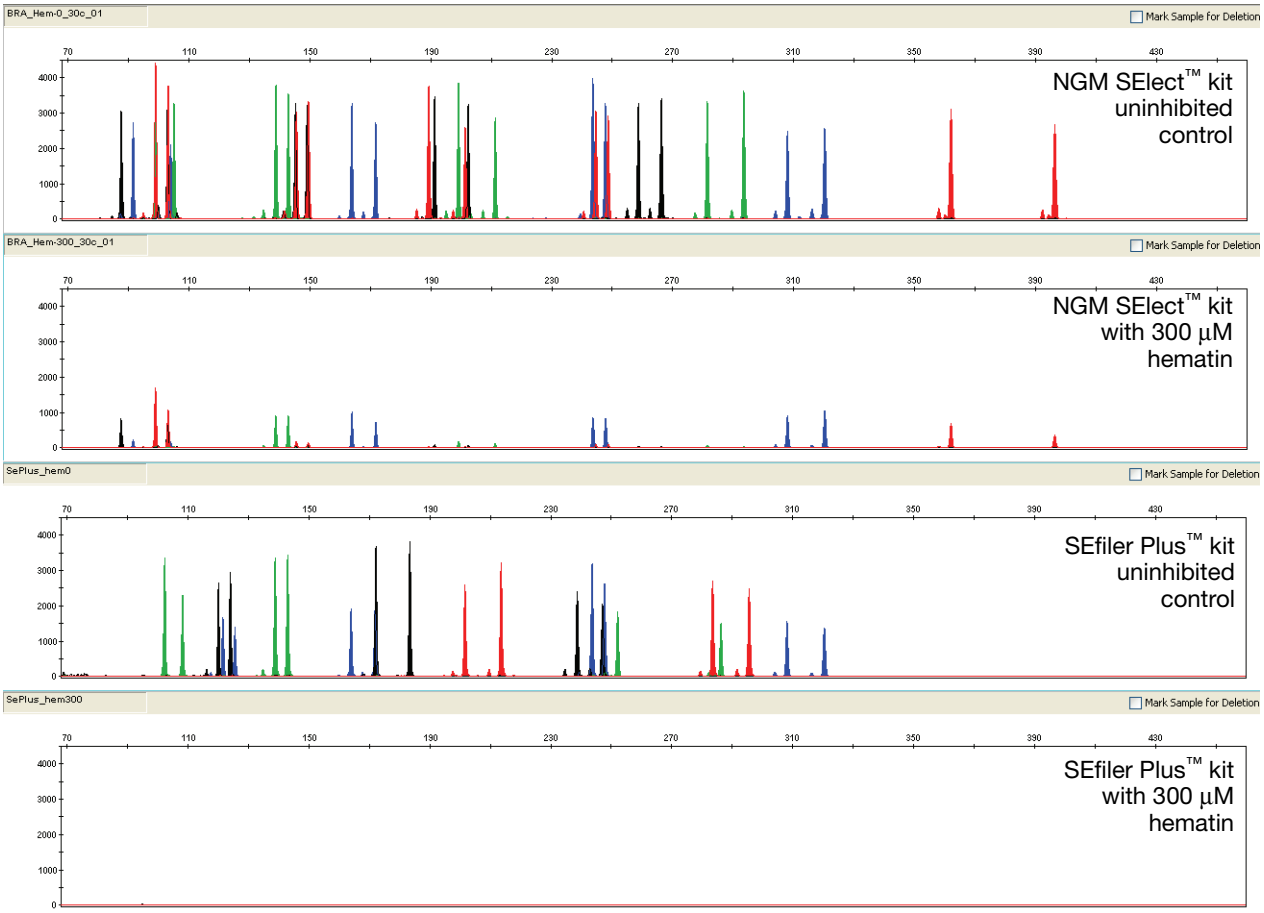

Figure 19 Electropherograms for the AmpFℓSTR® NGM SElect™ and SEfiler Plus™ kits show the improved performance of the NGM SElect™ kit in the presence of hematin compared with previous AmpFℓSTR® kits. The same set of inhibited samples was analyzed with the NGM SElect™ kit for both 29 and 30 PCR cycles of amplification (results shown for both kits are from the 30-cycle amplification).

Table 7 NGM SElect™ kit performance in simulated hematin inhibition (N = 3)

Hematin Concentration (µM)	Number of Alleles Detected‡
0	34, 34, 34
50	34, 34, 34
150	34, 34, 34
300	24, 29, 22

‡ Only those peaks >50 RFU were counted. A complete profile with control DNA 007 yields 34 peaks using the AmpFℓSTR® NGM SElect™ Kit.

Effect of inhibitors — humic acid

Traces of humic acid may inhibit the PCR amplification of DNA evidence collected from soil. Amplification of 1 ng of control DNA 007 in the presence of increasing amounts of humic acid was performed using the AmpFℓSTR® NGM SElect™ Kit for 29 and 30 cycles of amplification (see Figure 20). The concentrations of humic acid tested were 0, 25, 50, and 100 ng/µL (see Table 8 on page 122).

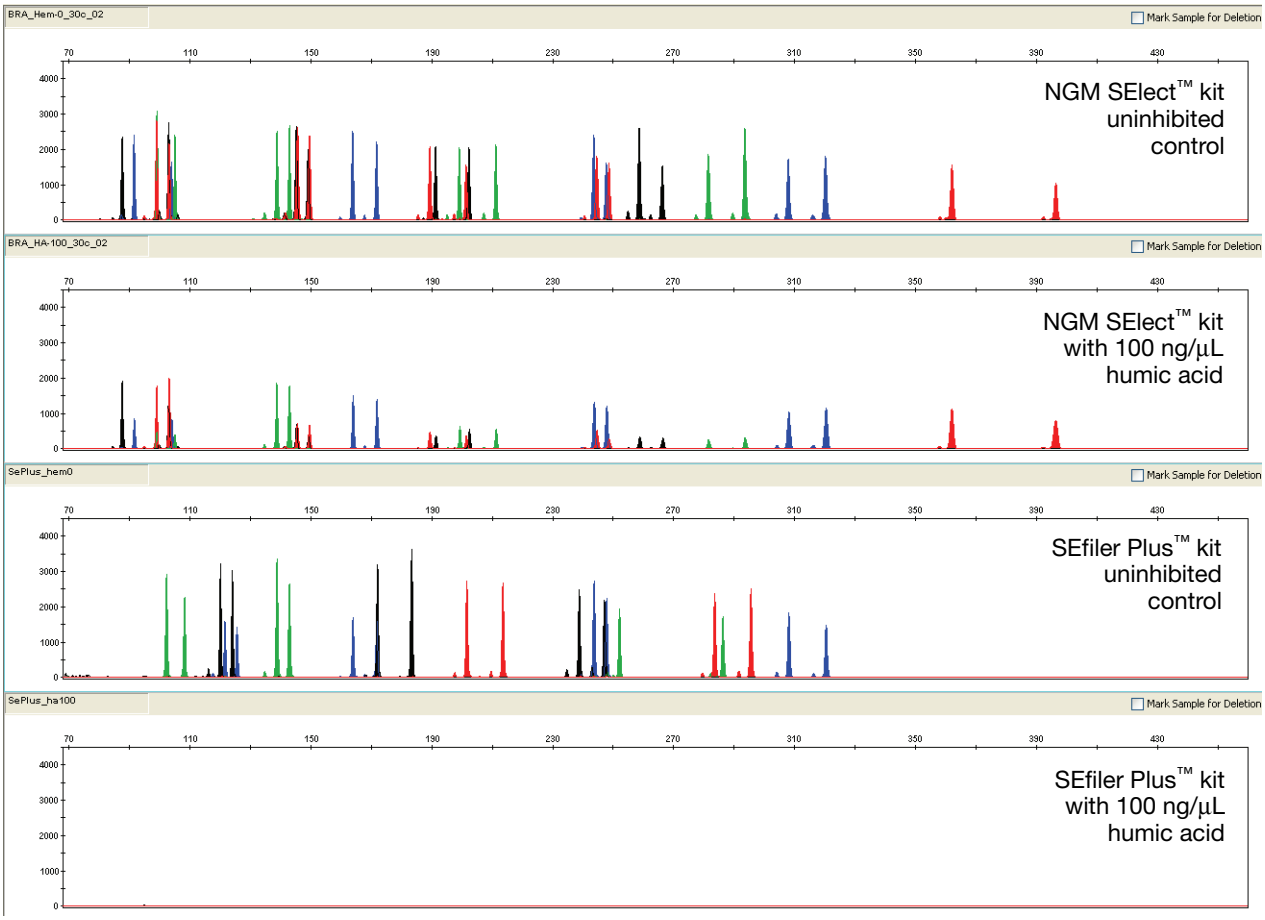

Figure 20 Electropherograms for the AmpFℓSTR® NGM SElect™ and SEfiler Plus™ kits show improved performance of the NGM SElect™ kit in the presence of humic acid compared to previous AmpFℓSTR® kits. The same set of inhibited samples was analyzed with the NGM SElect™ kit for both 29 and 30 PCR cycles of amplification (results shown for both kits are from the 30-cycle amplification).

Table 8 NGM SElect™ kit performance in simulated model of humic acid inhibition (n = 3)

Humic Acid Concentration (ng/µL)	Number of Alleles Detected‡
0	34, 34, 34
25	34, 34, 34
50	34, 34, 34
100	34, 34, 34

‡ Only those peaks >50 RFU were counted. A complete profile with control DNA 007 yields 34 peaks using the AmpFℓSTR® NGM SElect™ Kit.

Mixture studies

SWGDAM guideline 2.8

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

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

Mixture Studies

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

- The presence of more than two alleles at a locus
- The presence of a peak at a stutter position that is significantly greater in percentage than typically observed in a single-source sample
- Significantly imbalanced alleles for a heterozygous genotype

The peak height ratio is defined as the height of the lower peak (in RFU) divided by the height of the higher peak (in RFU), expressed as a percentage. Mean, median, minimum, and maximum peak height ratios observed for alleles in the AmpFℓSTR® NGM SElect™ Kit loci in unmixed population database samples are shown in Figure 21 on page 124.

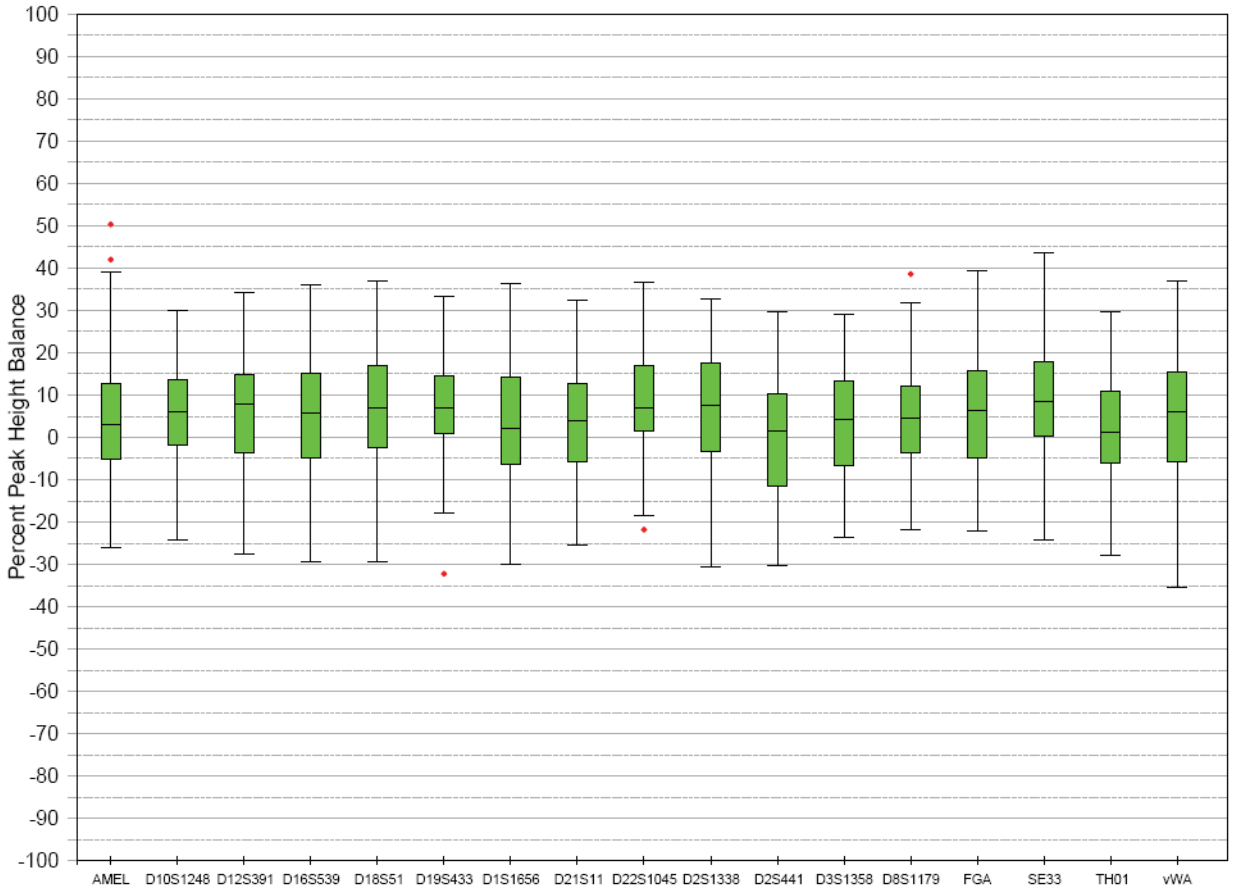

Figure 21 Heterozygote ratios for 1 ng of input DNA. The distribution of intra-locus peak height ratios are expressed as plus and minus percent, by locus. Green boxes show the middle 50% or interquartile range (IQR). Box halves below and above median show the second and third quartile, respectively. "Whiskers" indicate 1.5 IQR from the upper and lower margins of the IQR. Red diamonds are outlier data points more than 1.5 IQR from the median.

If an unusually low peak height ratio is observed for one locus, and there are no other indications that the sample is a mixture, the sample may be reamplified and reanalyzed to determine if the imbalance is reproducible. Possible causes of imbalance at a locus are:

- Degraded DNA
- Presence of inhibitors
- Extremely low amounts of input DNA
- A mutation in one of the primer binding sites
- Presence of an allele containing a rare sequence that does not amplify as efficiently as the other allele

Resolution of genotypes in mixed samples

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

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

Specific genotype combinations and input DNA ratios of the samples contained in a mixture determine whether or not it is possible to resolve the genotypes of the major and minor component(s) at a single locus.

The ability to obtain and compare quantitative values for the different allele peak heights on Applied Biosystems® instruments provides additional valuable data to aid in resolving mixed genotypes.

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

Limit of detection of the minor component

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

The results of the mixed DNA samples are shown in Figure 22 on page 126 where samples A and B were mixed according to the ratios indicated. The minor component allele calls at non-overlapping loci are highlighted. Detection of full profiles for the minor contributor was possible at ratios of 3:1 (0.750:0.250 ng) and 7:1 (0.875:0.125 ng). Generally, 15:1 ratios resulted in partial profiles for the minor component. The profiles of these samples are described in Table 9 on page 127.

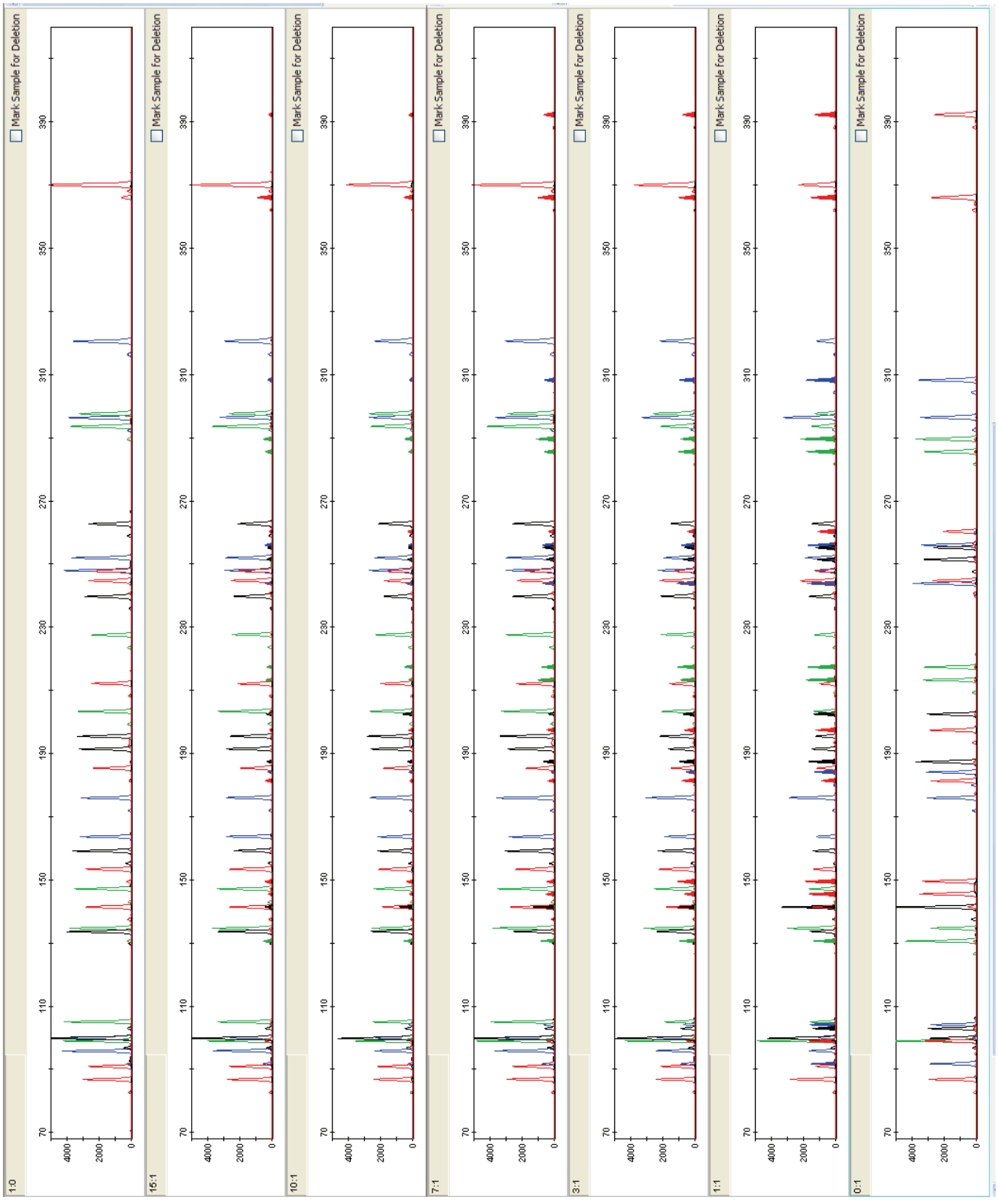

Figure 22 Amplification of DNA mixtures at various ratios. Panels show electropherograms for (top to bottom) 1:0 (major contributor only), 15:1 mixture, 10:1 mixture, 7:1 mixture, 3:1 mixture, 1:1 mixture, and 0:1 (minor contributor only).

Table 9 Genotypes of mixed DNA samples

Locus	Sample A Genotype	Sample B Genotype
D10S1248	13, 14	12, 15
vWA	14, 17	17, 19
D16S539	10, 11	9, 12
D2S1338	17, 23	17, 20
AMEL	X, Y	X
D8S1179	11,14	10,11
D21S11	29, 35	31.2, 32.2
D18S51	15, 16	13, 14
D22S1045	15	15, 16
D19S433	11, 17.2	13
TH01	7, 8	6, 9.3
FGA	19, 25	22, 23
D2S441	11, 12	11, 14
D3S1358	14, 17	15, 16
D1S1656	12, 18.3	11, 15
D12S391	18, 18.3	18, 22
SE33	19	18, 24.2

Population data

SWGDAM guideline 2.7

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

Overview

The AmpFℓSTR® NGM SElect™ PCR Amplification Kit contains loci for which extensive population data are available. For additional information on 11 loci shared between the kits, see the population data and additional studies section of the *AmpFℓSTR® SGM Plus® PCR Amplification Kit User's Manual* (PN 44309589).

Population samples used in these studies

The AmpFℓSTR® NGM SElect™ PCR Amplification Kit was used to generate the population data provided in this section. Whole blood samples, provided by the Interstate Blood Bank (Memphis, Tennessee) and Boca Biolistics (Coconut Creek, Florida), were collected in the United States (with no geographical preference) from randomly-selected individuals of known ethnicities. Ethnicities of sample donors were:

- African-American – 344 samples
- Caucasian – 346 samples
- Hispanic – 390 samples

DNA was extracted using an ABI PRISM® 6100 Nucleic Acid PrepStation.

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

Mutation rate

Estimating germ-line mutations

Estimation of spontaneous or induced germ-line mutation at genetic loci can be achieved by comparing the genotypes of offspring to those of their parents. From such comparisons the number of observed mutations are counted directly.

Genotyping analysis of three CEPH families (1333, 1340, and 1345) was conducted using the NGM SElect™ kit as part of the developmental validation process. These three-generation families, comprising 58 meioses altogether, showed three cases of apparent STR allele repeat number mutations, one in each family. Family 1333 had a mutation at the D12S391 locus that resulted in a change of allele 25 in a grandmother (individual 7341) to allele 24 in her daughter (individual 6987). Family 1340 had a mutation at the D8S1179 locus that resulted in a change of allele 10 in a grandfather (individual 6994) to allele 11 in his son (individual 7029). Family 1345 had a mutation at the SE33 locus that resulted in a change of allele 16 in a mother (individual 7348) to allele 15 in one of her sons (individual 7352). In all cases, the apparent mutation events resulted in either a gain or loss of a single repeat unit.

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

Additional mutation studies

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

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

Probability of identity

Table 10 shows the allele frequencies at AmpFℓSTR® NGM SElect™ Kit loci by population group. Table 11 shows the Probability of identity (P_I) values of the AmpFℓSTR® NGM SElect™ Kit loci individually and combined. The P_I value is the probability that two individuals selected at random will have an identical AmpFℓSTR® NGM SElect™ Kit genotype (Sensabaugh, 1982). The P_I values for the populations described in this section are then 8.12×10^{-22} (African-American), 2.35×10^{-21} (U.S. Caucasian) and 3.16×10^{-21} (U.S. Hispanic).

Table 10 Allele frequencies by population group for AmpFℓSTR® NGM SElect™ Kit STR loci. (Dashes indicate alleles not detected or, where values appear in parentheses, alleles not detected in significant quantities.)

Allele	African American (N = 344)	Caucasian (N = 346)	Hispanic (N = 390)
D10S1248			
6	-- (0.15)	--	--
7	-- (0.15)	--	--
8	--	--	-- (0.13)
9	-- (0.15)	--	-- (0.13)
10	-- (0.15)	--	-- (0.13)
11	3.63	-- (0.58)	-- (0.26)
12	13.95	3.47	4.23
13	22.67	29.05	25.51
14	28.2	29.77	36.03
15	18.6	19.65	22.95
16	9.88	13.44	8.08
17	2.18	3.76	2.56
18	-- (0.29)	-- (0.29)	--
19	--	--	--
20	--	--	--
D12S391			
13	--	--	-- (0.13)
14	--	--	-- (0.13)
15	7.12	4.19	3.97
15.1	-- (0.15)	--	--
16	5.09	3.47	5.13
16.1	-- (0.15)	--	--
17	15.7	10.55	7.31
17.1	-- (0.44)	--	-- (0.26)
17.3	-- (0.58)	2.02	1.15
18	25.73	16.18	20

Allele *(continued)*	African American (N = 344)	Caucasian (N = 346)	Hispanic (N = 390)
18.3	1.16	2.17	2.05
19	14.1	12.28	18.59
19.1	-- (0.58)	--	--
19.3	-- (0.29)	-- (0.58)	1.15
20	11.92	9.83	17.31
20.3	--	--	--
21	6.98	13.73	8.72
21.3	-- (0.15)	-- (0.14)	--
22	5.09	10.69	6.92
23	3.34	8.09	3.72
24	-- (0.58)	3.61	1.79
25	0.87	2.02	1.28
26	--	-- (0.29)	-- (0.26)
27	--	-- (0.14)	-- (0.13)
28	--	--	--
D16S539			
5	--	--	--
6	--	--	-- (0.13)
7	--	--	--
8	3.49	1.45	1.92
9	22.24	12.43	10.38
10	11.63	4.05	15.77
11	29.07	32.23	31.92
12	19.33	30.78	24.49
12.2	--	--	--
13	12.94	17.2	13.97
14	1.31	1.73	1.15
15	--	-- (0.14)	-- (0.26)
16	--	--	--
D18S51			
7	--	--	--
8	--	--	--
9	--	--	-- (0.13)
9.2	--	--	--
10	-- (0.29)	1.16	0.64
10.2	-- (0.15)	--	--
11	-- (0.44)	0.87	1.15
11.2	--	--	--

Allele *(continued)*	African American (N = 344)	Caucasian (N = 346)	Hispanic (N = 390)
12	6.25	14.74	10.77
12.2	--	--	--
13	4.07	11.85	11.54
13.2	-- (0.29)	--	--
14	5.81	17.49	15.77
14.2	-- (0.44)	--	-- (0.13)
15	17.3	15.32	12.31
15.2	--	--	-- (0.13)
16	18.31	11.85	12.82
16.2	--	--	--
17	15.7	10.98	17.05
17.2	--	--	--
18	14.1	8.53	8.46
18.2	--	--	--
19	9.16	4.34	3.46
19.2	--	--	--
20	4.22	1.3	1.79
20.2	-- (0.15)	--	--
21	2.03	1.01	2.31
21.2	--	--	--
22	1.02	-- (0.29)	0.64
22.2	--	--	--
23	-- (0.29)	-- (0.29)	-- (0.51)
23.2	--	--	--
24	--	--	-- (0.26)
25	--	--	-- (0.13)
26	--	--	--
27	--	--	--
D19S433			
9	-- (0.29)	--	--
9.2	--	--	--
10	1.16	-- (0.14)	-- (0.38)
10.2	-- (0.15)	--	--
11	9.74	--	1.54
11.2	-- (0.29)	--	-- (0.26)
12	10.76	7.23	8.46
12.1	--	-- (0.14)	--
12.2	3.78	-- (0.14)	1.41

Allele *(continued)*	African American (N = 344)	Caucasian (N = 346)	Hispanic (N = 390)
13	28.63	27.46	18.72
13.2	5.23	1.59	7.05
14	18.31	34.68	30.38
14.2	5.81	2.17	4.49
15	6.25	16.18	12.69
15.2	4.22	3.47	7.31
16	1.45	5.92	4.1
16.2	3.34	-- (0.29)	2.31
17	--	-- (0.29)	-- (0.51)
17.2	-- (0.58)	-- (0.14)	-- (0.38)
18	--	-- (0.14)	--
18.2	--	--	--
D1S1656			
9	-- (0.15)	--	-- (0.13)
10	1.31	-- (0.29)	-- (0.38)
11	5.52	5.92	3.85
12	7.99	16.04	9.49
13	11.19	6.65	7.18
14	24.27	6.36	11.03
14.3	1.02	-- (0.29)	-- (0.26)
15	17.44	15.17	16.03
15.3	1.89	8.53	2.95
16	10.32	9.97	15.26
16.1	--	--	-- (0.26)
16.3	7.27	4.91	5.26
17	2.62	4.91	6.92
17.1	--	-- (0.29)	--
17.3	5.96	12.72	15.26
18	-- (0.44)	-- (0.29)	0.77
18.3	1.89	5.92	4.36
19	-- (0.15)	--	--
19.3	-- (0.58)	1.73	0.64
20.3	--	--	--
D21S11			
23.2	--	--	--
24	--	--	--
24.2	--	--	-- (0.26)
25	--	--	--

Allele *(continued)*	African American (N = 344)	Caucasian (N = 346)	Hispanic (N = 390)
25.2	--	--	--
26	-- (0.29)	-- (0.58)	-- (0.38)
26.2	--	--	--
27	5.52	2.75	1.41
27.1	-- (0.15)	--	--
27.2	--	--	--
28	25.29	16.91	11.03
28.2	--	--	-- (0.13)
29	15.7	23.55	21.15
29.2	--	-- (0.14)	--
29.3	-- (0.15)	-- (0.14)	--
30	20.93	23.41	27.95
30.2	1.6	2.75	1.67
31	8.72	6.79	5
31.2	4.94	8.67	11.28
32	1.31	2.31	1.28
32.2	6.98	9.54	12.44
33	0.87	--	-- (0.13)
33.2	3.2	2.02	5.26
34	-- (0.15)	--	--
34.2	--	-- (0.43)	-- (0.13)
35	3.49	--	-- (0.26)
35.2	--	--	--
36	0.73	--	-- (0.13)
36.2	--	--	--
37	--	--	--
37.2	--	--	--
38	--	--	-- (0.13)
38.2	--	--	--
39	--	--	--
D22S1045			
5	--	--	--
6	--	--	--
7	--	--	--
8	0.73	--	--
9	--	--	--
10	4.07	-- (0.43)	0.64
11	14.53	13.58	7.82

Allele *(continued)*	African American (N = 344)	Caucasian (N = 346)	Hispanic (N = 390)
12	5.96	-- (0.58)	1.03
13	-- (0.29)	1.01	1.03
14	8.58	3.47	2.18
15	23.55	36.56	42.56
16	19.91	36.27	35.64
17	20.35	7.51	7.95
18	2.03	-- (0.58)	1.03
20	--	--	-- (0.13)
D2S1338			
13	-- (0.15)	-- (0.14)	--
14	--	-- (0.14)	--
15	-- (0.29)	-- (0.14)	--
16	5.23	4.19	3.59
17	10.03	18.79	17.69
18	4.8	8.38	6.54
19	15.99	14.31	17.82
20	10.03	15.46	13.72
21	12.79	2.75	3.59
22	12.65	1.73	6.28
23	9.3	10.12	14.87
24	8.58	9.97	8.85
25	6.98	11.85	5.38
26	2.47	1.73	1.41
27	0.73	-- (0.29)	-- (0.13)
28	--	--	-- (0.13)
29	--	--	--
D2S441			
8	-- (0.15)	--	--
9	--	-- (0.58)	-- (0.26)
10	8.87	19.8	31.15
11	35.03	33.82	31.67
11.3	3.34	5.06	4.36
12	20.06	4.05	3.97
12.3	-- (0.15)	-- (0.29)	-- (0.38)
13	3.63	3.18	1.92
13.3	--	--	--
14	26.89	28.32	22.56
14.3	--	--	--

Allele *(continued)*	African American (N = 344)	Caucasian (N = 346)	Hispanic (N = 390)
15	1.89	4.48	3.33
16	--	-- (0.43)	-- (0.38)
17	--	--	--
D3S1358			
9	-- (0.29)	--	-- (0.13)
11	--	-- (0.29)	--
12	-- (0.15)	--	-- (0.13)
13	-- (0.58)	-- (0.14)	-- (0.38)
14	9.16	15.17	9.49
15	28.34	27.31	34.49
15.2	-- (0.29)	--	--
16	32.85	23.99	26.54
16.2	--	--	--
17	22.09	19.8	17.95
17.2	--	--	--
18	5.81	11.85	10.13
18.2	--	--	--
19	-- (0.44)	1.45	0.77
20	--	--	--
D8S1179			
7	--	--	--
8	-- (0.44)	2.02	0.64
9	-- (0.29)	1.3	-- (0.26)
10	3.34	10.84	9.49
11	5.81	6.65	4.87
12	11.05	15.03	12.44
13	18.31	33.53	33.33
14	36.05	18.64	23.46
15	17.73	8.67	11.54
16	5.96	2.89	3.33
17	1.02	-- (0.43)	0.64
18	--	--	--
19	--	--	--
20	--	--	--
FGA			
16	--	-- (0.14)	--
16.1	-- (0.29)	--	--
16.2	--	--	--

Allele *(continued)*	African American (N = 344)	Caucasian (N = 346)	Hispanic (N = 390)
17	--	-- (0.14)	--
17.2	--	--	--
18	0.87	1.16	0.64
18.2	0.73	--	--
19	6.83	5.35	7.82
19.2	-- (0.44)	--	--
20	6.69	15.61	8.72
20.2	-- (0.29)	-- (0.43)	-- (0.26)
21	12.06	18.35	13.59
21.2	-- (0.15)	-- (0.29)	--
22	18.17	18.93	14.1
22.2	-- (0.15)	0.87	0.64
23	16.86	14.6	12.69
23.2	--	-- (0.43)	-- (0.38)
23.3	-- (0.29)	--	--
24	18.75	14.31	15.9
24.2	--	--	--
25	9.3	6.79	14.1
25.2	--	--	--
26	3.92	1.88	6.79
26.2	--	--	--
27	2.62	-- (0.58)	2.95
27.2	--	--	--
28	1.16	-- (0.14)	0.9
28.2	--	--	--
29	--	--	-- (0.38)
29.2	--	--	--
30	-- (0.15)	--	-- (0.13)
30.2	-- (0.15)	--	--
31	--	--	--
31.2	--	--	--
32	--	--	--
32.2	--	--	--
33.2	--	--	--
34.2	-- (0.15)	--	--
42.2	--	--	--
43.2	--	--	--
44.2	--	--	--

Allele *(continued)*	African American (N = 344)	Caucasian (N = 346)	Hispanic (N = 390)
45.2	--	--	--
46.2	--	--	--
47.2	--	--	--
48.2	--	--	--
49.2	--	--	--
50.2	--	--	--
51.2	--	--	--
SE33			
4.2	--	--	--
5	--	--	--
5.2	-- (0.15)	--	--
6	--	--	--
6.3	--	-- (0.14)	--
7	--	--	--
8	--	--	--
8.2	--	--	--
9	--	--	--
9.2	--	--	--
10	--	--	--
10.2	--	--	--
11	--	--	--
11.2	0.73	--	-- (0.13)
12	-- (0.15)	-- (0.43)	-- (0.13)
12.1	--	-- (0.14)	--
12.2	-- (0.29)	-- (0.14)	-- (0.13)
13	1.31	0.87	1.15
13.2	-- (0.44)	--	-- (0.13)
14	3.63	3.76	1.92
14.2	--	--	0.77
14.3	--	-- (0.14)	--
15	3.92	3.61	4.74
15.2	-- (0.29)	-- (0.14)	-- (0.13)
16	6.98	5.2	5.51
16.2	-- (0.29)	--	-- (0.26)
16.3	--	--	-- (0.13)
17	7.7	6.5	8.72
17.2	-- (0.15)	--	--
17.3	--	-- (0.14)	--

Allele *(continued)*	African American (N = 344)	Caucasian (N = 346)	Hispanic (N = 390)
18	11.05	8.24	10
18.2	-- (0.15)	--	-- (0.26)
19	15.26	8.09	7.95
19.2	-- (0.29)	-- (0.29)	--
19.3	--	-- (0.43)	--
20	9.3	4.91	4.49
20.2	0.87	0.87	0.77
21	5.81	2.02	3.33
21.1	--	--	--
21.2	1.02	1.16	1.28
22	1.74	-- (0.58)	1.03
22.2	1.45	3.32	2.18
23	-- (0.29)	--	--
23.2	-- (0.58)	2.6	2.69
23.3	--	--	-- (0.13)
24	-- (0.29)	-- (0.14)	-- (0.13)
24.2	1.6	4.48	2.18
25	--	--	--
25.2	2.33	3.9	3.46
26	-- (0.15)	--	-- (0.26)
26.2	5.67	4.19	6.92
27	--	--	--
27.2	5.81	7.08	6.79
27.3	--	--	-- (0.13)
28	--	--	--
28.2	4.07	7.8	5.9
29	--	--	--
29.2	2.47	7.8	6.28
30	--	--	--
30.2	1.45	4.91	3.97
31	--	--	-- (0.13)
31.2	1.02	2.89	2.18
32	--	-- (0.43)	--
32.2	0.73	1.59	2.05
33	--	-- (0.29)	-- (0.38)
33.2	-- (0.44)	-- (0.14)	-- (0.51)
34	--	-- (0.29)	-- (0.38)
34.2	-- (0.15)	--	-- (0.26)

Allele *(continued)*	African American (N = 344)	Caucasian (N = 346)	Hispanic (N = 390)
35	--	-- (0.14)	--
35.2	--	--	--
36	--	-- (0.14)	--
36.2	--	--	--
37	--	--	-- (0.13)
37.2	--	--	--
38	--	--	--
TH01			
3	--	--	--
4	--	--	--
5	-- (0.44)	-- (0.14)	--
5.3	--	--	--
6	15.41	21.68	27.95
6.1	-- (0.15)	--	--
6.3	--	--	--
7	37.06	17.77	31.92
7.3	--	--	--
8	21.22	11.42	8.46
8.3	--	--	--
9	15.84	17.05	12.56
9.3	8.43	31.07	17.69
10	1.45	0.87	1.41
10.3	--	--	--
11	--	--	--
12	--	--	--
13	--	--	--
13.3	--	--	--
vWA			
10	--	--	--
11	-- (0.44)	--	-- (0.13)
12	--	--	-- (0.26)
13	0.73	-- (0.14)	-- (0.13)
14	7.41	8.38	6.41
15	20.93	12.14	9.87
15.2	--	--	--
16	27.47	22.69	30
17	19.48	27.31	27.18
17.3	--	--	-- (0.13)

Allele *(continued)*	African American (N = 344)	Caucasian (N = 346)	Hispanic (N = 390)
18	13.81	18.06	18.46
18.2	--	--	--
19	6.98	9.97	6.67
20	2.03	1.3	0.77
21	-- (0.58)	--	--
22	--	--	--
23	-- (0.15)	--	--
24	--	--	--
25	--	--	--

Table 11 Probability of identity (P_I) values for the AmpFℓSTR® NGM SElect™ Kit STR loci

Locus	African-American N = 344	Caucasian N = 346	Hispanic N = 390
D10S1248	0.0695	0.0937	0.1114
D12S391	0.0392	0.0232	0.0319
D16S539	0.0717	0.1042	0.0816
D18S51	0.0314	0.0312	0.0279
D19S433	0.0396	0.0852	0.0478
D1S1656	0.0335	0.0224	0.0247
D21S11	0.0454	0.0520	0.0502
D22S1045	0.0556	0.1326	0.1611
D2S1338	0.0226	0.0316	0.0318
D2S441	0.1014	0.0980	0.1068
D3S1358	0.0998	0.0749	0.0952
D8S1179	0.0755	0.0635	0.0682
FGA	0.0327	0.0387	0.0283
SE33	0.0121	0.0085	0.0079
TH01	0.0944	0.0798	0.0905
vWA	0.0617	0.0654	0.0913
Combined	$8.12 \times e^{-22}$	$2.35 \times e^{-21}$	$3.16 \times e^{-21}$

Probability of paternity exclusion

Table 12 shows the Probability of paternity exclusion (P_E) values of the AmpFℓSTR® NGM SElect™ Kit STR loci individually and combined.

Table 12 Probability of paternity exclusion values for the AmpFℓSTR® NGM SElect™ Kit STR loci

Locus	African-American N = 344	Caucasian N = 346	Hispanic N = 390
D10S1248	0.6590	0.5680	0.4690
D12S391	0.7450	0.7990	0.6620
D16S539	0.5560	0.5790	0.5570
D18S51	0.7860	0.7580	0.7180
D19S433	0.6360	0.5070	0.6470
D1S1656	0.7450	0.8110	0.7230
D21S11	0.7270	0.7290	0.6920
D22S1045	0.6980	0.4550	0.3970
D2S1338	0.7980	0.7520	0.7380
D2S441	0.5250	0.4970	0.4770
D3S1358	0.4760	0.5220	0.4820
D8S1179	0.5870	0.6160	0.5570
FGA	0.7270	0.6660	0.7080
SE33	0.8630	0.9120	0.8690
TH01	0.5150	0.5020	0.5390
vWA	0.6030	0.6440	0.6380
PEi	$9.451788e^{-09}$	$1.455231e^{-08}$	$8.163126e^{-08}$
Combined	0.9999999905	0.9999999854	0.9999999184

The P_E value is the probability, averaged over all possible mother-child pairs, that a random alleged father will be excluded from paternity after DNA typing using the AmpFℓSTR® NGM SElect™ Kit STR loci (Chakraborty, Stivers, and Zhong, 1996).

Troubleshooting

A

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

Observation	Possible causes	Recommended actions
Faint or no signal from both the 007 control DNA and the DNA test samples at all loci	Incorrect volume or absence of either AmpFℓSTR® NGM SElect™ Master Mix or AmpFℓSTR® NGM SElect™ Primer Set	Repeat amplification using correct reagent volumes.
	No activation of enzyme	Repeat amplification, making sure to hold reactions initially at 95 °C for 11 min.
	Master Mix not vortexed thoroughly before aliquoting	Vortex Master Mix thoroughly.
	AmpFℓSTR NGM SElect™ Primer Set exposed to too much light	Store Primer Set protected from light.
	GeneAmp® PCR System malfunction	Refer to the thermal cycler user's manual and check instrument calibration.
	Incorrect thermal cycler parameters	Check the protocol for correct thermal cycler parameters.
	Tubes/plate not seated tightly in the thermal cycler during amplification	Push reaction tubes/plate firmly into contact with block after first cycle. Repeat test.
	Wrong PCR reaction tubes or plate	Use Applied Biosystems® MicroAmp® Reaction. Tubes with Caps or the MicroAmp® Optical 96-Well Reaction Plate for the GeneAmp® PCR System 9700 or Veriti® 96-Well Thermal Cycler.
	MicroAmp® Base used with tray/retainer set and tubes in GeneAmp® PCR System 9700	Remove MicroAmp® Base from tray/retainer set and repeat test.
	Insufficient PCR product electrokinetically injected	Mix 1.0 µL of PCR product and 10 µL of Hi-Di™ Formamide/GeneScan 600 LIZ™ Size Standard v2.0 solution.
	Degraded formamide	Check the storage of formamide; do not thaw and refreeze multiple times. Try Hi-Di™ Formamide.

Observation	Possible causes	Recommended actions
Positive signal from AmpFℓSTR® Control DNA 007 but partial or no signal from DNA test samples	Quantity of test DNA sample is below assay sensitivity	Quantify DNA and add 1.0 ng of DNA. Repeat test.
	Test sample contains high concentration of PCR inhibitor (for example, heme compounds, certain dyes	Quantify DNA and add minimum necessary volume. Repeat test.
		Wash the sample in a Centricon®-100 centrifugal filter unit. Repeat test.
	Test sample DNA is severely degraded	If possible, evaluate the quality of DNA sample by running an agarose gel. If DNA is degraded, reamplify with an increased amount of DNA or use the AmpFℓSTR® MiniFiler™ kit.
	Dilution of test sample DNA in water or wrong buffer (for example, TE formula with incorrect EDTA concentration)	Redilute DNA using low TE Buffer (with 0.1 mM EDTA).
More than two alleles present at a locus	Presence of exogenous DNA	Use appropriate techniques to avoid introducing foreign DNA during laboratory handling.
	Amplification of stutter product	Interpret according to laboratory procedures. **Note:** Additional information will be provided on completion of validation.
	Mixed sample	
	Incomplete 3´ A base addition (n-1 nt position)	Addition of excess DNA to the reaction will contribute to the occurrence of incomplete 3' base addition. Quantify DNA and add 1.0 ng of DNA to the reaction. Repeat test. Also be sure to include the final extension step of 60 °C for 10 min in the PCR.
	Signal exceeds dynamic range of instrument (off-scale data)	Ensure that you use the correct cycle number. Repeat PCR amplification using the recommended cycle number or reduce the amount of input DNA.
	Poor spectral separation (bad matrix)	Follow the steps for creating a spectral file.
		Confirm that Filter Set G5 modules are installed and used for analysis.
	Too much DNA in reaction	Use recommended amount of template DNA (1.0 ng).
	Incomplete denaturation of double stranded DNA	Use the recommended amount of Hi-Di™ Formamide and perform heat denaturation according to instructions on page 37 (3100/3100-*Avant* or 3130/3130*xl*) or page 40 (3500/3500xL).
Poor peak height balance	Incorrect thermal cycler parameters	Check the protocol for correct thermal cycler parameters.
	GeneAmp® PCR System 9700 with Aluminum 96-Well block or third-party thermal cyclers	Use Applied Biosystems® GeneAmp® PCR System 9700 with silver or gold-plated silver blocks or Veriti® 96-Well Thermal Cycler only.

Ordering Information B

How to order

To order kits and supplies, go to **www.appliedbiosystems.com**.

AmpFℓSTR® NGM SElect™ PCR Amplification Kit

The table below lists the AmpFℓSTR® NGM SElect™ PCR Amplification Kits available. For information on kit contents and storage, see "Kit contents and storage" on page 17.

Table 13 AmpFℓSTR® NGM SElect™ PCR Amplification Kits

Kit	Number of 25 µL reactions	Part number
AmpFℓSTR® NGM SElect™ PCR Amplification Kit	200	4457889
	1,000	4457890

IMPORTANT! The fluorescent dyes attached to the primers in the kit are light-sensitive. Protect the primer set from light when not in use. Amplified DNA, AmpFℓSTR® NGM SElect™ Allelic Ladder, and GeneScan™ 600 LIZ™ Size Standard v2.0 should also be protected from light. Keep freeze-thaw cycles to a minimum.

Materials and equipment not included

Tables 14 and **15** list required and optional equipment and materials not supplied with the AmpFℓSTR® NGM SElect™ Kit. Unless otherwise noted, many of the items are available from major laboratory suppliers (MLS).

Table 14 Equipment

Equipment	Source
Applied Biosystems® 3500/3500xL Genetic Analyzer for Human Identification	Contact your local Applied Biosystems sales representative
ABI PRISM® 3100/3100-*Avant* Genetic Analyzer	
Applied Biosystems® 3130/3130*xl* Genetic Analyzer	
Silver 96-Well GeneAmp® PCR System 9700 (with silver sample block)	N8050001
Gold-plated 96-Well GeneAmp® PCR System 9700 (with gold-plated silver sample block)	4314878
Silver 96-Well Sample Block	N8050251

Table 14 Equipment

Equipment *(continued)*	**Source**
Gold-plated Silver 96-Well Sample Block	4314443
Veriti® 96-Well Thermal Cycler	4375786
ProFlex™ 96-Well PCR System	4484075
Tabletop centrifuge with 96-Well Plate Adapters (optional)	MLS

Table 15 User-supplied materials‡

Item	**Source**
3500/3500xL Analyzer materials	
Anode buffer container (ABC)	4393927
Cathode buffer container (CBC)	4408256
POP-4™ polymer (960 samples) for 3500/3500xL Genetic Analyzers	4393710
POP-4™ polymer (384 samples) for 3500/3500xL Genetic Analyzers	4393715
Conditioning reagent	4393718
8-Capillary array, 36 cm for 3500 Genetic Analyzers	4404683
24-Capillary array, 36 cm for 3500xL Genetic Analyzers	4404687
96-well retainer & base set (Standard) 3500/3500xL Genetic Analyzers	4410228
8-Tube retainer & base set (Standard) for 3500/3500xL Genetic Analyzers	4410231
8-Strip Septa for 3500/3500xL Genetic Analyzers	4410701
96-Well Septa for 3500/3500xL Genetic Analyzers	4412614
Septa Cathode Buffer Container, 3500 series	4410715
For a complete list of parts and accessories for the 3500/3500xL instrument, refer to the *Applied Biosystems® 3500/3500xL Genetic Analyzer User Guide* (PN 4401661).	
3100/3100-*Avant* Analyzer materials	
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3100/3100-*Avant* Genetic Analyzer Capillary Array, 36 cm	4333464
POP-4™ Polymer for 3100/3100-*Avant* Genetic Analyzers	4316355
3100/3100-*Avant* Genetic Analyzer Autosampler Plate Kit, 96 well	4316471
GeneScan™ 600 LIZ™ Size Standard v2.0	4408399

Table 15 User-supplied materials‡

Item	Source
Running Buffer, 10X	402824
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
MicroAmp® Optical 96-Well Reaction Plate	N8010560
250 µL Glass Syringe (array-fill syringe)	4304470
5.0 mL Glass Syringe (polymer-reserve syringe)	628-3731
For a complete list of parts and accessories for the 3100 instrument, refer to Appendix B of the *ABI PRISM® 3100 Genetic Analyzer and 3100-Avant Genetic Analyzer User Reference Guide* (PN 4335393).	
3130/3130*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
3130/3130*xl* Genetic Analyzer Autosampler Plate Kit, 96 well	4316471
GeneScan™ 600 LIZ™ Size Standard v2.0	4322682
Running Buffer, 10X	402824
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
MicroAmp® Optical 96-Well Reaction Plate	N8010560
For a complete list of parts and accessories for the 3130*xl* instrument, refer to Appendix A of the *Applied Biosystems® 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide* (PN 4352716).	

Table 15 User-supplied materials‡

Item	Source
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
Hi-Di™ Formamide, 25 mL	4311320
Other user-supplied materials	
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 us, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Safety C

This appendix covers:

- Chemical safety 152
- Chemical waste safety 154
- Biological hazard safety 156
- Chemical alerts 157

Chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

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

Chemical safety guidelines

To minimize the hazards of chemicals:

- 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. (See “About SDSs” on page 152.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About SDSs

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

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

Obtaining SDSs

The SDSs for any chemical supplied by us is available to you free 24 hours a day. To obtain SDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **SDS**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the SDSs of chemicals not distributed by us, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

WARNING! HAZARDOUS WASTE. Refer to Safety Data Sheets and local regulations for handling and disposal.

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

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

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

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

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.

- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

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

Biological hazard safety

General biohazard

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:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* found at: **http://www.cdc.gov/biosafety/publications/index.htm**.
- 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

Chemical alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see “Safety alert words” on page 7.

General alerts for all chemicals

Avoid contact with skin, eyes, and/or clothing. Read the SDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Documentation

Related documentation

For additional documentation, see “Obtain support” on page 160.

Document title	Part number
Applied Biosystems® 3100/3100-Avant Data Collection v2.0 User Guide	4347102
Applied Biosystems® 3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0 User Bulletin	4350218
Applied Biosystems® 3100 Genetic Analyzer User Manual (Data Collection v1.1)	4315834
Applied Biosystems® 3100/3100-Avant Genetic Analyzers: Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products User Bulletin	4332345
AmpFℓSTR® NGM SElect™ PCR Amplification Kit - PCR Setup Quick Reference Card	4458842
AmpFℓSTR® NGM SElect™ PCR Amplification Kit - PCR Amplification and CE Quick Reference Card	4458843
Veriti® 96-Well Thermal Cycler AmpFℓSTR® Kit Validation User Bulletin	4440754
ProFlex™ PCR System Kit Validation User Bulletin	100031595
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® 3730/3730xl DNA Analyzers and 3130/3130xl Genetic Analyzers: AB Navigator Software Administrator Guide	4359472
Applied Biosystems® 3130/3100xl DNA Analyzers User Guide	4331468
Applied Biosystems® 3730/3730xl Genetic Analyzer Getting Started Guide	4359476
PrepFiler™ Forensic DNA Extraction Kit User Guide	4390932
GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide	4338775
Installation Procedures and New Features for GeneMapper® ID Software v3.2 User Bulletin	4352543

Document title	Part number
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 1.0.1/v1.1 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

Obtain support

For HID support:

- **In North America** – Send an email to **HIDTechSupport@lifetech.com**, or call **888.821.4443 option 1**.
- **Outside North America** – Contact your local support office.

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

www.lifetechnologies.com

At the website, you can:

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

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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