USER GUIDE

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AmpFℓSTR® NGM™ PCR Amplification Kit

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life technologies™

AmpFℓSTR® NGM™

PCR Amplification Kit

User's Guide

Applied Biosystems

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Preface

Safety information

Note: For general safety information, see this Preface and Appendix C, "Safety" on page 93. When a hazard symbol and hazard type appear by an instrument hazard, see the "Safety" Appendix for the complete alert. For all chemicals, read the SDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Safety alert words

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

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

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

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

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

SDSs

The Safety Data Sheets (SDSs) for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see "Obtaining SDSs" on page 95.

IMPORTANT! For the SDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

Overview 1

This chapter covers:

Product overview

Purpose

The AmpFℓSTR® NGM™ PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that amplifies 14 tetranucleotide repeat loci and one trinucleotide repeat locus, D22S1045. The kit simultaneously coamplifies the 10 loci contained in the AmpFℓSTR® SGM Plus® kit (D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01, and FGA) together with 2 highly polymorphic STR loci (D1S1656 and D12S391), 3 "mini" STR loci (D10S1248, D22S1045 and D2S441), and the gender determination locus Amelogenin. The AmpFℓSTR® NGM™ Kit delivers a 16-locus multiplex with a greater power of discrimination, better sensitivity, and improved robustness than earlier generation kits. The 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™ 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
- Applied Biosystems 310 Genetic Analyzer
- GeneAmp® PCR System 9700 with the Silver 96-Well Block
- GeneAmp® PCR System 9700 with the Gold-plated Silver 96-Well Block

About the primers

The AmpFℓSTR® NGM™ 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's signal-to-noise ratio 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™ 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™ Kit loci and alleles

Locus designation	Chromosome location	Alleles included in AmpFℓSTR® NGM™ Kit 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

Allelic ladder profile

Figure 1 shows the allelic ladder for the AmpFℓSTR® NGM™ Kit. See "Allelic ladder requirements" on page 34 for information on ensuring accurate genotyping.

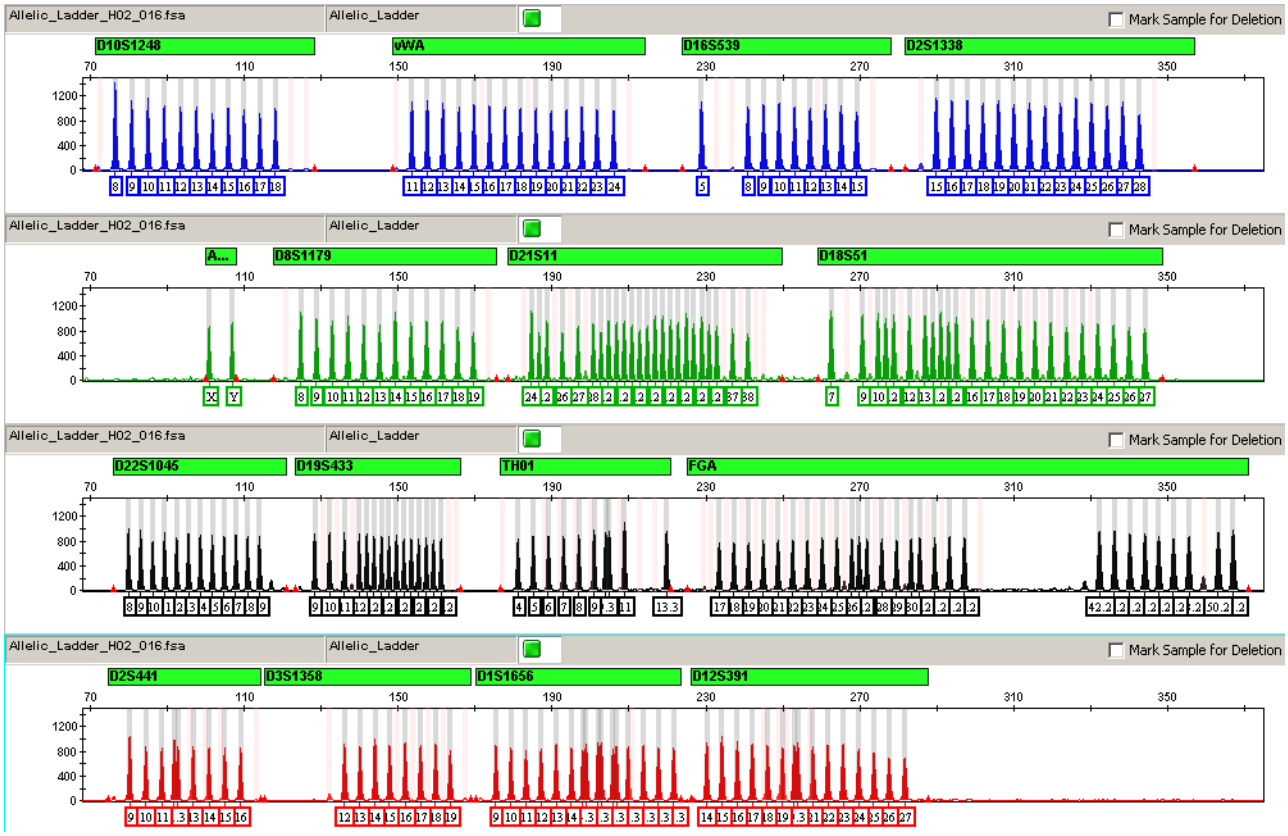

Figure 1 GeneMapper® *ID-X* Software plot of the AmpFℓSTR® NGM™ Kit Allelic Ladder

Control DNA 007 profile

Figure 2 shows amplification of Control DNA 007 using the AmpFℓSTR® NGM™ Kit.

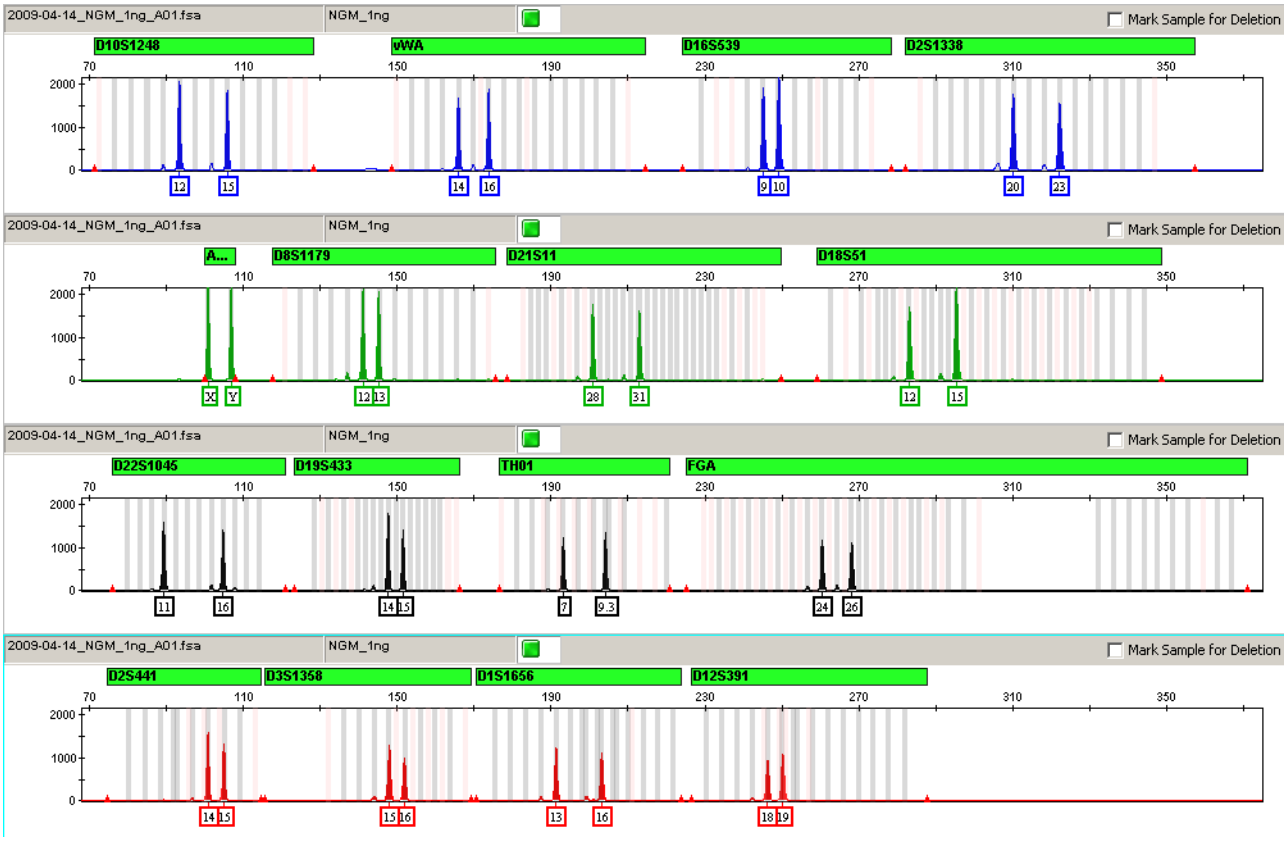

Figure 2 1 ng of Control DNA 007 amplified with the AmpFℓSTR® NGM™ Kit and analyzed on the Applied Biosystems 3130*xl* Genetic Analyzer

Workflow overview

Extract DNA

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

Quantify DNA

Quantifiler® Duo DNA Quantification Kit

Perform PCR

AmpFℓSTR® NGM™ PCR Amplification Kit

GeneAmp® PCR System 9700 Thermal Cycler*

* Amplification should be performed on silver- or gold-plated silver 96-well blocks only.

Perform Electrophoresis

ABI PRISM® 310 Genetic Analyzer

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

Applied Biosystems 3130/3130*xl* Genetic Analyzer

Applied Biosystems 3500/3500xL Genetic Analyzer for Human Identification

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™ 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	Operating system	Data Collection Software	Analysis software
3500/3500xL	• Windows® XP • Windows Vista®	3500 Series Data Collection Software v1.0	GeneMapper® *ID-X* Software v1.2
3130/3130*xl*‡	Windows® XP	3.0	• GeneMapper® *ID* Software v3.2.1 *and* • GeneMapper® *ID-X* Software v1.0.1 or higher
3100/3100-*Avant*	Windows NT®	1.1 (3100) 1.0 (3100-*Avant*)	
	Windows 2000	2.0	
310	Windows XP	3.1	
	Windows NT and Windows 2000	3.0	

‡ Applied Biosystems conducted validation studies for the AmpFℓSTR® NGM™ 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 5 different fluorescent dye colors into distinct spectral components. The 4 dyes used in the AmpFℓSTR® NGM™ PCR Amplification Kit to label samples are 6-FAM™, VIC®, NED™, and PET® dyes. The fifth dye, LIZ®, is used to label the GeneScan™ 500 LIZ® Size Standard.

How multicomponent analysis works

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

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

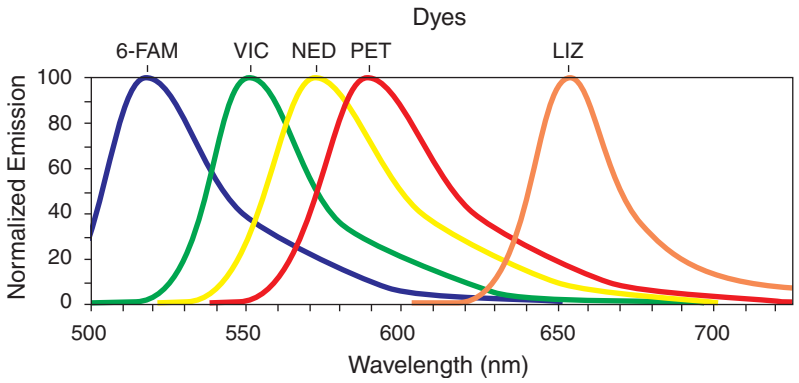

Figure 3 Emission spectra of the five dyes used in the AmpFℓSTR® NGM™ Kit

Materials and equipment

Kit contents and storage

The AmpFℓSTR® NGM™ PCR Amplification Kit contains materials sufficient to perform 200 (Part no. 4415020) or 1000 (Part no. 4415021) amplifications at a 25 µL reaction volume.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer set from light when not in use. Amplified DNA, AmpFℓSTR® NGM™ Allelic Ladder, and GeneScan™ 500 LIZ® Size Standard 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 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 Master Mix	Contains enzyme, salts, dNTPs, carrier protein, and 0.05% sodium azide.	2 tubes, 1.0 mL each	1 bottle, 10.0 mL	-15 to -25°C on receipt, 2 to 8 °C after initial use
AmpFℓSTR® NGM 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	-15 to -25°C on receipt, 2 to 8°C after initial use
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.

Standards for samples

For the AmpFℓSTR® NGM™ 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™ Allelic Ladder.
- **GeneScan™ 500 LIZ®** Size Standard – Standard used for obtaining sizing results. It contains 16 single-stranded labeled fragments of: 35, 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490, and 500 nucleotides. This standard, which has been evaluated as an internal size standard, yields precise sizing results for AmpFℓSTR® NGM™ Kit PCR products. Order the GeneScan™ 500 LIZ® Size Standard (Part no. 4322682) separately.

- **AmpFℓSTR® NGM™ Allelic Ladder** – Allelic ladder developed by Applied Biosystems for accurate characterization of the alleles amplified by the AmpFℓSTR® NGM™ Kit. The AmpFℓSTR® NGM™ Allelic Ladder contains most of the alleles reported for the 15 autosomal loci. Refer to Table 1 on page 11 for a list of the alleles included in the AmpFℓSTR® NGM™ Allelic Ladder.

Chapter 2

PCR Amplification

PCR Amplification 2

This chapter covers:

PCR work areas

Work area setup and lab design

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

- For AmpFℓSTR® NGM™ 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™ 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™ 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 additional information and references.

PCR-setup tools

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

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

Amplified DNA work area tools

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

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

Required user-supplied materials and reagents

Kit contents and storage

The AmpFℓSTR® NGM™ 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™ 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™ Kit is 1.0 ng in a maximum input volume of 10 µL amplified for 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

Applied Biosystems provides several kits for quantifying DNA in samples. See the references cited in the following table for details about these kits.

Product	Description	References
Quantifiler® Human DNA Quantification Kit (Part no.4343895) *and* Quantifiler® Y Human Male DNA Quantification Kit (Part no. 4343906)	**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® Human DNA Quantification Kits User's Manual* (Part no. 4344790)
Quantifiler® Duo DNA Quantification Kit (Part no. 4387746)	**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® Duo DNA Quantification Kit User's Manual* (Part no. 4391294)

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 (µL)
AmpFℓSTR® NGM™ Master Mix	10.0 µL
AmpFℓSTR® NGM™ 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™ Master Mix and the AmpFℓSTR® NGM™ 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 2–8°C and, therefore, do not require subsequent thawing. Do not refreeze the reagents.

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

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

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

6. Prepare the DNA samples:

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.

The final reaction should be 25 µL.

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.

Note: The AmpFℓSTR® NGM™ 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™ Kit.

Perform PCR

WARNING! PHYSICAL INJURY HAZARD. Thermal cycler.

1. Program the thermal cycling conditions.

IMPORTANT! When using the GeneAmp PCR System 9700 with either 96-well silver or gold-plated silver block, select the **9600 Emulation Mode**.

Initial incubation step	Cycle (29/30 cycles)		Final extension	Final hold
	Denature	Anneal		
HOLD	CYCLE		HOLD	HOLD
95°C 11 min	94°C 20 sec	59°C 3 min	60°C 10 min	4°C ¥

IMPORTANT! The NGM™ kit is validated for use at both 29 and 30 cycles. The optimum conditions for the NGM™ kit are 29 cycles of amplification with a 1 ng input DNA concentration. Laboratories choosing to use the NGM™ 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 (Part no. 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. Applied Biosystems studies indicate that a 1.2-mm bloodstained disc contains approximately 5–20 ng DNA. An appropriate cycle number for this high quantity of DNA is 24 cycles, determined by Applied Biosystems 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 4, 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.

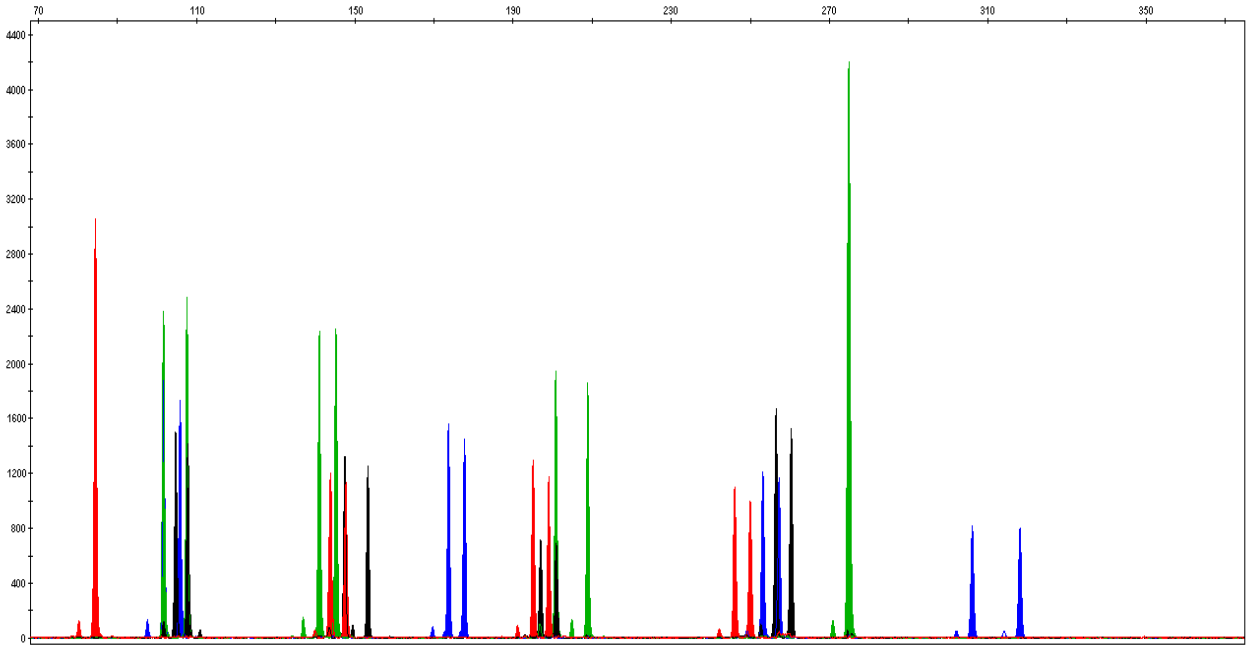

Figure 4 AmpFℓSTR® NGM™ PCR Amplification Kit results from a 1.2-mm FTA® bloodstain disc (24-cycle amplification), analyzed on the Applied Biosystems 3130*xl* Genetic Analyzer

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Chapter 3

Electrophoresis

Electrophoresis 3

This chapter covers:

Allelic ladder requirements

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

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

IMPORTANT! Variation in laboratory temperature can affect fragment migration speed and result in sizing variation. Applied Biosystems recommends the following 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 critical 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, "Ordering Information" on page 89 lists the required materials not supplied with the AmpFℓSTR® NGM™ PCR Amplification Kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the AmpFℓSTR® NGM™ Primer Set from light when not in use. Amplified DNA, AmpFℓSTR® NGM™ Allelic Ladder, and GeneScan™ 500 LIZ® Size Standard 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™ Kit PCR products. For details on the procedures, refer to the documents listed in the table.

Data Collection Software	Operating System	Run modules and conditions	References
3.0‡ (3130/3130*xl* Analyzer)	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* (Part no. 4363787)
2.0 (3100 Analyzer)	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* (Part no. 4350218)
1.1 (3100 Analyzer)	Windows NT®	• GeneScan36vb_DyeSetG5Module Injection condition: 3kV/10 sec • GS500Analysis.gsp	*ABI PRISM® 3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR PCR Amplification Kit PCR Products User Bulletin* (Part no. 4332345)
1.0 (3100-*Avant* Analyzer)	Windows NT®	• GeneScan36Avb_DyeSetG5Module Injection condition: 3 kV/ 5sec • GS500Analysis.gsp	*ABI PRISM® 3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR PCR Amplification Kit PCR Products User Bulletin* (Part no. 4332345)

‡ Applied Biosystems conducted validation studies for the AmpFℓSTR® NGM™ 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™ 500 LIZ® Internal Size Standard needed to prepare the samples, using the table below.

Reagent	Volume per reaction (µL)
GeneScan™ 500 LIZ® Size Standard	0.5 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. Pipet 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:
   a. 10 µL of the formamide: size standard mixture
   b. 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, "Ordering Information" on page 89 lists the required materials not supplied with the AmpFℓSTR® NGM™ PCR Amplification Kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the AmpFℓSTR® NGM™ Primer Set from light when not in use. Amplified DNA, AmpFℓSTR® NGM™ Allelic Ladder, and GeneScan™ 500 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™ 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* (Part no. 4401661) *3500 and 3500xL Genetic Analyzers Quick Reference Card* (Part no. 4401662)
Applied Biosystems 3500xL			• HID36_POP4 Injection conditions: 1.2kV/24 sec • Dye Set G5	

Prepare samples for electrophoresis on the 3500/3500xL instrument

Prepare the samples for capillary electrophoresis on the 3500/3500xL instrument immediately before loading.

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

Reagent	Volume per reaction (µL)
GeneScan™ 500 LIZ® Size Standard	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. Pipet 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:
   a. 10 µL of the formamide: size standard mixture
   b. 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.

Section 3.3 310 Instrument

Set up the 310 instrument for electrophoresis

Reagents and parts

Appendix B, "Ordering Information" on page 89 lists the required materials not supplied with the AmpFℓSTR® NGM™ PCR Amplification Kit.

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

310 instrument requirements

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

Data Collection Software	Operating System	Run modules and conditions	References
3.1‡ *or* 3.0‡	Windows XP *or* Windows NT® and Windows 2000	• GS STR POP4 (1mL) G5 v2.md5 Injection condition: 15 kV/5 sec	ABI PRISM® *310 Genetic Analyzer User's Manual (Windows)* (Part no. 4317588) ABI PRISM® *310 Protocols for Processing AmpFℓSTR PCR Amplification Kit Products with Microsoft Windows NT Operating System: User Bulletin* (Part no. 4341742)

‡ Applied Biosystems conducted concordance studies for the AmpFℓSTR® NGM™ Kit using this configuration.

Prepare samples for electrophoresis on the 310 instrument

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

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

Reagent	Volume per reaction (µL)
GeneScan™ 500 LIZ® Size Standard	0.75 µL
Hi-Di Formamide	24.25 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 0.2-mL or 0.5-mL sample tube, add:
   a. 25 µL of the formamide: size standard mixture
   b. 1.5 µL of PCR product or allelic ladder

   Note: For blank wells, add 25 µL of Hi-Di™ Formamide.
5. Seal the tubes with the appropriate septa, then briefly centrifuge to ensure that the contents of each tube are mixed and collected at the bottom.
6. Heat the tubes in a thermal cycler for 3 minutes at 95°C.
7. Immediately place the tubes on ice for 3 minutes.
8. Place the sample tray on the autosampler.
9. Ensure that an injection list is prepared.
10. Start the electrophoresis run.

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Chapter 4

Data Analysis

Data Analysis 4

This chapter covers:

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 an .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 the GeneMapper® *ID-X* Software to perform Human Identification (HID) analysis with AmpFℓSTR kits, go to "Set up GeneMapper® ID-X Software for data analysis" on page 64 or refer to the *GeneMapper® ID-X Software Version 1.0 Human Identification Analysis Getting Started Guide* (Part no. 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 49.
- 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 the *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (Part no. 4335523) for more information.
- Define custom views of plots.

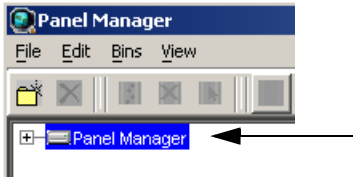
  Refer to the *GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial* (Part no. 4335523) for more information.

Import panels and bins

To import the AmpFℓSTR® NGM™ Kit panel and bin set from the Applied Biosystems web site 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 **NGM 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* (Part no. 4338775).
3. Select **Tools ▸ Panel Manager**.
4. Find, then open the folder containing the panels, bins, and marker stutter:
   a. Select **Panel Manager** in the navigation pane.

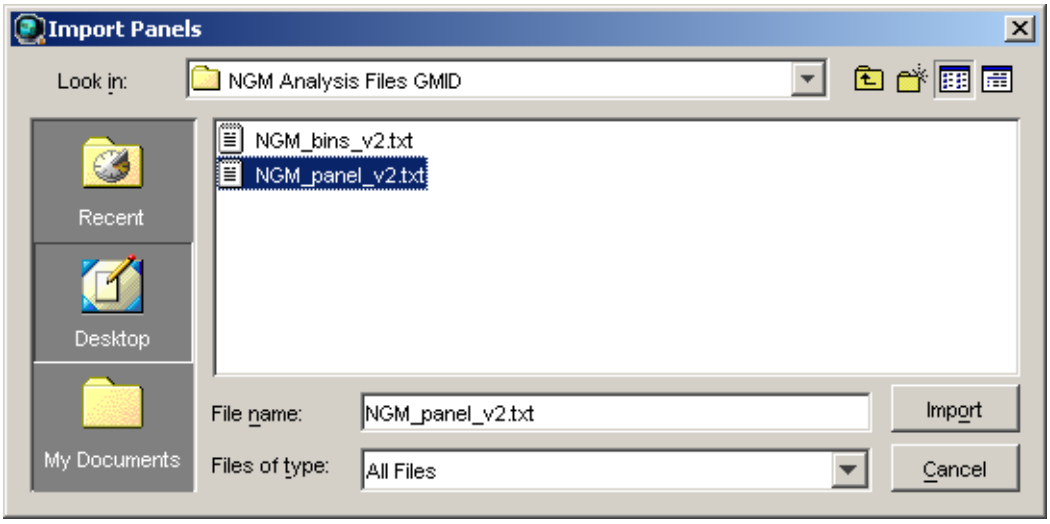


   b. Select **File ▸ Import Panels** to open the Import Panels dialog box.

c. Navigate to, then open the **NGM Analysis Files GMID** folder that you unzipped in step 1 on page 49.

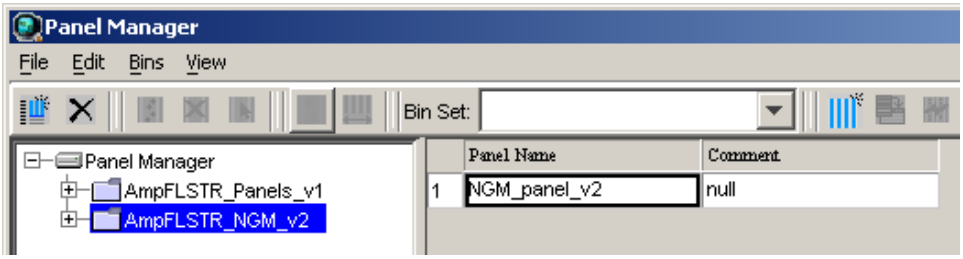
5. Select **NGM_panel_v2**, then click **Import**.

Note: Importing this file creates a new folder in the navigation pane of the Panel Manager, AmpFLSTR_NGM_v2. This folder contains the panel and associated markers.



6. Import **NGM_bins_v2**:

a. Select the **AmpFLSTR_NGM_v2** folder in the navigation pane.

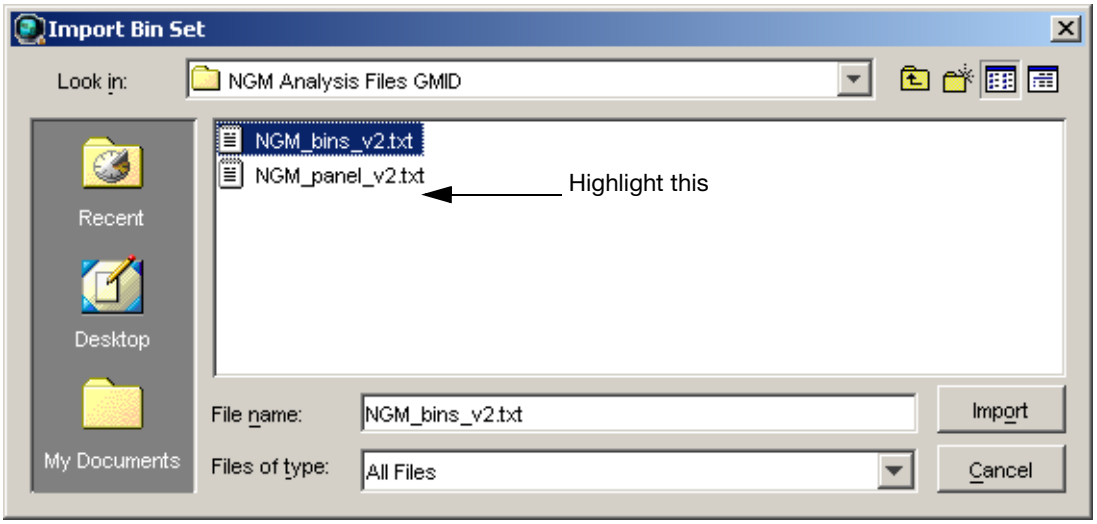


b. Select **File ▸ Import Bin Set** to open the Import Bin Set dialog box.

c. Navigate to, then open the **NGM Analysis Files GMID** folder.

d. Select **NGM_bins_v2**, then click **Import**.

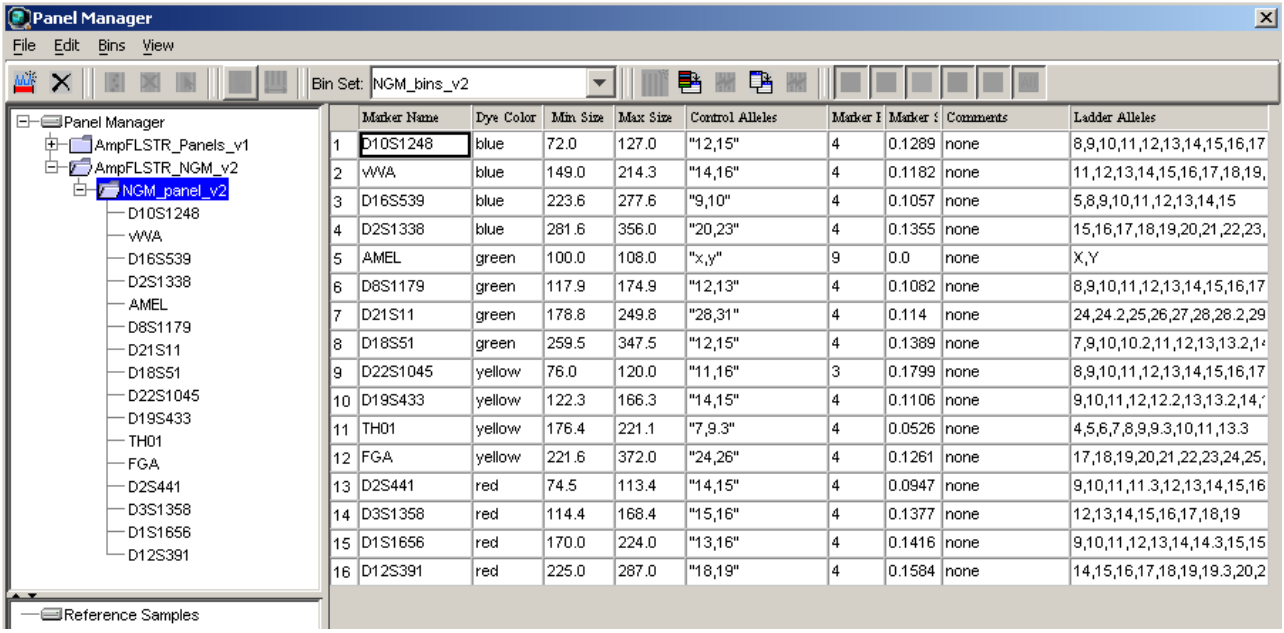
Note: Importing this file associates the bin set with the panels in the NGM_panel_v2 folder.



7. View the imported panels in the navigation pane:

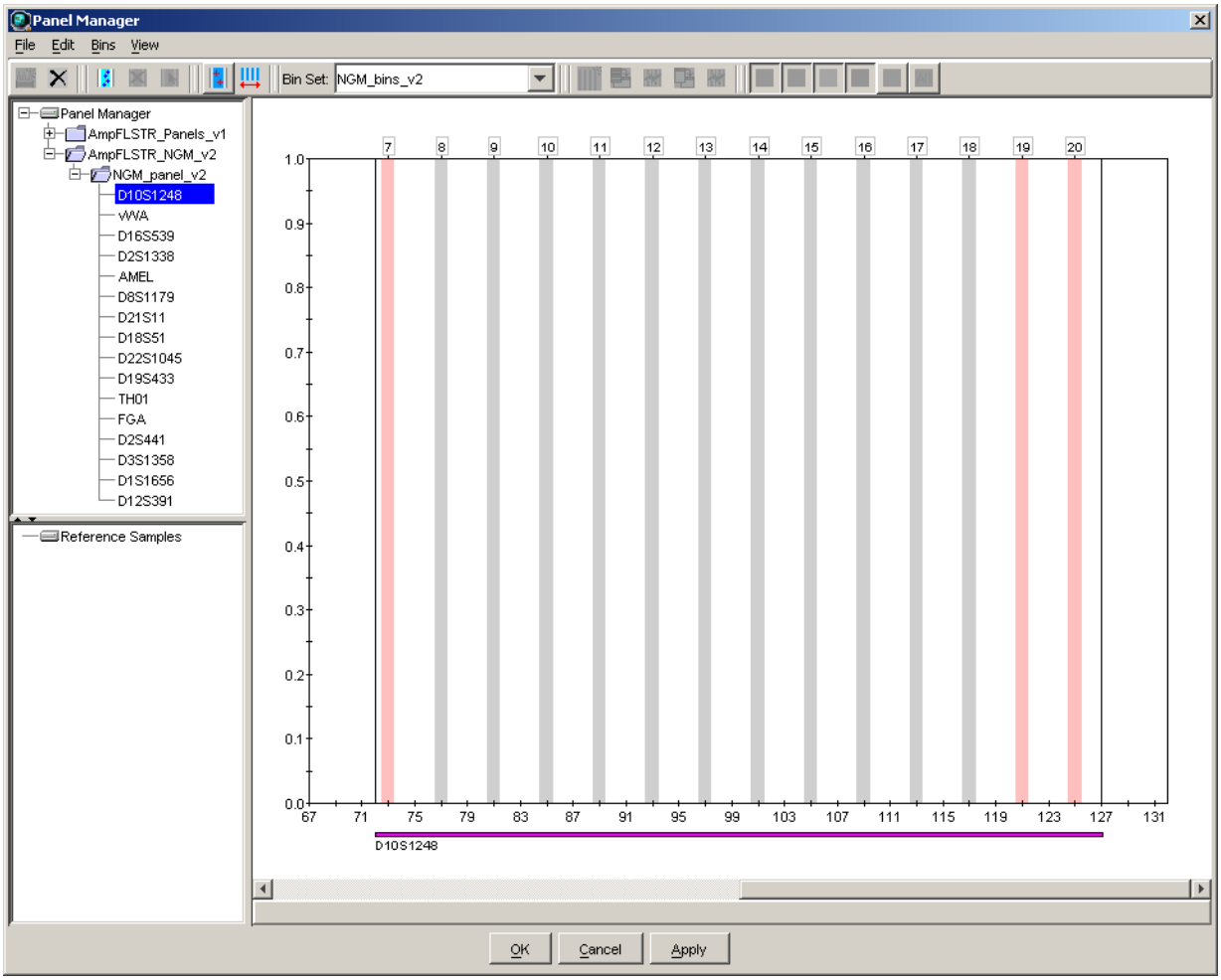
   a. Double-click the **AmpFLSTR_NGM_v2** folder to view the **NGM_panel_v2** folder.

   b. Double-click the **NGM_panel_v2** folder to display the panel information in the right pane.



	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	0.1289	none	8,9,10,11,12,13,14,15,16,17
2	vWA	blue	149.0	214.3	"14,16"	4	0.1182	none	11,12,13,14,15,16,17,18,19,
3	D16S539	blue	223.6	277.6	"9,10"	4	0.1057	none	5,8,9,10,11,12,13,14,15
4	D2S1338	blue	281.6	356.0	"20,23"	4	0.1355	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	0.1082	none	8,9,10,11,12,13,14,15,16,17
7	D21S11	green	178.8	249.8	"28,31"	4	0.114	none	24,24.2,25,26,27,28,28.2,29
8	D18S51	green	259.5	347.5	"12,15"	4	0.1389	none	7,9,10,10.2,11,12,13,13.2,1
9	D22S1045	yellow	76.0	120.0	"11,16"	3	0.1799	none	8,9,10,11,12,13,14,15,16,17
10	D19S433	yellow	122.3	166.3	"14,15"	4	0.1106	none	9,10,11,12,12.2,13,13.2,14,
11	TH01	yellow	176.4	221.1	"7,9.3"	4	0.0526	none	4,5,6,7,8,9,9.3,10,11,13.3
12	FGA	yellow	221.6	372.0	"24,26"	4	0.1261	none	17,18,19,20,21,22,23,24,25,
13	D2S441	red	74.5	113.4	"14,15"	4	0.0947	none	9,10,11,11.3,12,13,14,15,16
14	D3S1358	red	114.4	168.4	"15,16"	4	0.1377	none	12,13,14,15,16,17,18,19
15	D1S1656	red	170.0	224.0	"13,16"	4	0.1416	none	9,10,11,12,13,14,14.3,15,15
16	D12S391	red	225.0	287.0	"18,19"	4	0.1584	none	14,15,16,17,18,19,19.3,20,2

8. Select **D10S1248** to display the Bin view for the marker in the right pane.



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

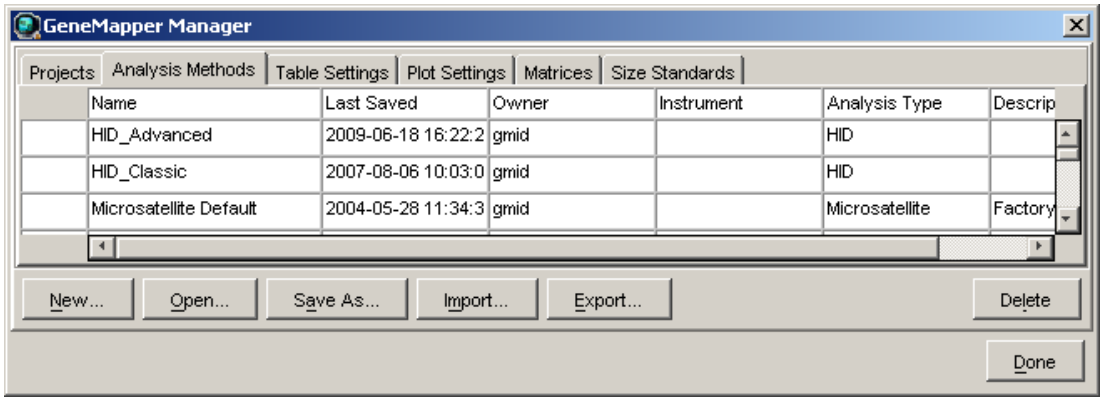
IMPORTANT! If you close the Panel Manager without clicking OK, the panels and bins are not imported into the GeneMapper® *ID* Software database.

Create a HID analysis method

The HID Advanced analysis method for the AmpFℓSTR® NGM™ Kit uses the NGM_bins_v2 file described in step 6 on page 50.

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

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



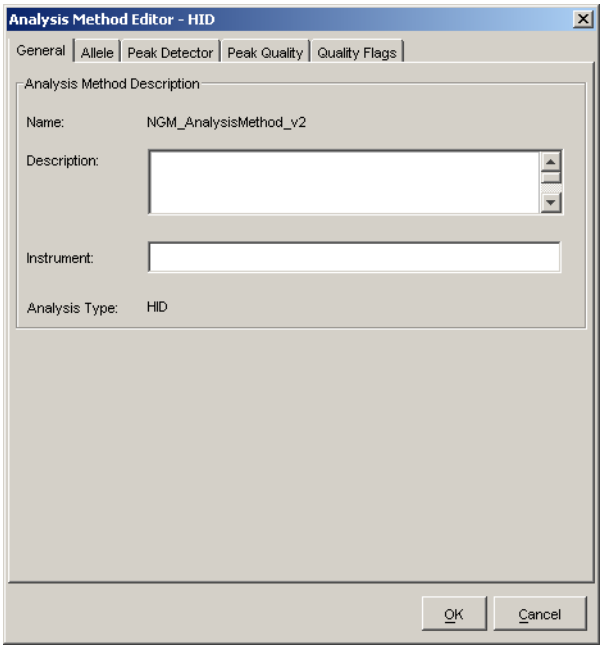
2. Select the **Analysis Methods** tab, then click **New** to open the New Analysis Method dialog box.

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.

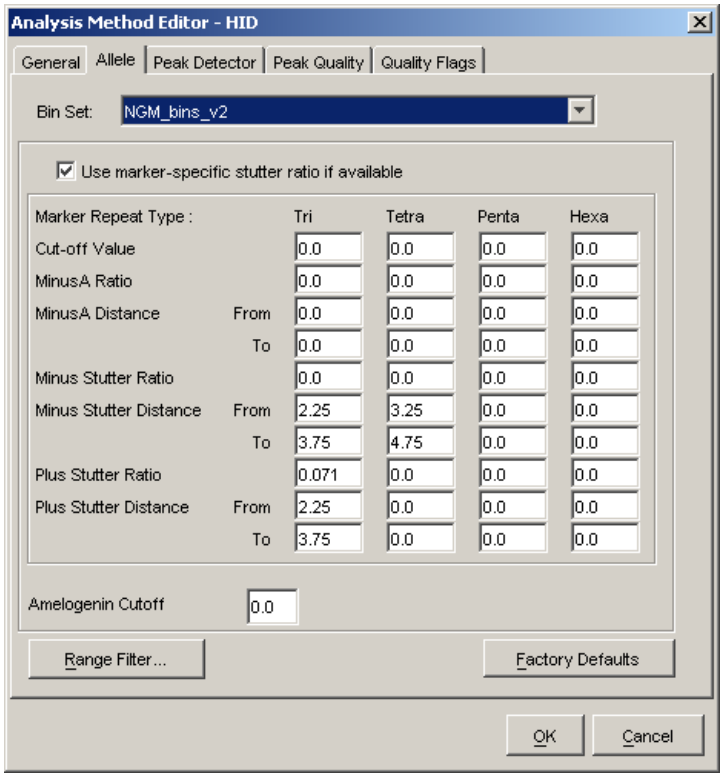
Note: The Analysis Method Editor closes when you save your settings (See step 5 on page 58). To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.

- General tab settings



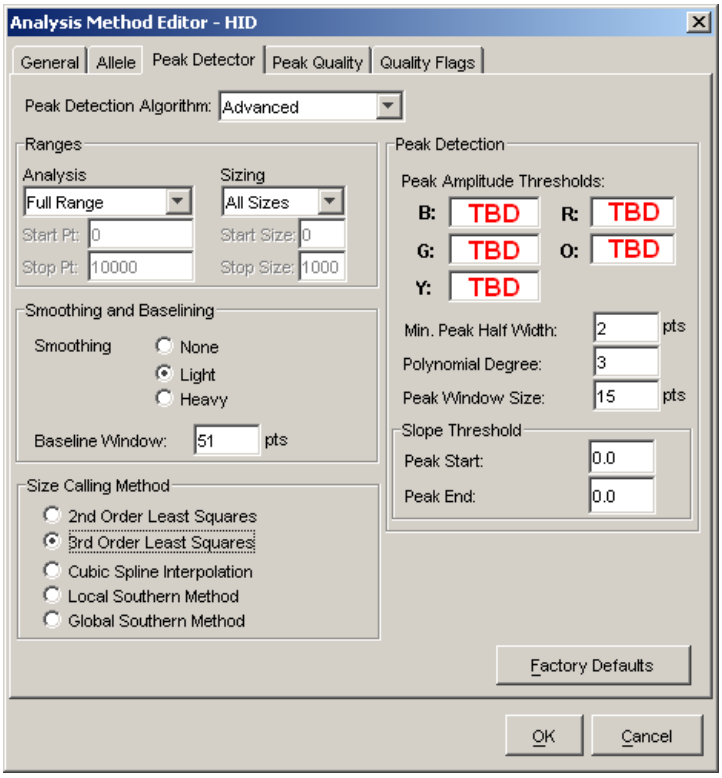
In the Name field, either type the name as shown for consistency with files supplied with other AmpFℓSTR® kits, or enter a name of your choosing. In the Security Group field, select the Security Group appropriate to your software configuration from the drop-down list. The Description and Instrument fields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

- Allele tab settings



  - In the Bin Set field, select the **NGM_bins_v2** 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 NGM_panel_v2 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™ 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™ 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 at Applied Biosystems. However, we recommend that users determine the settings appropriate for use in their laboratory during internal validation studies.

- Peak Detector tab settings



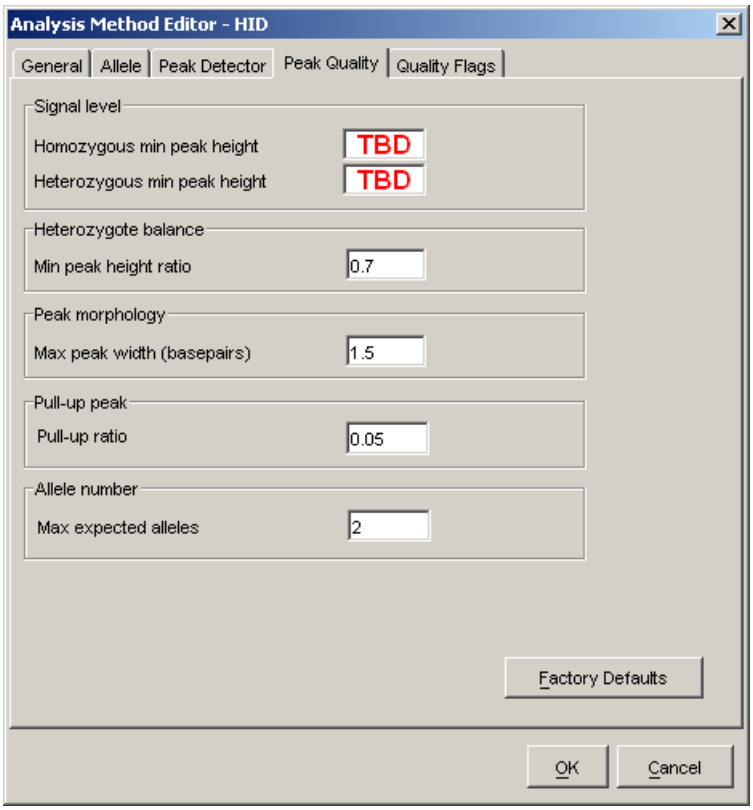
IMPORTANT! To be determined (TBD) indicates values to be determined in your laboratory. Laboratories must perform the appropriate internal studies to determine the peak amplitude thresholds for interpretation of NGM™ kit data.

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.

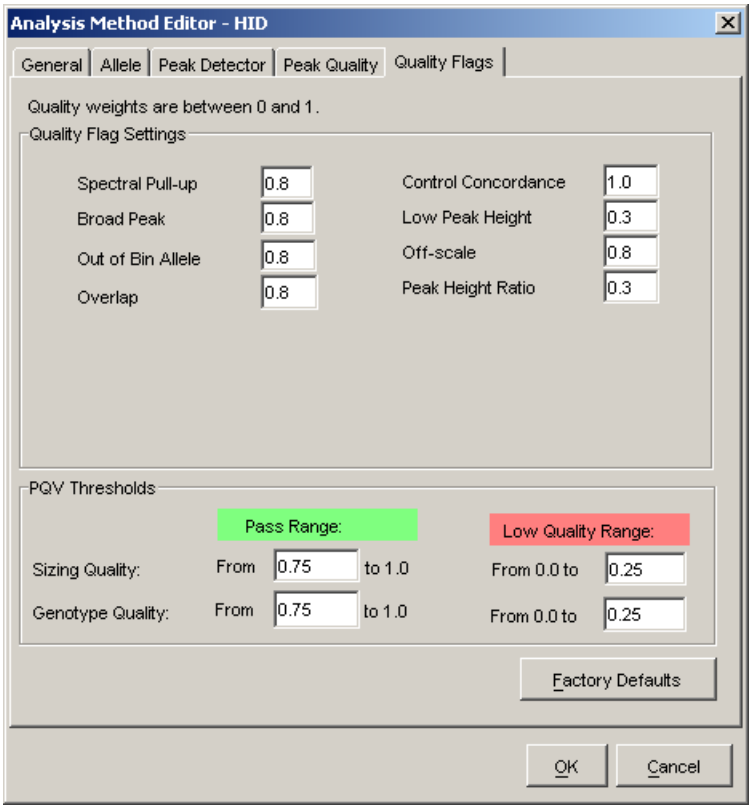
– **Size calling method** – The NGM™ kit has been validated using the 3rd Order Least Squares sizing method in combination with the GeneScan™-500 LIZ® size standard. Alternative sizing methods should be selected only after extensive evaluation as part of an internal validation study in the user's laboratory.

- Peak Quality tab settings



IMPORTANT! To be determined (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™ Kit data.

- Quality Flags tab settings



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

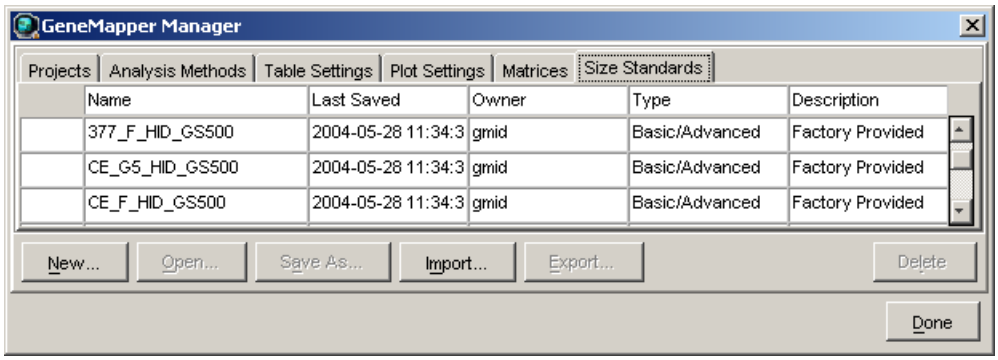
5. Click **Save**.

Create a HID size standard

The size standard for the AmpFℓSTR® NGM™ PCR Amplification Kit uses the following GeneScan™ 500 LIZ® Size Standard peaks in its sizing algorithm: 75, 100, 139, 150, 160, 200, 300, 350, 400, and 450.

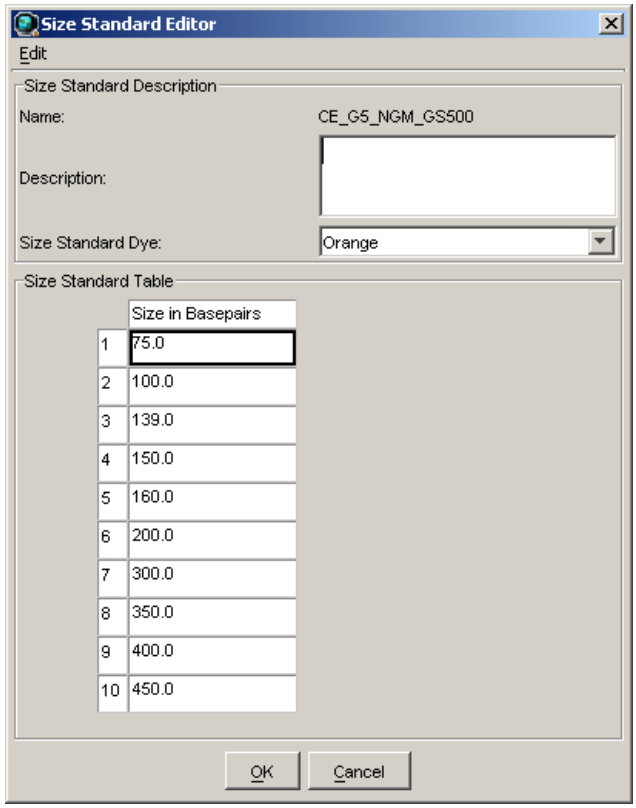
Use the following procedure to create the size standard for the AmpFℓSTR® NGM™ Kit.

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

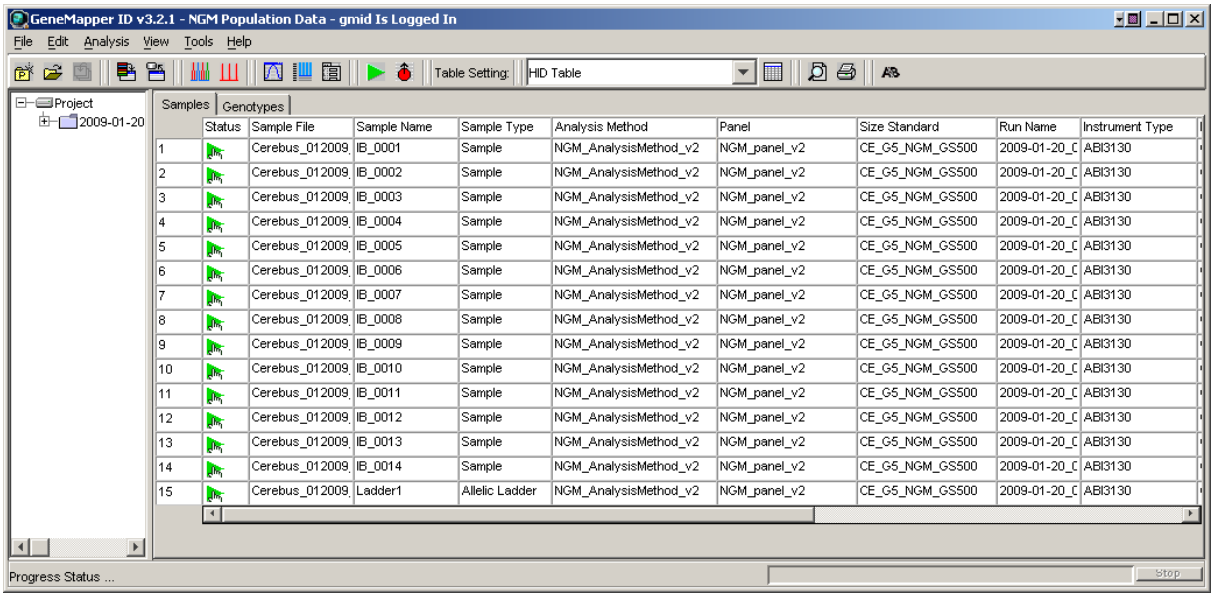
1. In the Project window, select **File** ▸ **Add Samples to Project**, then navigate to the disk or directory containing the sample files.

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 named the settings differently, select the name you specified.

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	**NGM_AnalysisMethod_v2**
Panel	**NGM_panel_v2**
Size Standard	**CE_G5_NGM_GS500**

   - 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* (Part no. 4335617).
   - CE_G5_NGM_GS500 (size standard fragments defined in the AmpFℓSTR® NGM™ Kit): 75, 100, 139, 150, 160, 200, 300, 350, 400, and 450. For additional information about size standards, refer to the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (Part no. 4338775).
   - CE_G5_NGM_GS500: Neither the 250-nt nor the 340-nt peak is included in the size standard definition. These peaks can be used as an indicator of precision within a run.

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 both:
     – 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 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 (Part no. 4335523)*
- *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide (Part no. 4338775)*
- *Installation Procedures and New Features for GeneMapper® ID Software Version v3.2 User Bulletin* (Part no. 4352543)

Section 4.1 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 an .fsa file (for 310 and 31xx CE instruments) or an .hid file (for 3500 and 3500xL instruments). Files in .fsa format can be analyzed by any version of GeneMapper *ID-X* software (that is v1.0 or higher); .hid files can only be analyzed by GeneMapper *ID-X* v1.2 or higher.

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 69.
- Create a size standard, as explained in "Create a size standard" on page 74.
- Define custom views of analysis tables.

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

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

Import panels, bins, and marker stutter

To import the AmpFℓSTR® NGM™ Kit panels, bin sets, and marker stutter from the Applied Biosystems web site into the GeneMapper® *ID-X* Software 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 **NGM Analysis Files GMIDX**.

   b. Unzip the file.

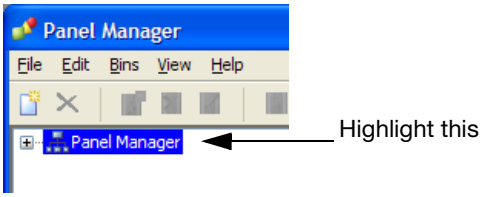
2. Start the GeneMapper® *ID-X* Software, then log in with the appropriate user name and password.

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

3. Select **Tools ▸ Panel Manager**.

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

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

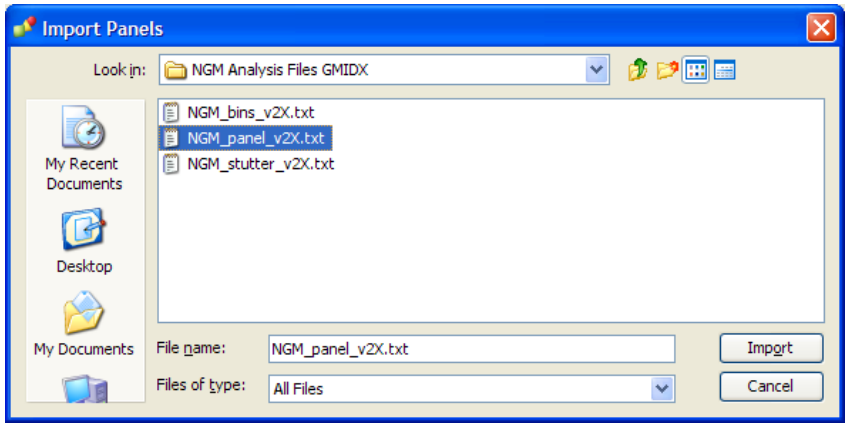
   

   b. Select **File ▸ Import Panels** to open the Import Panels dialog box.

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

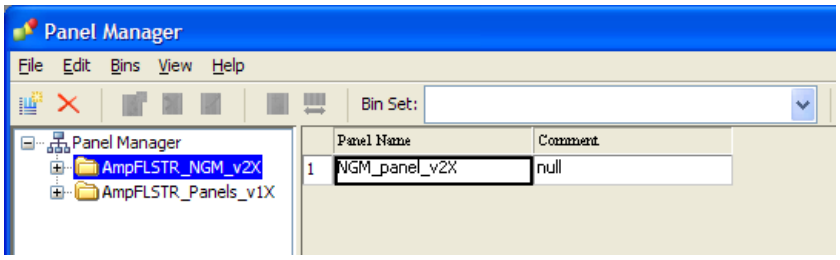
5. Select **NGM_panel_v2X**, then click **Import**.

Note: Importing this file creates a new folder in the navigation pane of the Panel Manager "AmpFLSTR_NGM_v2X". This folder contains the panel and associated markers.



6. Import NGM_bins_v2X:

a. Select the **AmpFLSTR_NGM_v2X** folder in the navigation pane.

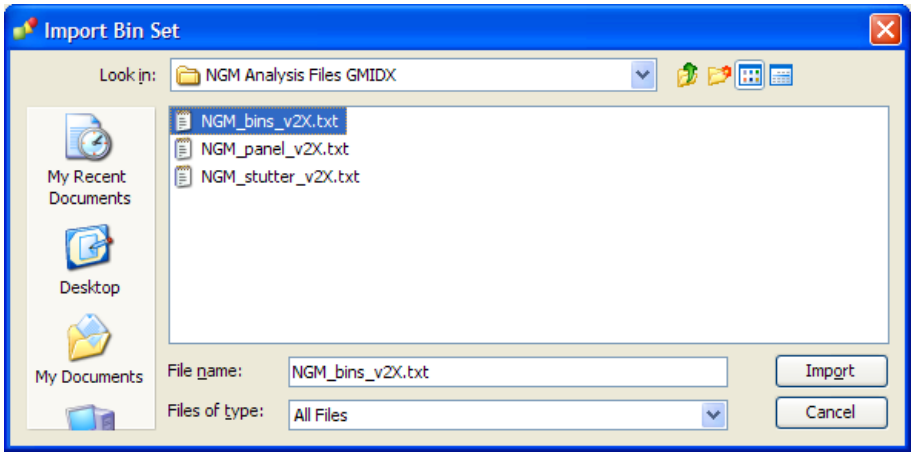


b. Select **File** ▸ **Import Bin Set** to open the Import Bin Set dialog box.

c. Navigate to, then open the **NGM Analysis Files GMIDX** folder.

d. Select **NGM_bins_v2X**, then click **Import**.

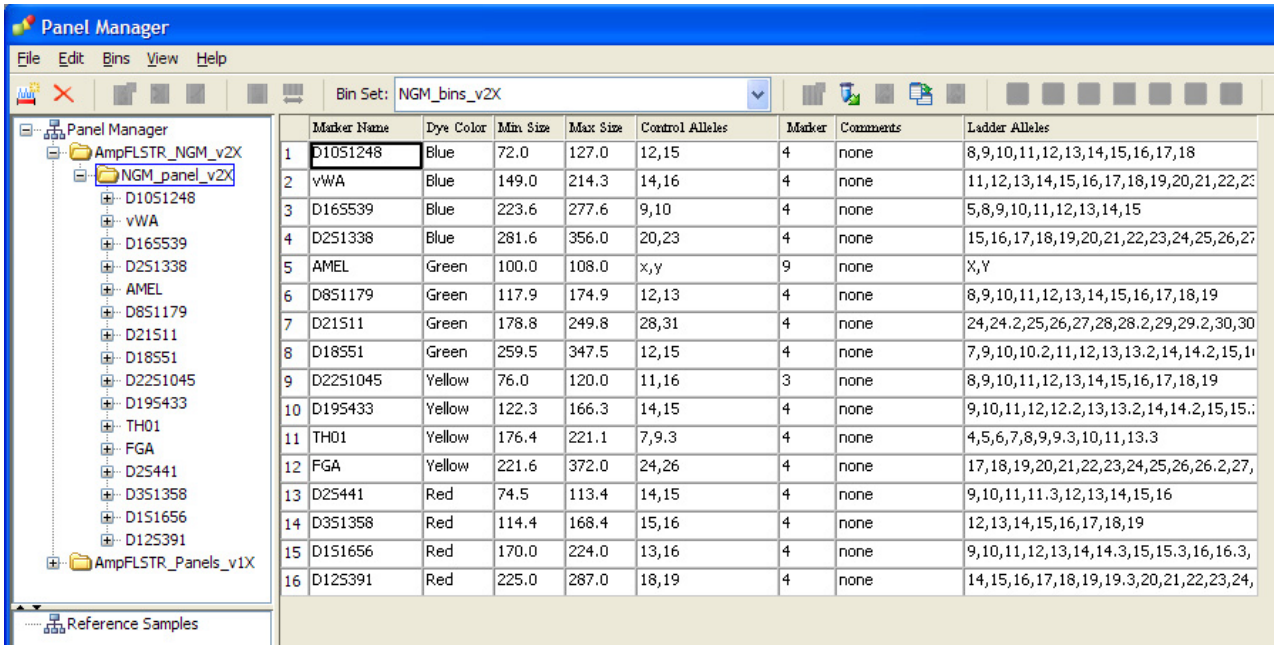
Note: Importing this file associates the bin set with the panels in the NGM_panel_v2X folder.



7. View the imported panels in the navigation pane:

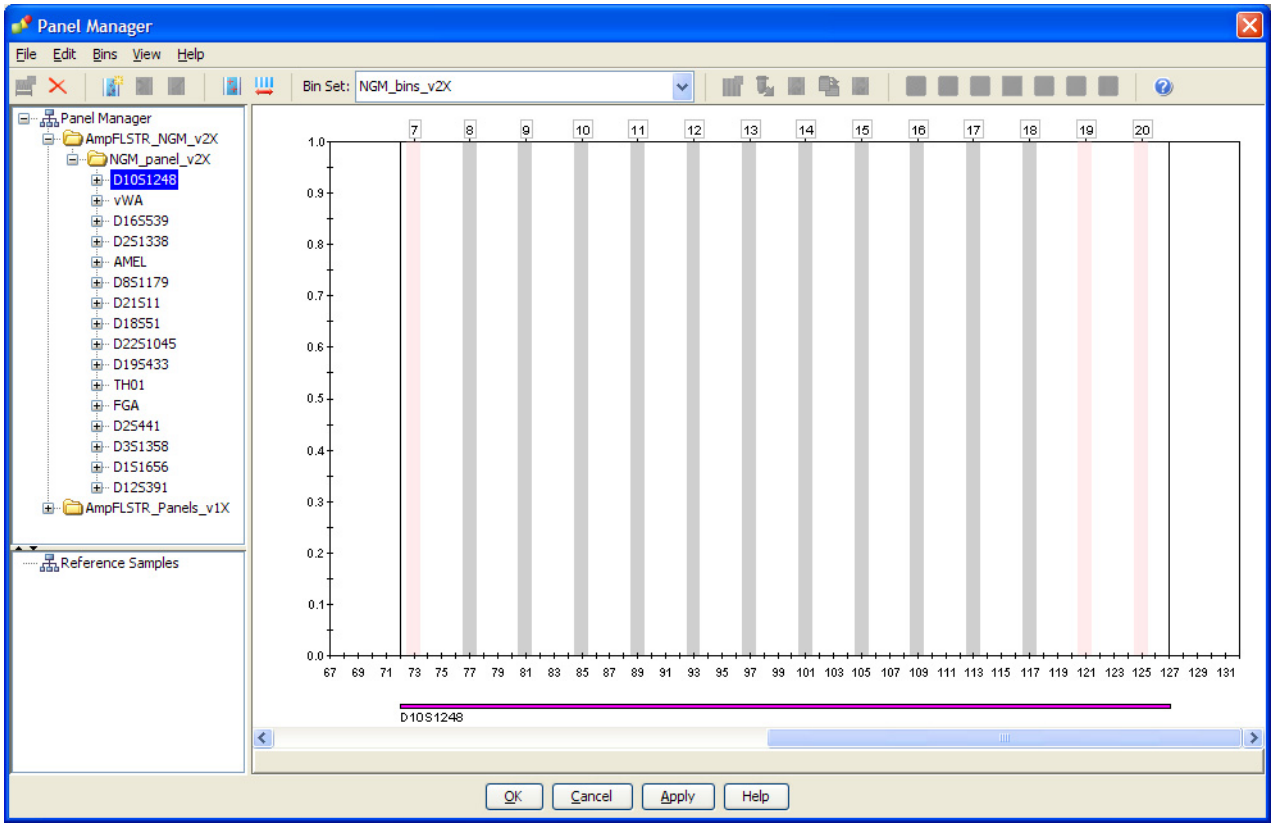
   a. Double-click the **AmpFLSTR_NGM_v2X** folder to view the NGM_panel_v2X folder.

   b. Double-click the **NGM_panel_v2X** folder to display the panel information in the right pane and the markers below it.



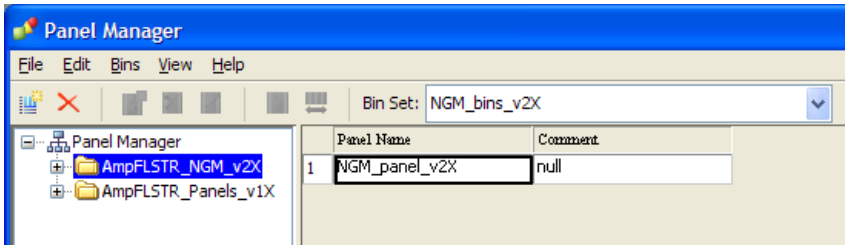
	Marker Name	Dye Color	Min Size	Max Size	Control Alleles	Marker	Comments	Ladder Alleles
1	D10S1248	Blue	72.0	127.0	12,15	4	none	8,9,10,11,12,13,14,15,16,17,18
2	vWA	Blue	149.0	214.3	14,16	4	none	11,12,13,14,15,16,17,18,19,20,21,22,23
3	D16S539	Blue	223.6	277.6	9,10	4	none	5,8,9,10,11,12,13,14,15
4	D2S1338	Blue	281.6	356.0	20,23	4	none	15,16,17,18,19,20,21,22,23,24,25,26,27
5	AMEL	Green	100.0	108.0	x,y	9	none	X,Y
6	D8S1179	Green	117.9	174.9	12,13	4	none	8,9,10,11,12,13,14,15,16,17,18,19
7	D21S11	Green	178.8	249.8	28,31	4	none	24,24.2,25,26,27,28,28.2,29,29.2,30,30
8	D18S51	Green	259.5	347.5	12,15	4	none	7,9,10,10.2,11,12,13,13.2,14,14.2,15,1
9	D22S1045	Yellow	76.0	120.0	11,16	3	none	8,9,10,11,12,13,14,15,16,17,18,19
10	D19S433	Yellow	122.3	166.3	14,15	4	none	9,10,11,12,12.2,13,13.2,14,14.2,15,15.
11	TH01	Yellow	176.4	221.1	7,9.3	4	none	4,5,6,7,8,9,9.3,10,11,13.3
12	FGA	Yellow	221.6	372.0	24,26	4	none	17,18,19,20,21,22,23,24,25,26,26.2,27,
13	D2S441	Red	74.5	113.4	14,15	4	none	9,10,11,11.3,12,13,14,15,16
14	D3S1358	Red	114.4	168.4	15,16	4	none	12,13,14,15,16,17,18,19
15	D1S1656	Red	170.0	224.0	13,16	4	none	9,10,11,12,13,14,14.3,15,15.3,16,16.3,
16	D12S391	Red	225.0	287.0	18,19	4	none	14,15,16,17,18,19,19.3,20,21,22,23,24,

8. Select **D10S1248** to display the Bin view for the marker in the right pane.



9. Import NGM_stutter_v2X:

   a. Select the **AmpFLSTR_NGM_v2X** folder in the navigation panel.

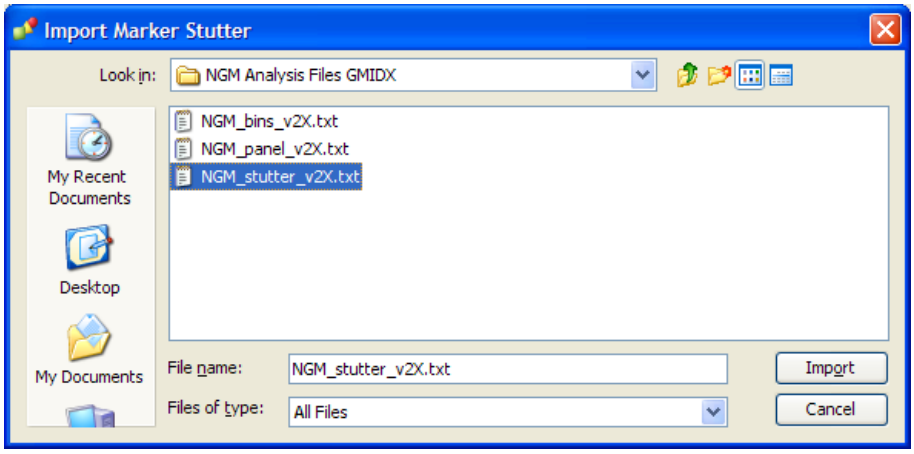


   b. Select File ▸ **Import Marker Stutter** to open the Import Marker Stutter dialog box.

   c. Navigate to, then open the **NGM Analysis Files GMIDX** folder.

   d. Select **NGM_stutter_v2X**, then click **Import**.

   Note: Importing this file associates the marker stutter ratio with the bin set in the NGM_bins_v2X folder.



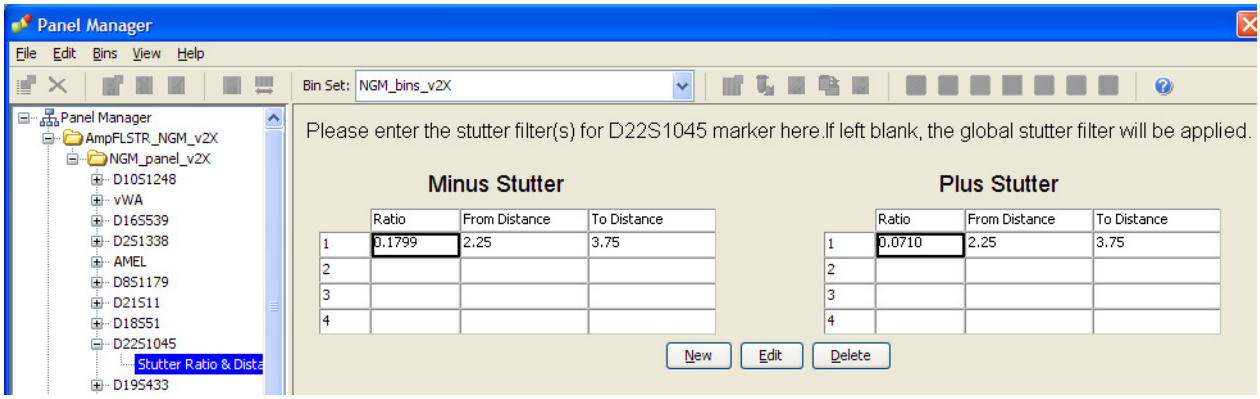
10. View the imported marker stutters in the navigation pane:

    a. Select the **NGM_panel_v2X** folder to display its list of markers in the right pane.

    b. Double-click the **NGM_panel_v2X** folder to display its list of markers below it.

    c. Double-click **D22S1045** to display the Stutter Ratio & Distance view for the marker in the right pane.

    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.



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

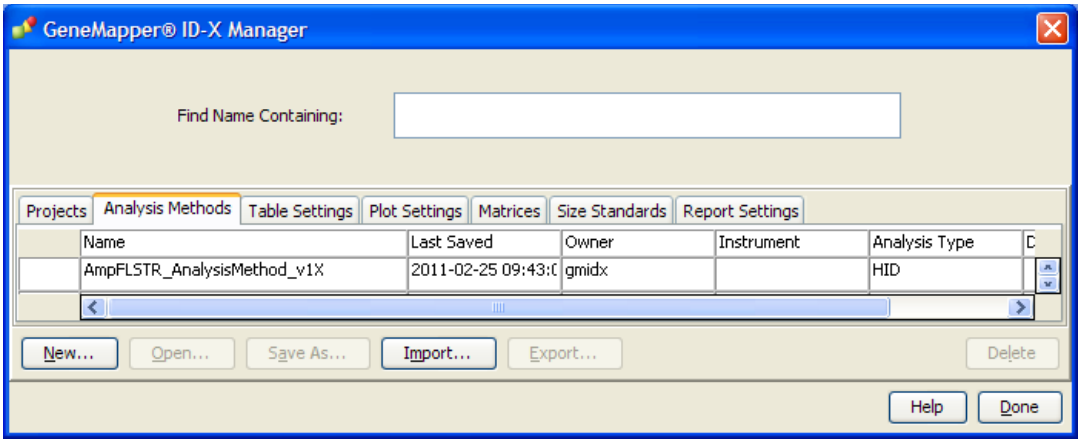
IMPORTANT! If you close the Panel Manager without clicking **Apply**, the panels, bin sets, and marker stutter will not be imported into the GeneMapper® *ID-X* Software database.

Create an analysis method

Use the following procedure to create an analysis method for the AmpFℓSTR® NGM™ 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 v1.0, v1.1 or with GeneMapper® *ID* Software version 3.2.1.

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

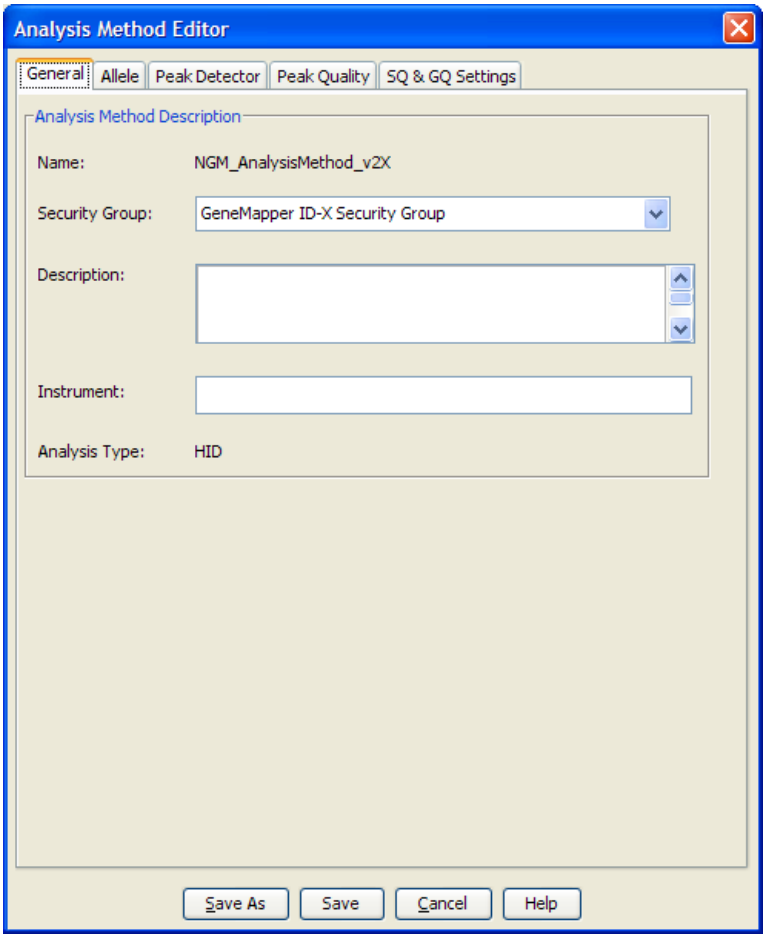


2. Select the **Analysis Methods** tab, then click **New** to open the Analysis Method Editor with the **General** tab selected.

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.

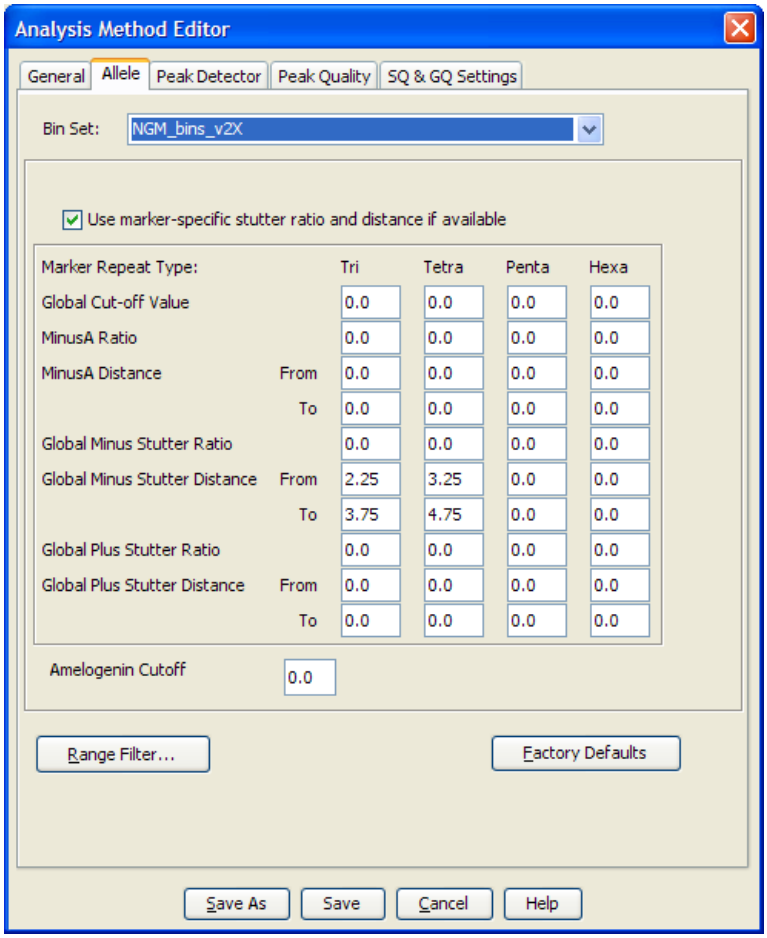
   Note: The Analysis Method Editor closes when you save your settings (see step 4 on page 74). To complete this step quickly, do not save the analysis method until you finish entering settings in all of the tabs.

- 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 drop-down list. The Description and Instrument fields are optional.

- Allele tab settings

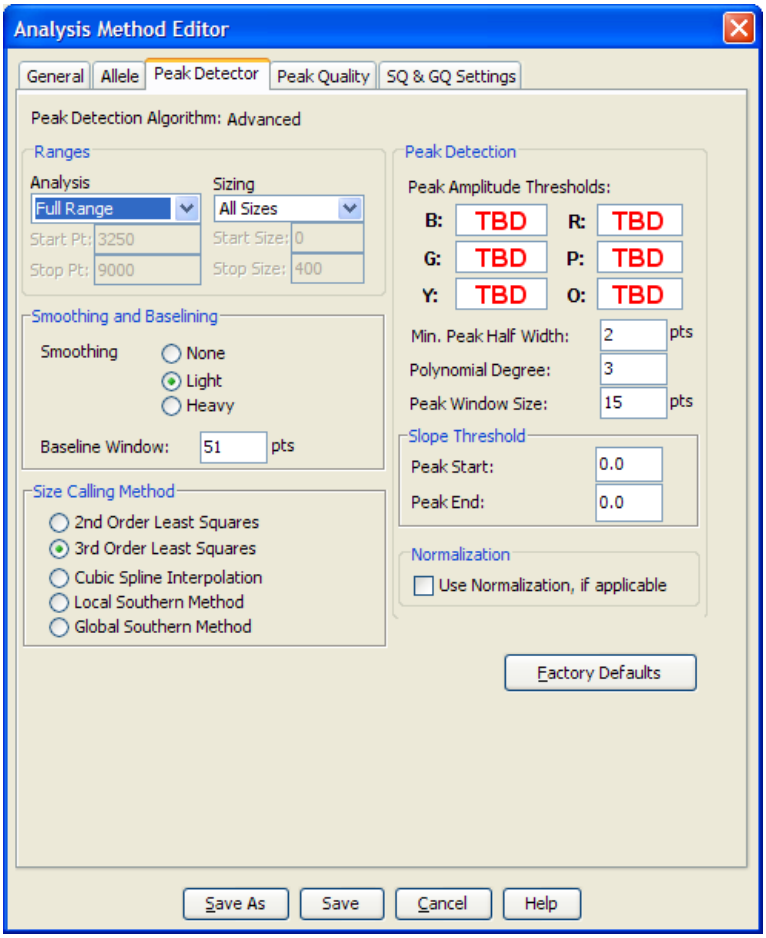


  – In the Bin Set field, select the **NGM_bins_v2X** bin set imported previously and configure the stutter distance parameters as shown.

  – GeneMapper® *ID-X* Software 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 NGM_stutter_v2X file.

- Peak Detector tab settings



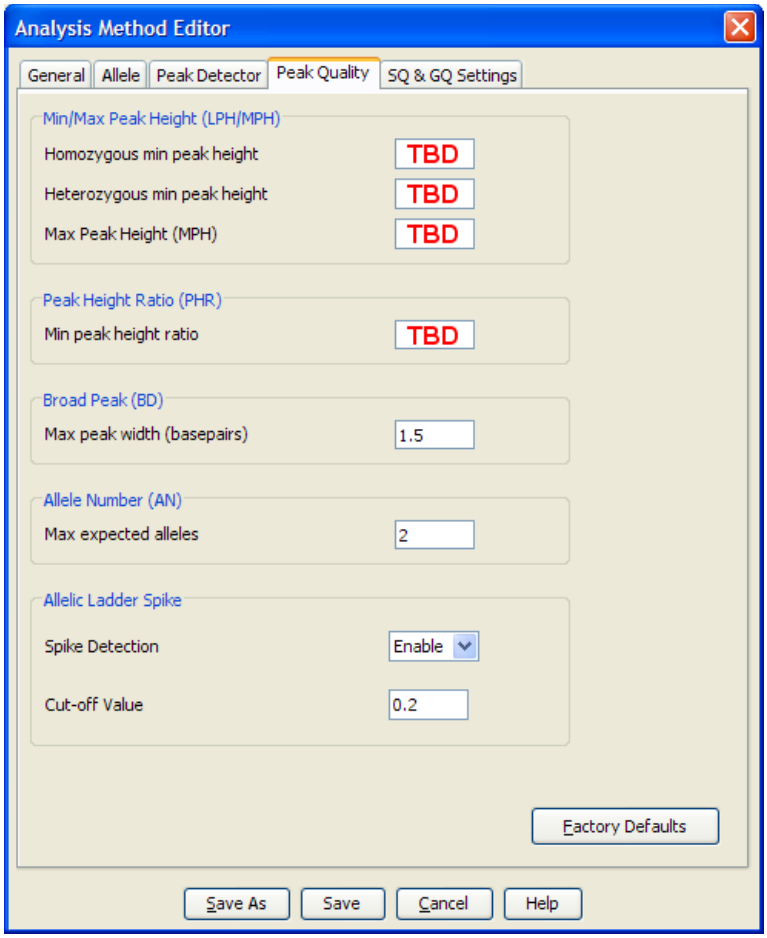
IMPORTANT! To be determined (TBD) indicates values to be determined in your laboratory. Laboratories must perform the appropriate internal studies to determine the appropriate peak amplitude thresholds for interpretation of AmpFℓSTR® NGM™ Kit data.

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™ kit has been validated using the 3rd Order Least Squares sizing method in combination with the GeneScan™-500 LIZ® size standard. Alternative sizing methods should be selected only after extensive evaluation as part of an internal validation study in the user's laboratory.

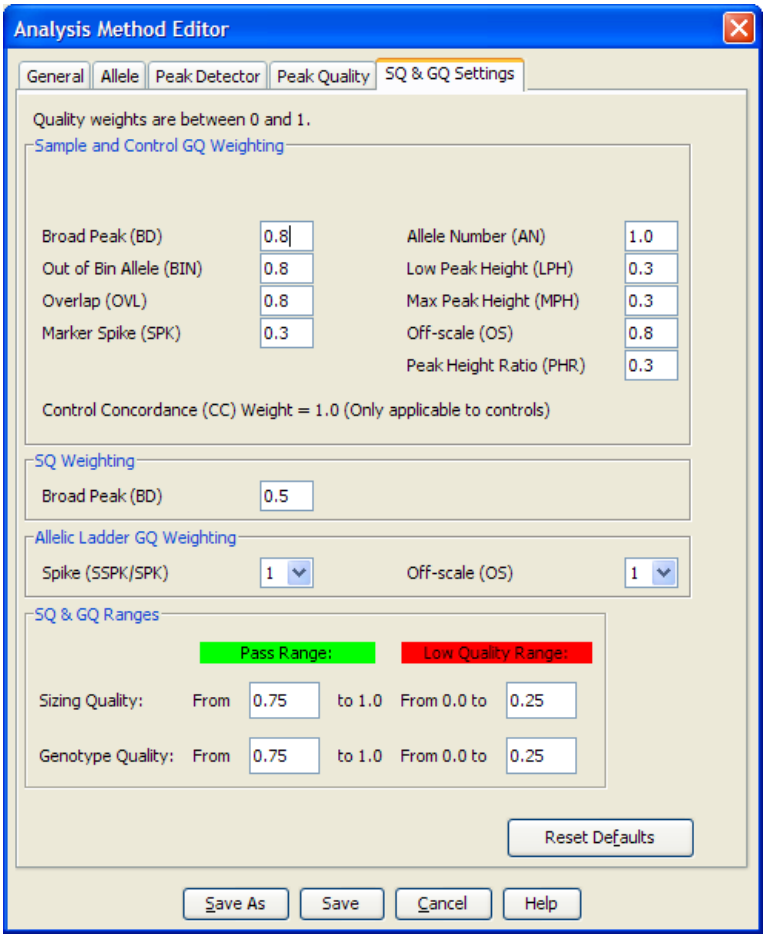
– **Normalization** – a Normalization checkbox is available on this tab in GeneMapper® *ID-X* Software v1.2 or higher for use in conjunction with data run on the Applied Biosystems 3500 Series Genetic Analyzers. Users of this version of software should perform laboratory evaluations to determine whether to use the Normalization feature for analysis of NGM™ kit data.

- Peak Quality tab settings



IMPORTANT! To be determined (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™ Kit data.

- SQ & GQ tab settings



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

4. Click **Save**.

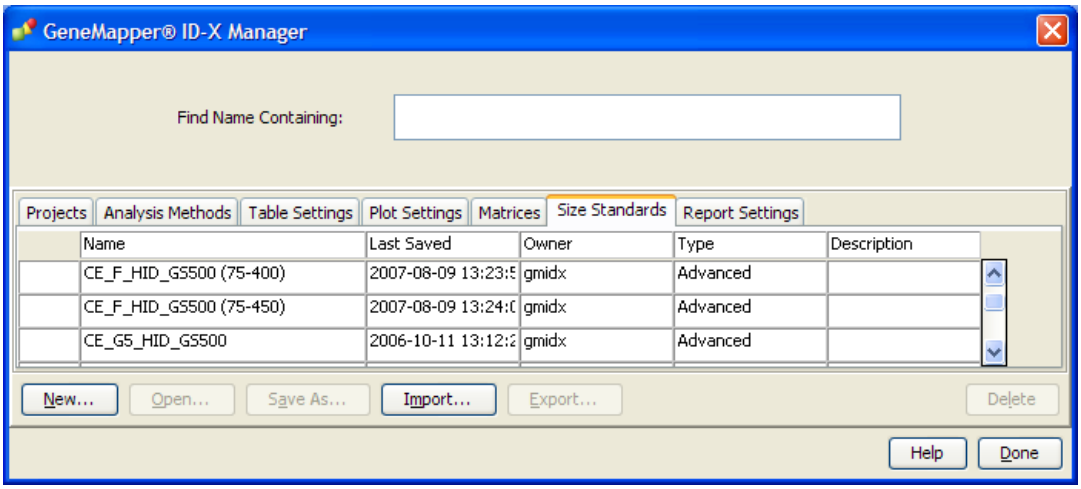
Create a size standard

The size standard for the AmpFℓSTR® NGM™ PCR Amplification Kit uses the following GeneScan™ 500 LIZ® Size Standard peaks in its sizing algorithm: 75, 100, 139, 150, 160, 200, 300, 350, 400, and 450.

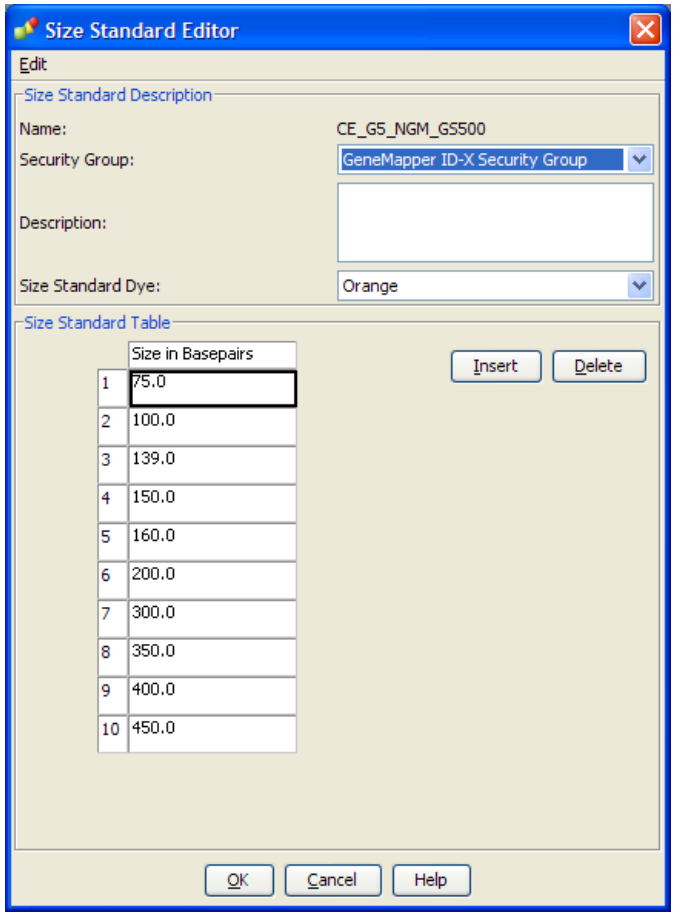
Use the following procedure to create the size standard for the AmpFℓSTR® NGM™ Kit.

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 drop-down 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 74.



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 navigate to the disk or directory containing the sample files.

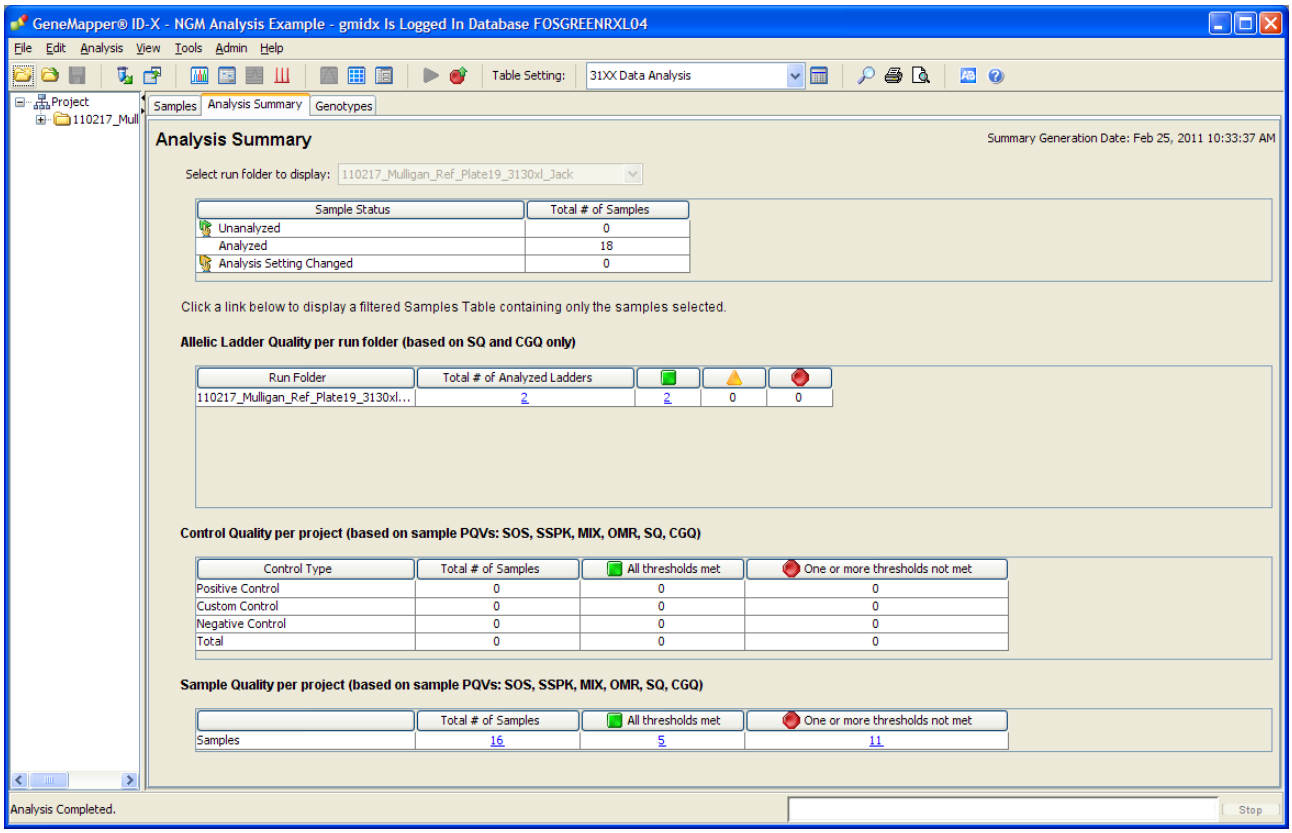
2. Apply analysis settings to the samples in the project. The names of the settings shown are the names suggested in the sections above. If you named the settings differently, select the name you specified.

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	NGM_AnalysisMethod_v2X
Panel	NGM_panel_v2X
Size Standard	CE_G5_NGM_GS500

   - 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* (Part no. 4335617).
   - CE_G5_NGM_GS500 (size standard fragments defined in the AmpFℓSTR® NGM™ Kit): 75, 100, 139, 150, 160, 200, 300, 350, 400, and 450. For additional information about size standards, refer to the *GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide* (Part no. 4338775).
   - CE_G5_NGM_GS500: Neither the 250-nt nor the 340-nt peak is included in the size standard definition. These peaks can be used as an indicator of precision within a run.

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. The figure below shows the 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* (Part no. 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* (Part no. 4375574)
  - *GeneMapper® ID-X Software Version 1.0 Quick Reference Guide* (Part no. 4375670)
  - *GeneMapper® ID-X Software Version 1.0 Reference Guide* (Part no. 4375671)
  - *GeneMapper® ID-X Software Version 1.1 (Mixture Analysis Tool) Getting Started Guide* (Part no. 4396773)

- *GeneMapper® ID-X Software Version 1.1 (Mixture Analysis Tool) Quick Reference Guide* (Part no. 4402094)
- *GeneMapper® ID-X Software Version 1.2 Quick Reference Guide* (Part no. 4426482)

Part Number 4466844 Rev. A 04/2011

Chapter 5

Experiments and Results

Experiments and Results 5

Content in this chapter to come at PRC.

Part Number 4466844 Rev. A 04/2011

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 and the DNA test samples at all loci	Incorrect volume or absence of either AmpF*ℓ*STR® NGM™ Master Mix or AmpF*ℓ*STR® NGM™ 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.
	AmpF*ℓ*STR NGM™ 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.
	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	**For ABI PRISM® 3100-*Avant* or Applied Biosystems 3100/3130*xl* runs:** Mix 1.0 µL of PCR product and 10 µL of Hi-Di™ Formamide/ GeneScan™ 500 LIZ® Size Standard solution. **For Applied Biosystems 3500/ 3500xL instrument runs:** Mix 1.0 µL of PCR product and 10 µL of Hi-Di™ Formamide/ GeneScan™ 500 LIZ® Size Standard solution. **For ABI PRISM® 310 instrument runs:** Mix 0.75 µL of PCR product and 24.25 µL of Hi-Di™ Formamide/ GeneScan™ 500 LIZ® Size Standard solution.
Faint or no signal from both the 007 and the DNA test samples at all loci (*continued*)	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.
	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.	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 cycle number is optimized according to instructions on page 26. Repeat PCR amplification using fewer PCR cycles or use your laboratory's SOP to analyze off-scale data.
	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) at 29 cycles; 500 pg at 30 cycles.
	Incomplete denaturation of double stranded DNA	Use the recommended amount of Hi-Di™ Formamide and perform heat denaturation according to instructions on page 36.
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 only.

Ordering Information B

Materials and equipment not included

The tables below list optional equipment and materials not supplied with the AmpFℓSTR® NGM™ Kit. Unless otherwise noted, many of the items are available from major laboratory suppliers (MLS).

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	
Applied Biosystems 310 Genetic Analyzer	
GeneAmp® PCR System 9700 with the Silver 96-Well Block	N8050001
GeneAmp® PCR System 9700 with the Gold-plated Silver 96-Well Block	4314878
Silver 96-Well Sample Block	N8050251
Gold-plated Silver 96-Well Sample Block	4314443
Tabletop centrifuge with 96-Well Plate Adapters (optional)	MLS

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
Note: For a complete list of parts and accessories for the 3500/3500xL instrument, refer to the *Applied Biosystems 3500/3500xL Genetic Analyzer User Guide* (Part no. 4401661)	
AmpFℓSTR® NGM™ PCR Amplification Kit (200x/1000x)	4415020/4415021

Item	Source
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™ 500 LIZ® Size Standard	4322682
Running Buffer, 10×	402824
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
MicroAmp® Optical 96-Well Reaction Plate	N8010560
250-µL Glass Syringe (array-fill syringe)	4304470
5.0-mL Glass Syringe (polymer-reserve syringe)	628-3731
Note: 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* (Part no. 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™ 500 LIZ® Size Standard	4322682
Running Buffer, 10×	402824
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
MicroAmp® Optical 96-Well Reaction Plate	N8010560
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* (Part no. 4352716).	
310 Analyzer materials	
310 DNA Analyzer Capillary Array, 47-cm	402839
0.5 mL Sample Tray	5572
96-Well Tray Adaptor (for 9700 thermal cycler trays)	4305051
GeneScan™ 500 LIZ® Size Standard	4322682
Running Buffer, 10×	4335643
Genetic Analyzer Septa Retainer Clips for 96-Tube Sample Tray	402866
Genetic Analysis Sample Tubes (0.5-mL)	401957
Septa for 0.5-mL Sample Tubes	401956
DS-33 Matrix Standard Set (6-FAM™, VIC®, NED™, PET®, and LIZ® dyes) for ABI PRISM® 310/377 systems	4318159
MicroAmp® 8-Tube Strip, 0.2-mL	N8010580
MicroAmp® 96-Well Base (holds 0.2-mL reaction tubes)	N8010531

Item	Source
MicroAmp® 96-Well Full Plate Cover	N8010550
MicroAmp® 96-Well Tray/Retainer Set	403081
POP-4™ Polymer for the 310 Genetic Analyzer	402838
For a complete list of parts and accessories for the 310 instrument, refer to Appendix B of the *ABI PRISM® 310 Genetic Analyzer User Guide* (Part no. 4317588).	
PCR Amplification	
MicroAmp® 96-Well Tray	N8010541
MicroAmp® Reaction Tube with Cap, 0.2-mL	N8010540
MicroAmp® 8-Tube Strip, 0.2-mL	N8010580
MicroAmp® 8-Cap Strip	N8010535
MicroAmp® 96-Well Tray/Retainer Set	403081
MicroAmp® 96-Well Base	N8010531
MicroAmp® Clear Adhesive Film	4306311
MicroAmp® Optical Adhesive Film	4311971
MicroAmp® Optical 96-Well Reaction Plate	N8010560
Other user-supplied materials	
Hi-Di™ Formamide, 25-mL	4311320
Aerosol resistant pipette tips	MLS
Microcentrifuge tubes	MLS
Pipettors	MLS
Tape, labeling	MLS
Tube, 50-mL Falcon	MLS
Tube decapper, autoclavable	MLS
Deionized water, PCR grade	MLS
Tris-HCL, pH 8.0	MLS
EDTA, 0.5 M	MLS
Vortex	MLS

Safety

C

This appendix covers:

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 94.)
- 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 SDS for any chemical supplied by Applied Biosystems 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 Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

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

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

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

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the 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* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**)
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

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.

Specific chemical alerts

CAUTION! CHEMICAL HAZARD. AmpFℓSTR® NGM™ PCR Amplification Kit may cause eye, skin, and respiratory tract irritation. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

WARNING! CHEMICAL HAZARD. POP-4™ Polymer for 3130/3130*xl* Genetic Analyzers causes skin, eye, and respiratory tract irritation.

WARNING! CHEMICAL HAZARD. Running Buffer, 10 causes skin, eye, and respiratory tract irritation.

WARNING! CHEMICAL HAZARD. Hi-Di™ Formamide is harmful if swallowed, inhaled or absorbed through skin, and causes irritation to skin, eyes, and respiratory tract. It affects the central nervous system and may affect the reproductive system.

WARNING! CHEMICAL HAZARD. POP-4™ Polymer for 3100/3100-*Avant* Genetic Analyzers is irritating to eyes, respiratory system, and skin. It causes adverse cardiovascular effects. It contains a known or suspected reproductive toxin and a known or suspected mutagen.

WARNING! CHEMICAL HAZARD. POP-7™ Polymer for the 3730 Genetic Analyzer is harmful by inhalation and if swallowed and irritating to eyes, respiratory system, and skin.

Documentation

Related documentation

For additional documentation, see "How to obtain support" on page 102.

Document title	Part number
ABI PRISM® 3100/3100-Avant Data Collection v2.0 User Guide	4347102
ABI PRISM® 3100/3100-Avant Genetic Analyzers Using Data Collection Software v2.0 User Bulletin	4350218
ABI PRISM® 3100 Genetic Analyzer User Manual (Data Collection v1.1)	4315834
ABI PRISM® 3100/3100-Avant Genetic Analyzers Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products User Bulletin	4332345
AmpFℓSTR® NGM™ PCR Amplification Kit - PCR Setup Quick Reference Card	4442401
AmpFℓSTR® NGM™ PCR Amplification Kit - CE Quick Reference Card	4442693
Applied Biosystems 3130/3100xl Genetic Analyzers Using Data Collection Software v3.0 User Bulletin	4363787
Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide	4352715
Applied Biosystems 3130/3130xl Genetic Analyzers Maintenance, Troubleshooting, and Reference Guide	4352716
Applied Biosystems 3130/3130xl Genetic Analyzers Quick Reference Card	4362825
Applied Biosystems 3130/3130xl Genetic Analyzers AB Navigator Software Administrator Guide	4359472
Applied Biosystems 3130/3100xl DNA Analyzers User Guide	4331468
Applied Biosystems 3730/3730xl Genetic Analyzer Getting Started Guide	4359476
Quantifiler® Kits: Quantifiler® Human DNA Quantification Kit and Quantifiler® Y Human Male DNA Quantification Kit User's Manual	4344790
PrepFiler™ Forensic DNA Extraction Kit User Guide	4390932
GeneMapper® ID Software Version 3.1 Human Identification Analysis User Guide	4338775
GeneMapper® ID Software Versions 3.1 and 3.2 Human Identification Analysis Tutorial	4335523
Installation Procedures and New Features for GeneMapper® ID Software v3.2 User Bulletin	4352543
GeneMapper® ID-X Software Version 1.0 Getting Started Guide	4375574
GeneMapper® ID-X Software Version 1.0 Quick Reference Guide	4375670
GeneMapper® ID-X Software Version 1.0 Reference Guide	4375671
GeneMapper® ID-X Software Version 1.1 (Mixture Analysis) Getting Started Guide	4396773

Document title	Part number
GeneMapper® ID-X Software Version 1.1 (Mixture Analysis) Quick Reference Guide	4402094

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

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

How to obtain support

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

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

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

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