applied biosystems

CLA GlobalFiler™ PCR Amplification Kit

USER GUIDE

for Cell Line Authentication

Catalog Number A65909

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Revision B.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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Revision history: MAN0018827 B.0 (English)

Revision	Date	Description
B.0	2 February 2024	• The kit catalog number was changed: Cat. No. A44662 is now A65909. • Storage conditions were updated for the primer mix and master mix ("Contents and storage" on page 14). • Compatible instruments and software were updated (throughout the user guide). • Copy edits were made to align with the current documentation style.
A.0	26 March 2020	New document for the CLA GlobalFiler™ PCR Amplification Kit.

The information in this guide is subject to change without notice.

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Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The CLA GlobalFiler™ PCR Amplification Kit is a 6-dye, 24-locus short tandem repeat (STR) analysis kit with 3 sex-discriminating markers. The kit:

- Uses genomic DNA.
- Can identify sample cross-contamination, sample mix-up, and sample mixtures.
- Is compatible with all cell line databasing loci standards and global databasing loci standards.
- Simplifies the amplification step during the authentication of cell lines.
- Provides reduced amplification time and increased discrimination power compared to the AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kit and AmpFℓSTR™ Identifiler™ Direct PCR Amplification Kit.

Use in HSA and MSA

Cell line authentication using STR analysis kits can be used for human sample authentication (HSA) and mixed sample analysis (MSA).

- In HSA, you can use the kits to verify the DNA profile of the sample or to perform a quality check.
- In MSA, you can use the kits to deconvolute the presence of multiple contributors in a sample (chimerism) by identifying more than one DNA genotype in the sample.

Related products

Additonal CLA kits are available:

- CLA Identifiler™ Plus PCR Amplification Kit is a 5-dye, 16-locus short tandem repeat (STR) analysis kit with 1 sex-discriminating marker. The kit uses genomic DNA. The kit amplifies the same loci as the AmpFℓSTR™ Identifiler™ kit.
- CLA Identifiler™ Direct PCR Amplification Kit is a 5-dye, 16-locus short tandem repeat (STR) analysis kit with 1 sex-discriminating marker. The kit uses buccal samples collected on swabs or blood/buccal samples collected on treated or untreated FTA paper. The kit amplifies the same loci as the AmpFℓSTR™ Identifiler™ Plus kit and does not require DNA extraction or purification.

Identifying the presence of contaminating cells in a culture

One objective of cell line authentication is to determine whether a cell line is contaminated with unrelated cells. Because contaminating cells are likely to have a different STR profile than the parent cell line, use of CLA STR kits is a useful tool to flag the presence of contamination.

In a mixture of cell lines, the final DNA profile reflects the genomic makeup of all cells present. Therefore, data must be examined carefully.

- One peak at a locus could represent:
  - Homozygous parent cell DNA
  - Homozygous parent cell and homozygous contaminating cell DNA
  - Homozygous parent cell DNA and a deletion at the locus for contaminating cell DNA
- Two peaks at a locus could represent:
  - Heterozygous parent cell DNA
  - Both cell types homozygous for different loci
  - Both cell types heterozygous for the same loci
- Three or more peaks at a locus could represent:
  - Mixture of two cell types, with one homozygous for one allele, the other heterozygous for different alleles
  - Three or more different cell lines
  - Drift of a cell line's allelic make-up over continual passage
  - A combination of the above

The interpretation of aberrant peaks at a single locus can be challenging and ambiguous. However, with the analysis of 24 loci and 3 sex-discriminating markers in the CLA GlobalFiler™ PCR Amplification Kit, it can be possible to identify distinct peaks that clearly point to the presence of a contaminating cell line, even if the genomic makeup of the contaminating cell line is not fully discernible.

Dyes used in the kit

Table 1 CLA GlobalFiler™ kit dyes

Dye	Color	Label
6-FAM™	Blue	Samples, allelic ladders, and controls
VIC™	Green	
NED™	Yellow	
TAZ™	Red	
SID™	Purple	
LIZ™	Orange	GeneScan™ 600 LIZ™ Size Standard v2.0

Loci amplified by the kit

Table 2 CLA GlobalFiler™ kit loci and alleles

Locus designation	Chromosome location	Alleles included in the allelic ladder	Dye label	DNA Control 007
D3S1358	3p21.31	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20	6-FAM™	15, 16
vWA	12p13.31	11,12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24		14, 16
D16S539	16q24.1	5, 8, 9, 10, 11, 12,13, 14, 15		9, 10
CSF1PO	5q33.3-34	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		11, 12
TPOX	2p23-2per	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15		8, 8
Y indel	Yq11.221	1, 2	VIC™	2
Amelogenin	X: p22.1-22.3 Y: p11.2	X, Y		X, Y
D8S1179	8q24.13	5, 6, 7, 8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 19		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		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		12, 15
DYS391	Yq11.21	7, 8, 9, 10, 11, 12, 13		11
D2S441	2p14	8, 9, 10, 11, 11.3, 12, 13, 14, 15, 16, 17	NED™	14, 15
D19S433	19q12	6, 7, 8, 9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, 19.2		14, 15

Table 2 CLA GlobalFiler kit loci and alleles *(continued)*

Locus designation	Chromosome location	Alleles included in the allelic ladder	Dye label	DNA Control 007
TH01	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3	NED™	7, 9.3
FGA	4q28	13, 14, 15, 16, 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		24, 26
D22S1045	22q12.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	TAZ™	11, 16
D5S818	5q21-31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18		11, 11
D13S317	13q22-31	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16		11, 11
D7S820	7q11.21-22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		7, 12
SE33	6q14	4.2, 6.3, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20.2, 21, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 27.2, 28.2, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35, 35.2, 36, 37		17, 25.2
D10S1248	10q26.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	SID™	12, 15
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		13, 16
D12S391	12p13.2	14, 15, 16, 17, 18, 19, 19.3, 20, 21, 22, 23, 24, 25, 26, 27		18, 19
D2S1338	2q35	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28		20, 23

Standards and controls in the kit

The CLA GlobalFiler™ kit requires the following standards and controls for PCR amplification, PCR product sizing, and genotyping:

Item	Description	Included in the kit
DNA Control 007	A positive control for evaluating the efficiency of the amplification step and STR genotyping of the kit allelic ladder. See “DNA Control 007 profile” on page 11.	Yes
GlobalFiler™ Allelic Ladder	Developed for accurate characterization of the alleles amplified in the kit. The allelic ladder allows automatic genotyping of most of the reported alleles for the loci in the kit. See “Loci amplified by the kit” on page 9 and “Allelic ladder profile” on page 12.	Yes
GeneScan™ 600 LIZ™ Size Standard v2.0	Used for obtaining sizing results. This standard, which has been evaluated as an internal size standard, yields precise sizing results for PCR products. See “GeneScan™ 600 LIZ™ Size Standard v2.0” on page 13.	Yes

DNA Control 007 profile

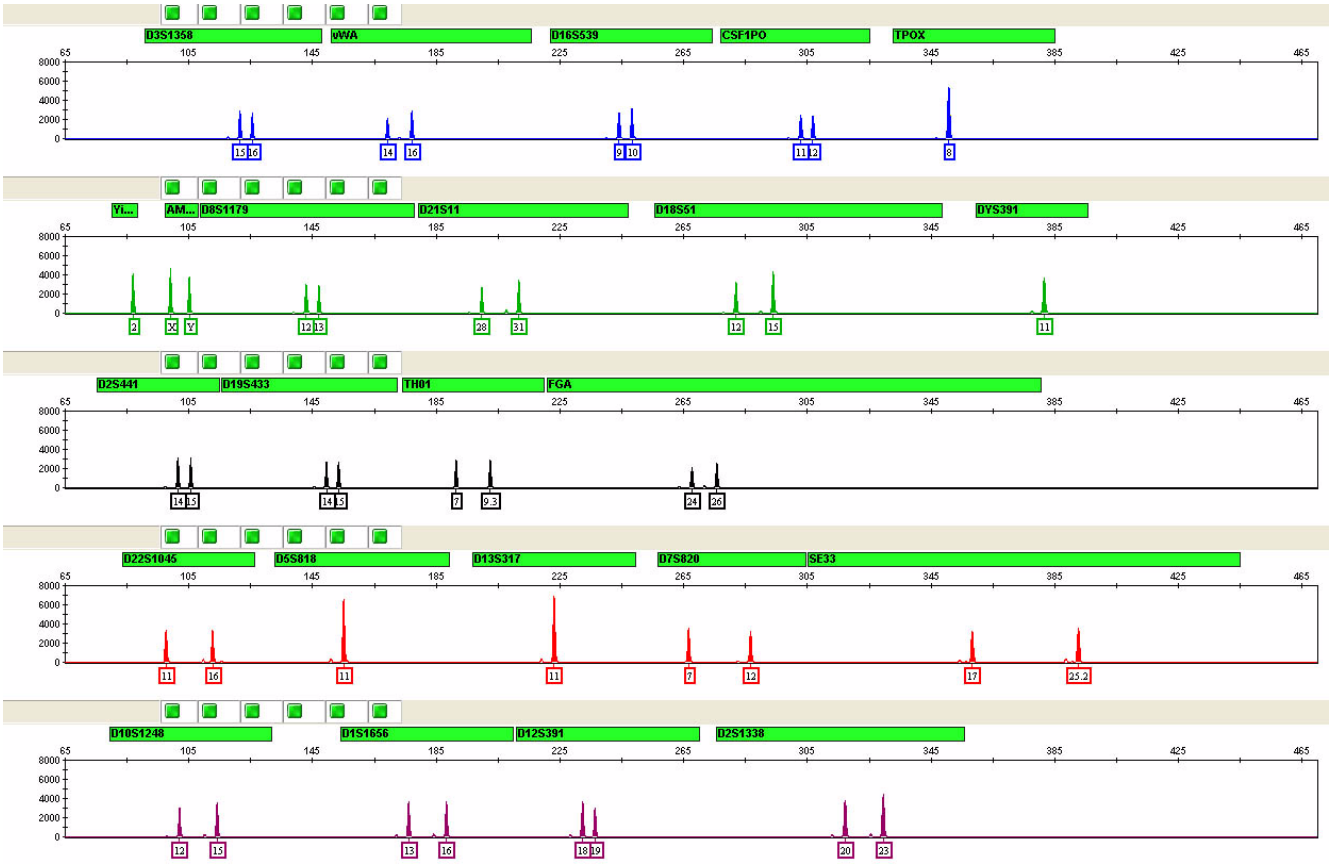


Figure 1 DNA Control 007 (1 ng) amplified with the CLA GlobalFiler™ kit and analyzed on a 3500xL Genetic Analyzer (Y-axis scale 0–8,000 RFU).

Allelic ladder profile

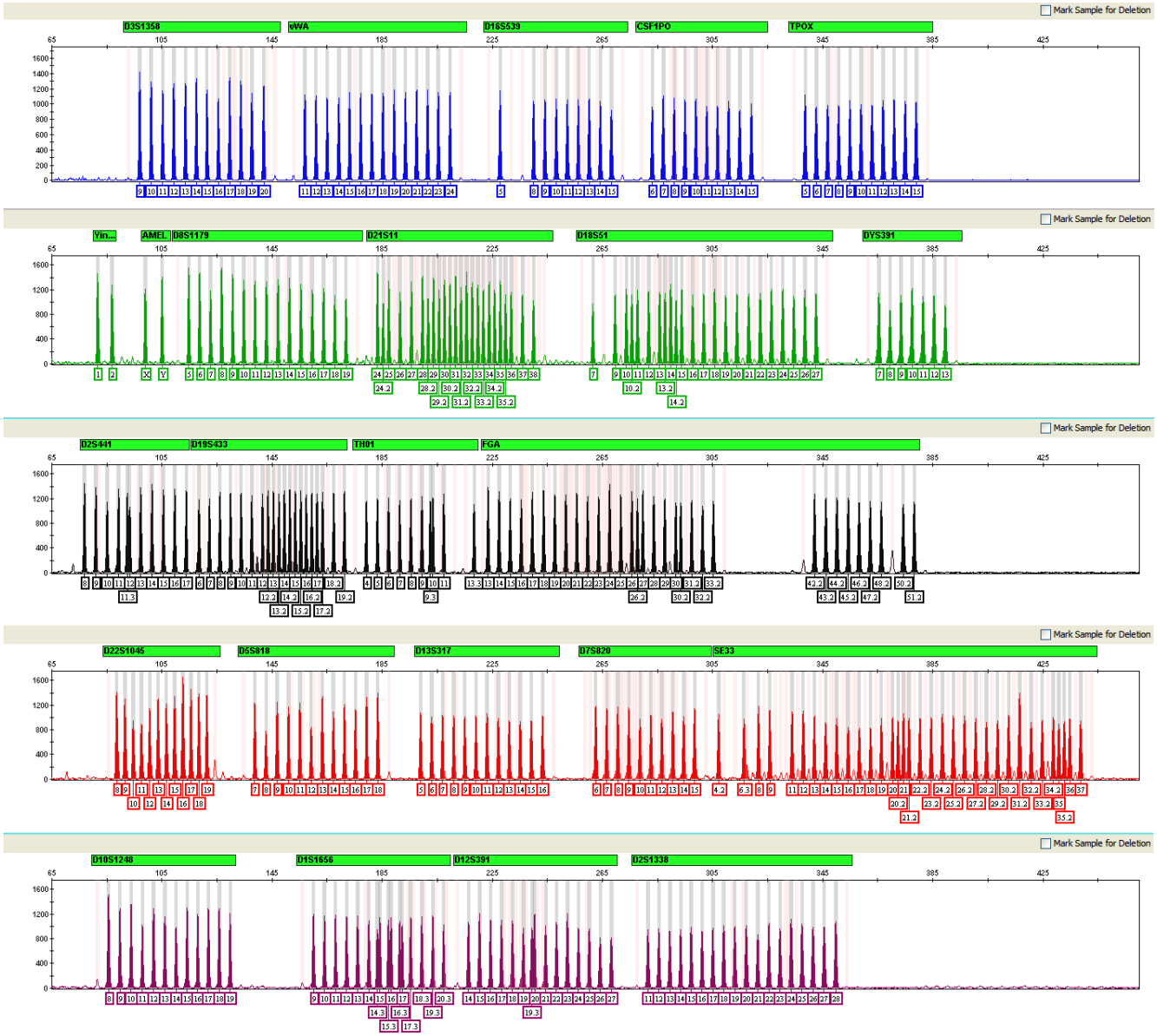


Figure 2 GeneMapper™ Software plot of the GlobalFiler™ Allelic Ladder

GeneScan™ 600 LIZ™ Size Standard v2.0

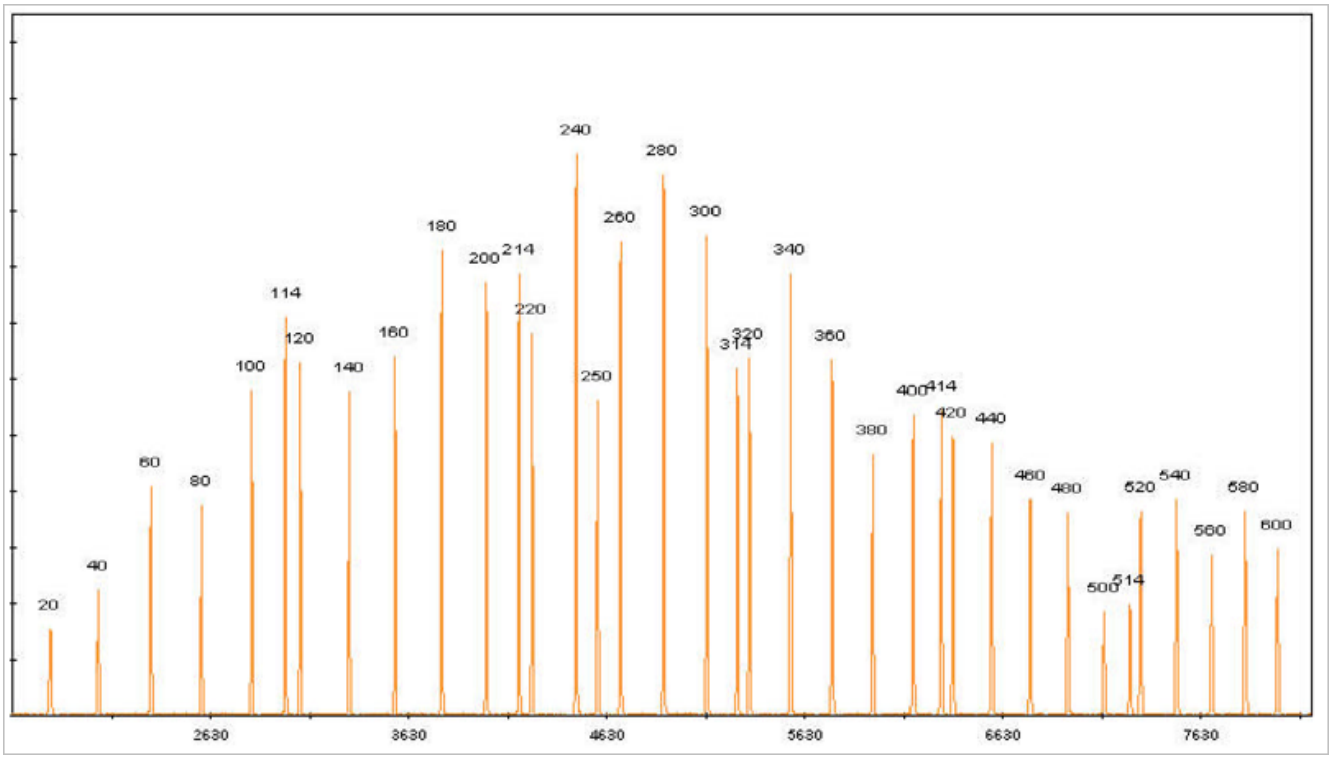


Figure 3 GeneMapper™ Software plot of the GeneScan™ 600 LIZ™ Size Standard v2.0

Contents and storage

The CLA GlobalFiler™ kit contains sufficient quantities of the reagents for 200 amplification reactions at 25 µL/reaction.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer mix, amplified DNA, allelic ladder, and size standard from light when not in use.

IMPORTANT! Do not refreeze kit components after thawing.

Item	Description	Amount	Storage[1]
		200 reactions (Cat. No. A65909)	
GlobalFiler™ Master Mix	Contains enzyme, salts, dNTPs, bovine serum albumin, and 0.05% sodium azide in buffer and salt.	1 × 1.5 mL	–25°C to –15°C on receipt. 2–8°C after first use, up to the expiration date stated on the kit.
DNA Control 007	Contains 0.1 ng/µL human male genomic DNA from cell line in 0.05% sodium azide and buffer.[2] See "DNA Control 007 profile" on page 11 for information.	1 × 0.3 mL	
GlobalFiler™ Primer Mix	Contains forward and reverse primers to amplify human DNA targets.	1 × 0.5 mL	–25°C to –15°C on receipt. 2–8°C after first use, up to the expiration date stated on the kit. Store protected from light.
GlobalFiler™ Allelic Ladder	Contains amplified alleles. See "Allelic ladder profile" on page 12 for information.	1 × 0.065 mL	
GeneScan™ 600 LIZ™ Size Standard v2.0	Contains 36 single-stranded labeled fragments of: 20, 40, 60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, 460, 480, 500, 514, 520, 540, 560, 580, and 600 nucleotides.	2 × 200 µL (~800 reactions) [3]	2–8°C, protected from light. Do not freeze.

[1] See packaging for expiration date. Do not use expired product.

[2] DNA Control 007 is included at a concentration that is appropriate for use as an amplification control (that is, to provide confirmation of the capability of the kit reagents to generate a profile of expected genotype). It is not designed for use as a DNA quantification control. If you quantify aliquots of Control 007, the concentration may differ from the labeled concentration.

[3] The total number of reactions will vary depending on the specific application. This number is based on the volumes specified in this document.

Required materials not supplied

See Appendix A, "Materials required but not supplied".

Instrument and software compatibility

Note: Compatible instruments and software that have been discontinued are not listed in this user guide.

Thermal cyclers

- VeritiPro™ Thermal Cycler, 96-well
- ProFlex™ 96-well PCR System
- ProFlex™ 2 × 96-well PCR System
- ProFlex™ 3 × 32-Well PCR System

Genetic analyzers and data collection software

Genetic analyzer	Data collection software
SeqStudio™ Flex Series Genetic Analyzer	SeqStudio™ Flex Series Instrument Software v1.0, v1.0.2, or v1.1.1
SeqStudio™ Genetic Analyzer	SeqStudio™ Data Collection Software v1.2.4
	SeqStudio™ Data Collection Software v1.2.1
	SeqStudio™ Data Collection Software v1.2
3500/3500xL Genetic Analyzer	3500 Series Data Collection Software v3.3 (Windows™ 10 operating system)
	3500 Series Data Collection Software v 3.1 (Windows™ 7 operating system)
3730/3730*xl* DNA Analyzer **Note:** We recommend the 48-capillary array for the 3730*xl* instrument. The close proximity of capillaries in the 96-capillary array can introduce cross-talk between capillaries and interfere with data interpretation.	• 3730*xl* Data Collection Software 5 (Windows™ 10 operating system) • 3730/3730*xl* Data Collection Software 4.1 (Windows™ 10 operating system)
	3730/3730*xl* Data Collection Software 4.0 (Windows™ 7 operating system)

Analysis software

- Microsatellite Analysis Software (accessible from **apps.thermofisher.com**)
- GeneMapper™ Software v6 Software (Windows™ 10 operating system)
- GeneMapper™ Software v5 Software (Windows™ 7 operating system)

For more information

- For testing information on specific platforms, see the instrument or software user documentation.
- For ordering information, see Appendix A, “Materials required but not supplied”.

Workflow

CLA GlobalFiler™ PCR Amplification Kit

Extract DNA

1. Go to thermofisher.com, then search for gDNA purification solutions.

Perform PCR

1. “Prepare the amplification kit reactions” on page 19
2. “Perform PCR” on page 20

Perform capillary electrophoresis

1. “(Before first use of the kit) Set up the capillary electrophoresis instrument” on page 22
2. “Prepare samples for electrophoresis and start the run” on page 26

Analyze data

- Chapter 4, “Analyze data with Microsatellite Analysis Software”
- Chapter 5, “Analyze data with GeneMapper™ Software”

2 Perform PCR: GlobalFiler™ kit

Guidelines for cell line preparation, extraction, and purification

Follow your standard laboratory operating protocol and consider the following guidelines:

- The required DNA input amount is 1 ng of genomic DNA extracted from cells.
- If a cell line is to be used for research and the identity is unknown, expand the cell line before use.
- Follow cell culture best practices.
- Handle cell lines under quarantine conditions if the cell line identity is unknown.
- One T-25 flask (for attached cells) or a 125 mL Erlenmeyer (for suspension) provides enough material for DNA extraction.
- Obtain cells at an early passage.
- Continue growing cells while STR analysis is performed. Consider quarantine until the STR identification is available. After obtaining the STR identification, proceed with preparing freeze stocks or continued use of the cells for additional experiments.
- Clean the work surface area thoroughly.
- Prepare samples one at a time.
- Clearly label samples with cell line information.
- Extract, purify, and quantify the DNA, see **www.thermofisher.com/gdnaprep**.

Effect of DNA quantity on results

The required DNA input amount is 1.0 ng for 29-cycle PCR or 500 pg for 30-cycle PCR.

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

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

To address these problems, rerun the amplification reaction using less DNA.

If too little DNA is added to the PCR reaction, the total number of allele copies added to the PCR reaction could be extremely low. Unbalanced amplification of the alleles can occur because of stochastic fluctuation.

Before you begin

(Optional) Prepare low-TE buffer

For optimal results, we recommend using low-TE buffer for sample preparation. Prepare the low-TE buffer as described in this procedure or purchase TE Buffer (Cat. No. 12090015).

1. Mix the buffer components 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 proportionally for specific needs.
2. Aliquot, then autoclave the solutions.
3. Store the aliquots at room temperature.

(Before first use of the kit) Thaw reagents

Thaw the master mix and primer mix.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer mix, amplified DNA, allelic ladder, and size standard from light when not in use.

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

Prepare the amplification kit reactions

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer mix, amplified DNA, allelic ladder, and size standard from light when not in use.

1. Vortex the master mix and primer mix for 3 seconds. Before opening the tubes or bottles, remove droplets from the caps by briefly centrifuging the tubes or tapping the bottles on the bench.

2. Pipet the required volumes of reaction mix components into an appropriately-sized, clear (non-colored), polypropylene tube.

Component	Amount per reaction
Master mix	7.5 µL
Primer set	2.5 µL

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

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

4. Pipet 10 µL of the reaction mix into each well of a MicroAmp™ Optical 96-Well Reaction Plate or each MicroAmp™ tube.

5. *(If needed)* Adjust the sample input amount and volume.
   - If the total sample input amount is >1.0 ng for 29 cycles or >500 pg for 30 cycles, dilute with low-TE buffer to achieve a 15-µL input volume.
   - If the total sample input volume is <15 µL, bring to volume with low-TE buffer to achieve a 15-µL input volume.

6. Prepare the samples and controls as shown in the following table, then add to the appropriate wells or tubes. The final reaction volume is 25 µL.

Component	Amount per reaction	
	29-cycle protocol	30-cycle protocol
Negative control	15 µL of low-TE buffer	15 µL of low-TE buffer
Test sample	15 µL of DNA	15 µL of DNA
Positive control	Combine, then add to the reaction well or tube: • 10 µL of control DNA (0.1 ng/µL) • 5 µL of low-TE buffer	Combine, then add to the reaction well or tube: • 5 µL of control DNA (0.1 ng/µL) • 10 µL of low-TE buffer

7. Seal the plate with MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical Adhesive Film, or cap the tubes.

   IMPORTANT! We recommend adhesive film for plate sealing to provide a consistent seal across all wells and prevent evaporation. Do not use caps for the plate, which may not provide a consistent seal across all wells.

8. Centrifuge the tubes or plate at 3,000 rpm for ~20 seconds in a tabletop centrifuge (with plate holders, if using 96-well plates).

Proceed to "Perform PCR" on page 20.

Perform PCR

1. Program the thermal cycler.
   a. Set the ramping mode to **9700 Simulation**.
   b. Set the thermal cycling conditions as shown in the following table.

Initial incubation step	Cycle (29 or 30 cycles)		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE		HOLD	HOLD
95°C, 1 minute	94°C, 10 seconds	59°C, 90 seconds	60°C, 10 minutes	4°C, Up to 24 hours[1]

[1] The infinity (∞) setting allows an unlimited hold time.

2. Load the plate or tubes into the thermal cycler, close the heated cover, then start the run.

3. When the run is complete, store the amplified DNA.

Storage time	Temperature
<2 weeks	2–8°C
>2 weeks	–25°C to –15°C

IMPORTANT! Protect the amplified DNA from light.

3 Perform electrophoresis

Allelic ladder requirements for electrophoresis

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

Instrument	Number of allelic ladders to run	One injection equals	Number of samples per allelic ladder
SeqStudio™ 24 Flex Genetic Analyzer	1 per injection	24 samples	23 samples + 1 allelic ladder
SeqStudio™ 8 Flex Genetic Analyzer	1 per 3 injections	8 samples	23 samples + 1 allelic ladder
SeqStudio™ Genetic Analyzer	1 per 6 injections	4 samples	23 samples + 1 allelic ladder
3500xL Genetic Analyzer	1 per injection	24 samples	23 samples + 1 allelic ladder
3500 Genetic Analyzer	1 per 3 injections	8 samples	23 samples + 1 allelic ladder
3730[1]	2 per injection	48 samples	46 samples + 2 allelic ladders

[1] We recommend the 48-capillary array for the 3730*xl* instrument. The close proximity of capillaries in the 96-capillary array can introduce cross-talk between capillaries and interfere with data interpretation.

IMPORTANT! Variation in laboratory temperature can cause changes in fragment migration speed and sizing variation between runs. Follow the guidelines in the preceding table, which should account for normal variation in run speed. To facilitate accurate genotyping of all samples in your laboratory environment, perform internal validation studies to verify the required allelic ladder injection frequency.

Materials required for electrophoresis

Appendix A, “Materials required but not supplied” lists the required materials that are not supplied with the kit.

IMPORTANT! The fluorescent dyes attached to the primers are light-sensitive. Protect the primer mix, amplified DNA, allelic ladder, and size standard from light when not in use.

(Before first use of the kit) Set up the capillary electrophoresis instrument

Data collection software setup

To analyze PCR products generated by the kit, you can use the data collection software and run parameters provided in this section. See the appropriate table for your instrument.

Note: For detailed procedures, see the appropriate user documentation for your instrument, as listed in Appendix C, “Documentation and support”.

Table 3 Software setup: SeqStudio™ Flex Series Genetic Analyzer

SeqStudio™ Flex Data Collection Software	*(Optional)* Additional software	Run parameters
v1.0, v1.0.2, or v1.1.1	• SAE Administrator Console v2.1 • SeqStudio™ Plate Manager Software v2.1, v2.1.1 • SeqStudio™ Flex Remote Monitoring Software	**Size standard:** GS600LIZ(60–600)
		Run module—Factory default run modules: • FragmentAnalysis36_POP7(xl) • FragmentAnalysis50_POP7(xl)
		Injection protocol: • Fragment_Protocol_36_POP7(xl) • Fragment_Protocol_50_POP7(xl)
		Injection conditions and run conditions: Per the factory default run modules
		Dye set: J6 (DS-36)
		Analysis settings: Fragment_Default

Table 4 Software setup: SeqStudio™ Genetic Analyzer

SeqStudio™ Data Collection Software	*(Optional)* Additional software	Plate setup
v1.1.4 or later	• SAE Administrator Console v2.0, v2.1 • SeqStudio™ Plate Manager Software v1.2, V2.0	**Run module:** FragAnalysis
		Dye set: J6 (DS-36)

Table 5 Software setup: 3500/3500xL Genetic Analyzer

Operating system	3500 Data Collection Software	Run parameters
Windows™ 10	v3.3	**Size standard:** GS600LIZ(60–600)
		Run module—Factory default run modules: • FragmentAnalysis36_POP7(xl) • FragmentAnalysis50_POP7(xl)
		Injection protocol: • Fragment_Protocol_36_POP7(xl) • Fragment_Protocol_50_POP7(xl)
		Injection conditions and run conditions: Per the factory default run modules
		Dye set: J6 (DS-36)
		Analysis settings: Fragment_Default
Windows™ 7	v3, v3.1	Same conditions as for 3500 Data Collection Software v3.3

Table 6 Software setup for the 3730/3730*xl* DNA Analyzer

Operating system	3730/3730*xl* Data Collection Software	Run parameters
Windows™ 10	• *(3730xl only)* v5 • v4.1	**Run module:** • GeneMapper_36_POP7_1 • GeneMapper_50_POP7_1
		Injection conditions: 1.2 kV/15 seconds (xl: 24 seconds)
Windows™ 7	v4.0	**Run conditions:** 13 kV/1,550 seconds
		Dye set: J6

Note: We recommend the 48-capillary array for the 3730*xl* instrument. The close proximity of capillaries in the 96-capillary array can introduce cross-talk between capillaries and interfere with data interpretation.

Spectral calibration

Ensure that the spectral calibration is current: DS-36 Matrix Standard Kit (Dye set J6, 6-dye). If the spectral calibration is not current, see the user documentation for your instrument for instructions on performing the calibration.

Capillary-to-plate mapping

SeqStudio™ Flex Series Genetic Analyzer

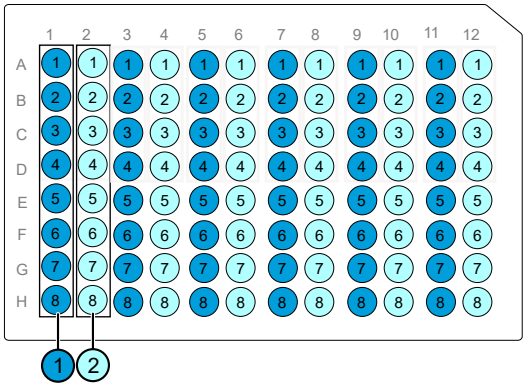


Figure 4 SeqStudio™ Flex 96-well plate and strip-tube capillary-to-plate mapping (8 capillary)

- Injection 1, 3, 5, 7, 9, 11 (wells A–H)
- Injection 2, 4, 6, 8, 10, 12 (wells A–H)

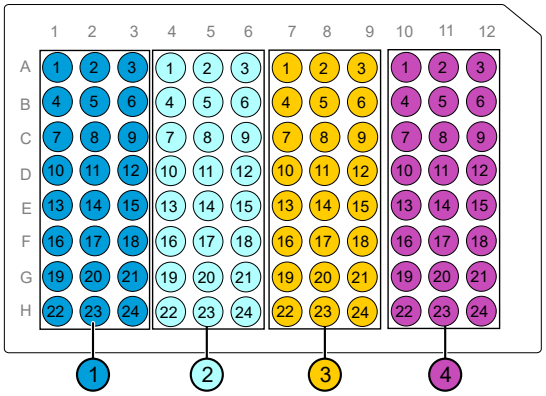


Figure 5 SeqStudio™ Flex 96-well plate and strip-tube capillary-to-plate mapping (24 capillary)

- Injection 1 (wells A1–H3)
- Injection 2 (wells A4–H6)
- Injection 3 (wells A7–H9)
- Injection 4 (wells A10–H12)

SeqStudio™ Genetic Analyzer

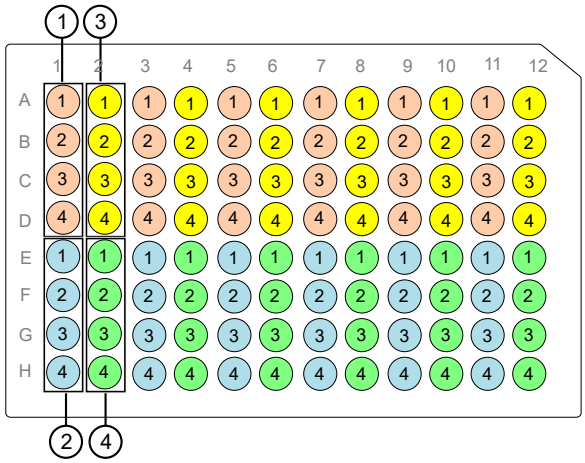


Figure 6 SeqStudio™ capillary-to-plate mapping

- Injection 1 (wells A1–D1)
- Injection 2 (wells E1–H1)
- Injection 3 (wells A2–D2)
- Injection 4 (wells E2–H2)

3500/3500xL Genetic Analyzer

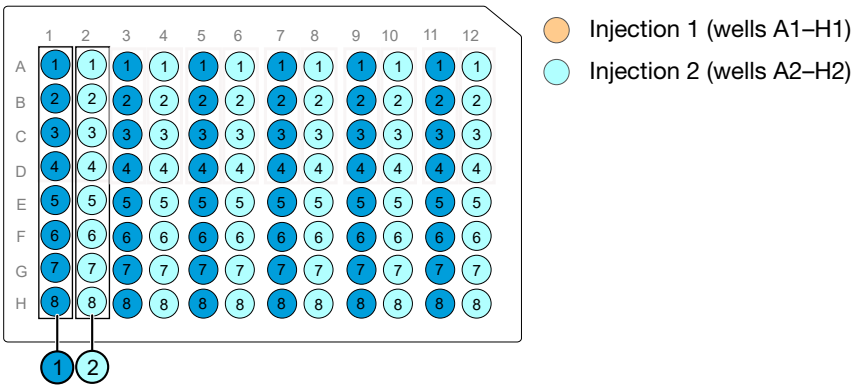


Figure 7 3500 96-well plate capillary-to-plate mapping (8 capillary)

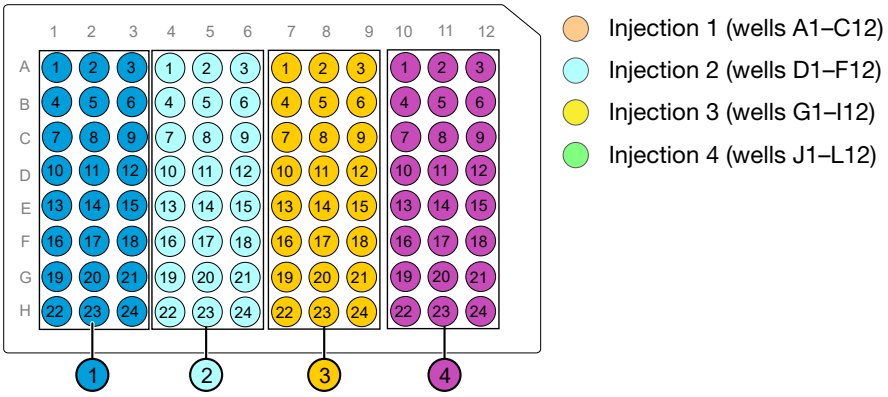


Figure 8 3500xL 96-well plate capillary-to-plate mapping (24 capillary)

3730/3730*xl* DNA Analyzer

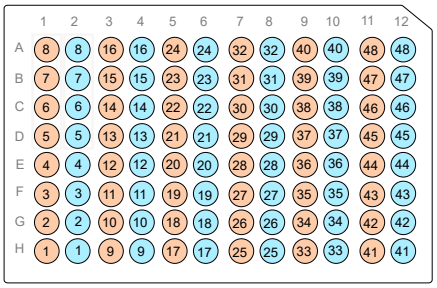


Figure 9 3730/3730*xl* capillary-to-plate mapping (48-capillary array)

Prepare samples for electrophoresis and start the run

Prepare the samples for electrophoresis immediately before loading.

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

Component	Amount per reaction
GeneScan™ 600 LIZ™ Size Standard v2.0	0.5 µL
Hi-Di™ Formamide	8.5 µL

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

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

2. Vortex the tube, then briefly centrifuge.

3. Pipet the required volumes of components into each well of a MicroAmp™ Optical 96-Well Reaction Plate.

Component	Amount per reaction
Formamide/size standard mixture	9 µL
PCR product or allelic ladder	1 µL

Note: For blank wells, add 10 µL of Hi-Di™ Formamide.

4. Seal the reaction plate with film, then briefly vortex and centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.

5. Heat the reaction plate in a thermal cycler at 95°C for 3 minutes.

6. Immediately place the plate on ice for 3 minutes.

7. Remove the film, then seal with the appropriate septa.

8. Place the sample tray on the autosampler, then start the electrophoresis run.

4 Analyze data with Microsatellite Analysis Software

This chapter contains brief procedures. For more information on using the software, click ? (Help) in the top right of any Microsatellite Analysis Software screen.

For information on known artifacts in STR kits, see “Application notes” on page 115.

Overview of Microsatellite Analysis Software

Microsatellite Analysis Software is a microsatellite genotyping module available on the Thermo Fisher™ Connect Platform (**thermofisher.com/connect**).

This software is used to analyze a mixture of DNA fragments, separated by size. The analysis provides a profile of the separation, precisely calculates the sizes of the fragments, and determines the microsatellite alleles present in the sample.

The software allows you to view, edit, analyze, print, and export microsatellite marker data generated using Applied Biosystems™ genetic analyzers.

Note: You may observe minor differences in results if you analyze the same data in the GeneMapper™ Software.

Allelic ladder requirements for CLA data analysis

- CLA analysis requires at least one allelic ladder sample per project. Perform the appropriate internal validation studies before you use multiple allelic ladder samples in a project.

  For multiple allelic ladder samples, the software calculates allelic bin offsets by using an average of all allelic ladders that use the same panel in a project.
- Allelic ladder samples must be labeled as "**Allelic Ladder**" in the **Sample Type** column in a project. Analysis will fail if the **Allelic Ladder Sample Type** is not specified.
- 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 allelic ladders do exist. Off-ladder (OL) alleles can contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the 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 protocol.

Names and versions of settings files used in this section

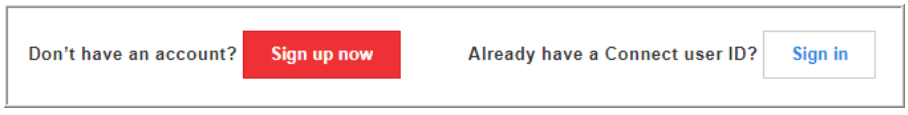
The names and version numbers of the settings files that are shown in this section may differ from the names and version numbers that you see when you download or import files.

Access the Microsatellite Analysis Software and import files

Before you begin, save FSA files from an instrument run to your computer, to your Connect account on **thermofisher.com/connect**, or to a Dropbox™ account. The FSA files can be analyzed or unanalyzed.

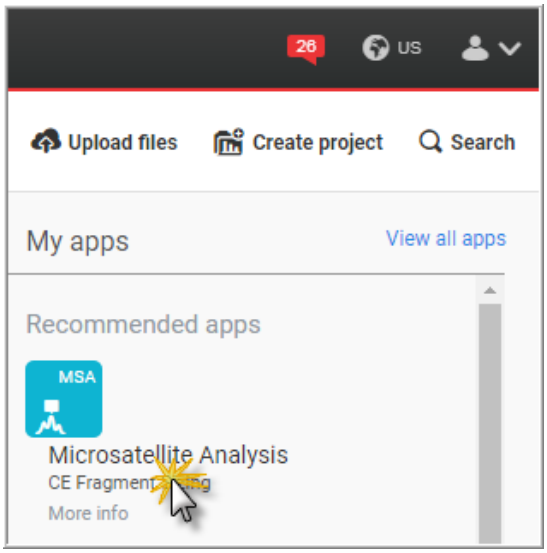
This section uses example data files collected on the SeqStudio™ Genetic Analyzer. Example data files may be available for download. For information, see “(If needed) Download newer versions of settings files” on page 35.

1. Go to **thermofisher.com/connect**.
2. Scroll down to the sign in options, then sign in.

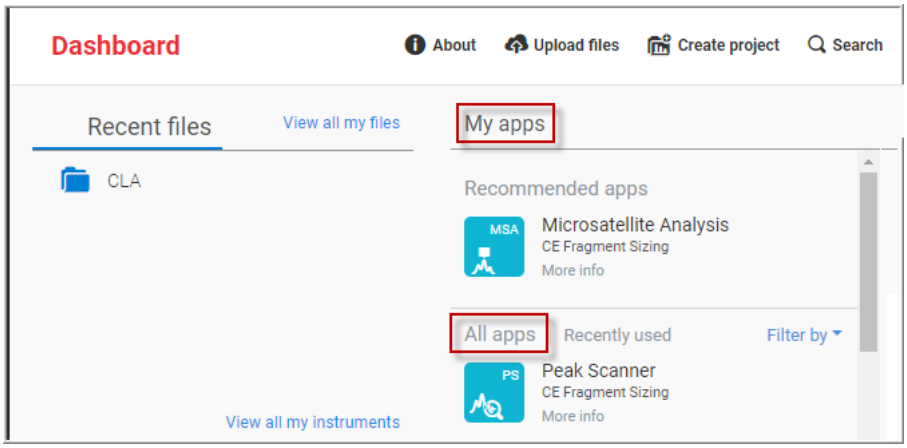


3. In the **My Apps** list at the top right of the screen, click **Microsatellite Analysis**.

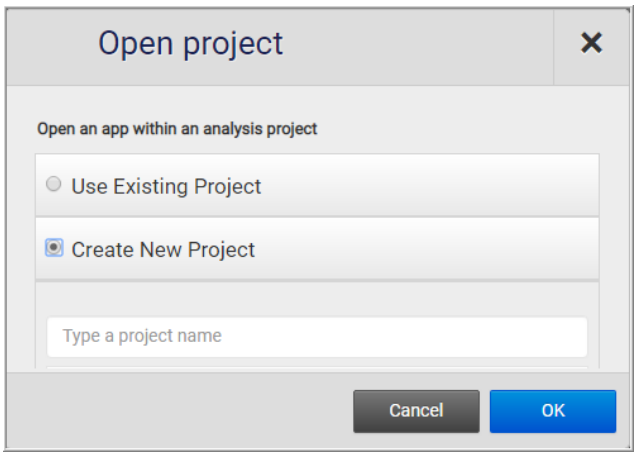
IMPORTANT! Click the text, do not click the icon.



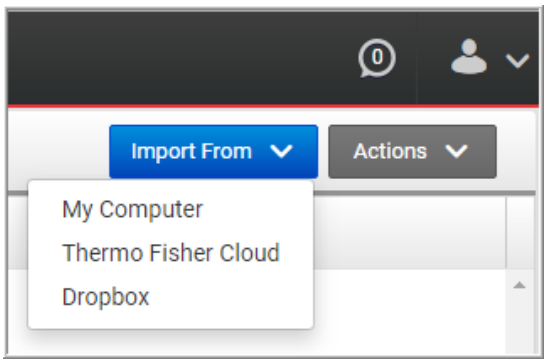
If **Microsatellite Analysis** is not listed under **My Apps**, scroll down in the **All Apps** list.



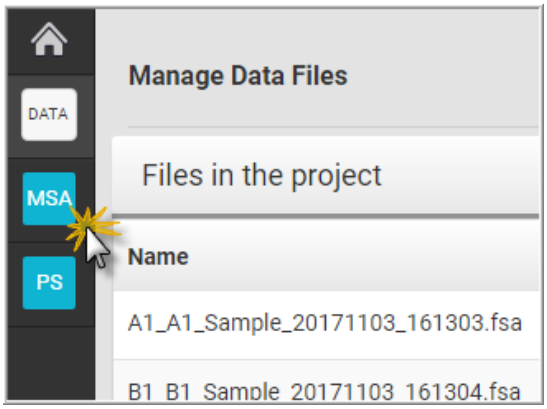
4. In the **Open Project** dialog box, select or create a project, then click **OK**.



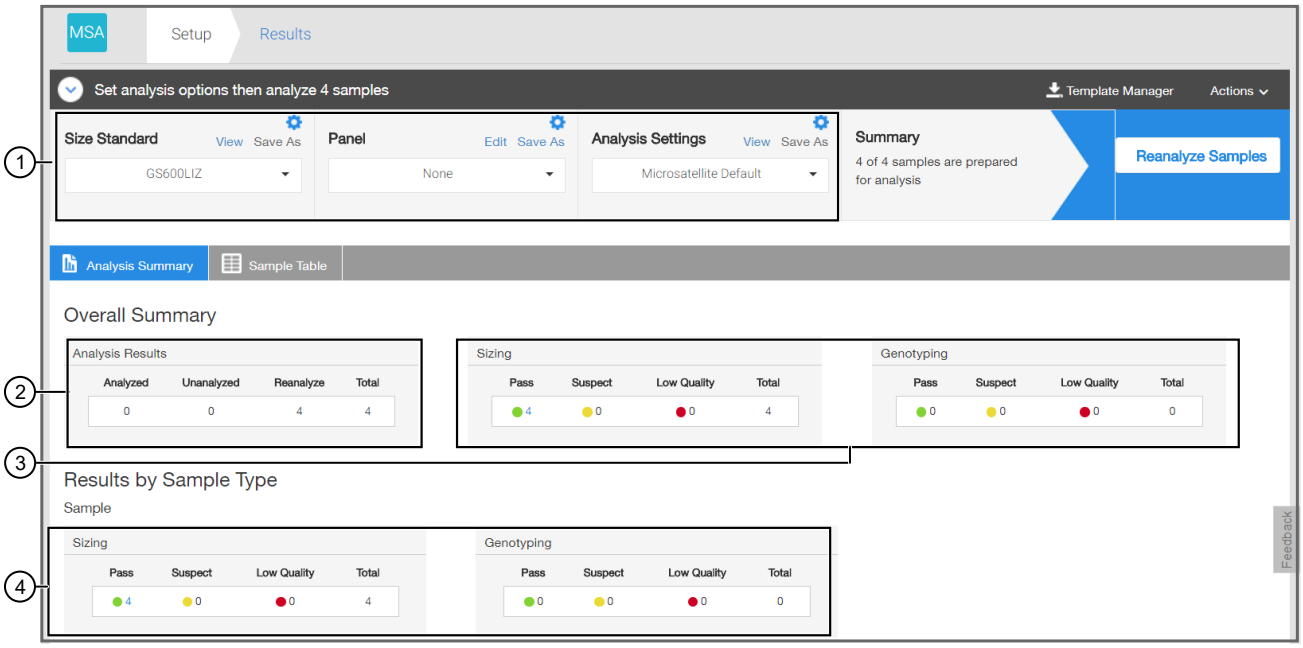
5. In the top right of the **Manage Data Files** screen, click **Import From**, select the location of the files, then select the FSA data files of interest.



6. In the top left of the **Manage Data Files** screen, click MSA.



The **Setup** screen is displayed.



① Analysis options for all samples in the project.
② Analysis Results lists the total number of samples in the project and the number of samples in each analysis status category.
③ Sizing and genotyping results for all samples in the project.
④ Sizing and genotyping results for all samples in the project, organized by sample type.

7. If this is the first time you are using the software, or if you are unsure if the latest settings are available in the software, continue to "Set up the Microsatellite Analysis Software for analysis (before first use of the kit)" on page 33.

   If you know that the latest settings are available in the software, skip to page 42.

Set up the Microsatellite Analysis Software for analysis (before first use of the kit)

About importing files into the Microsatellite Analysis Software database

Before you use Microsatellite Analysis Software to analyze CLA data for the first time, you must import the latest settings files (see "(If needed) Download newer versions of settings files" on page 35).

IMPORTANT! Settings files are instrument-specific. Ensure that you import files for the instrument that corresponds to the data you will analyze.

Note: The names and version numbers of the files that are shown in this section may differ from the file names and version numbers that you see when you download or import files.
If you need help to determine the correct files to use, contact your local field applications scientist or technical support.

File to import	File type	Description	See page
MSA Settings Template (preferred method)	ZIP	Includes all settings required for analysis: size standard, panels and bins, and analysis method. It also retains the plot and table settings of the project from which it was exported.	36
Optional individual files to import if you did not import a template (alternative method)			
Panel and bins (Created with Microsatellite Analysis Software)	ZIP	Defines the markers (loci) that are being interrogated, and includes bins (location of expected alleles) for each marker.	38
Size Standard	XML	Defines the sizes of the fragments present in the size standard.	40
Analysis settings	XML	Defines the settings for peak detection, allele calling, and peak quality flags.	41

Workflow: Set up Microsatellite Analysis Software

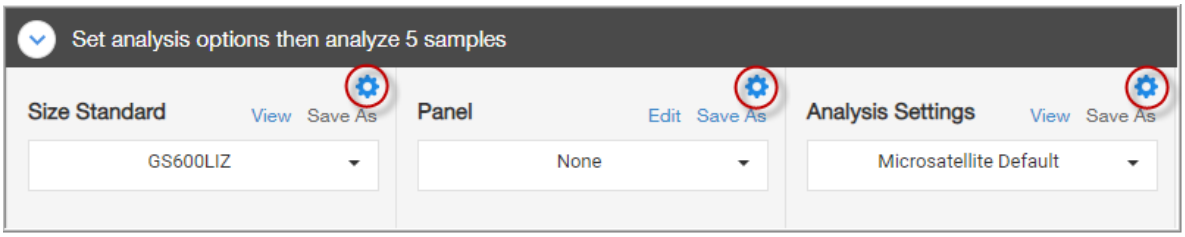
Before you use Microsatellite Analysis Software to analyze data for the first time, you must do the following:

Set up Microsatellite Analysis Software

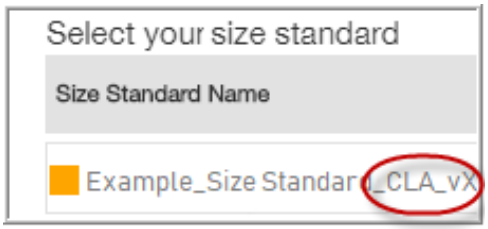
- **Check settings file versions on your computer**
- **(If needed) Download newer versions of settings files**
- **Import an MSA settings template (preferred method)**

Check settings file versions on your computer

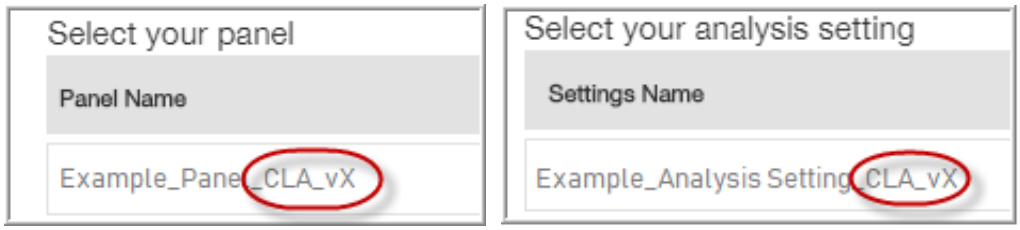
1. Access the Microsatellite Analysis Software as described in “Access the Microsatellite Analysis Software and import files” on page 29.
2. Click ⚙ next to **Size Standard**.



3. Look for settings with a **_CLA** suffix and check the version of files.



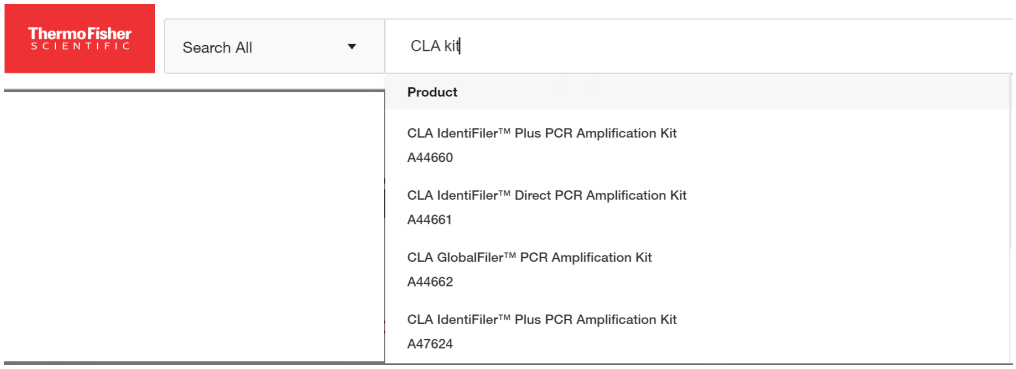
4. Check the version of files for **Panel** and **Analysis Settings**.



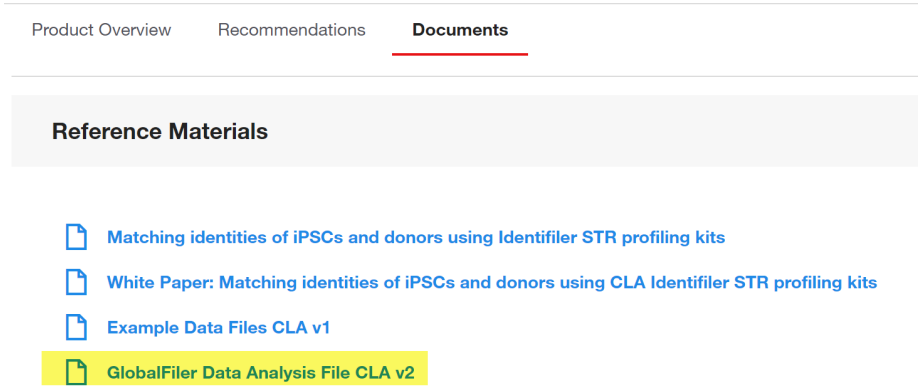
5. Check for newer versions of the files on the website as described in the next procedure.

(If needed) Download newer versions of settings files

1. Go to **www.thermofisher.com**.
2. In the search field, type **CLA kit**, then select the kit of interest.



3. Select the **Documents** tab, then scroll down to the **Reference Materials** section.



4. If this is the first time you are using the Microsatellite Analysis Software, or if a newer version of the download file is available, click the file name to download the file.

5. Unzip the file.

   The ZIP file contains a separate folder for each instrument type. Each instrument folder contains a folder with Microsatellite Analysis Software settings and GeneMapper™ Software settings (ignore this folder). The Microsatellite Analysis Software folder contains:

   - **MSA_Template_CLA_vX.zip**—Contains the settings needed to analyze data in the Microsatellite Analysis Software. *Do not unzip the MSA ZIP file*. The ZIP file is a template that contains size standard, panel and bins and analysis settings.
   - **MSA_Analysis_Settings_CLA_vX folder**—Optional individual settings that are also in the template. These files are not needed if you import the template.

Import an MSA settings template (preferred method)

An MSA settings template ZIP file is created by exporting a template from the Microsatellite Analysis Software. For information on exporting a template, click ?.

A settings template ZIP file can include all files required for analysis: size standard, panels and bins, and analysis method. It also retains the plot and table settings of the project from which it was exported.

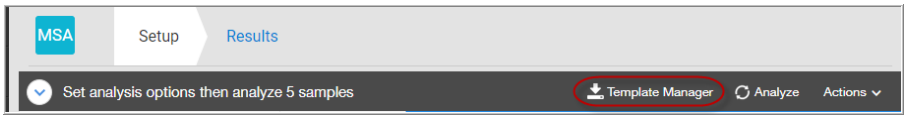
If the size standard, panel, and analysis method libraries do not contain CLA settings, or if you downloaded a new version of the template ZIP file, follow this procedure to import the template ZIP file into the Microsatellite Analysis Software.

Note: Alternatively, you can import individual settings. See “Import individual settings (alternative method)” on page 38.

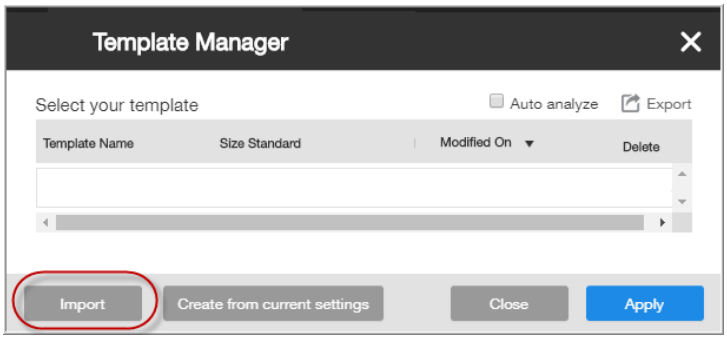
---

1. Access the Microsatellite Analysis Software as described in “Access the Microsatellite Analysis Software and import files” on page 29.

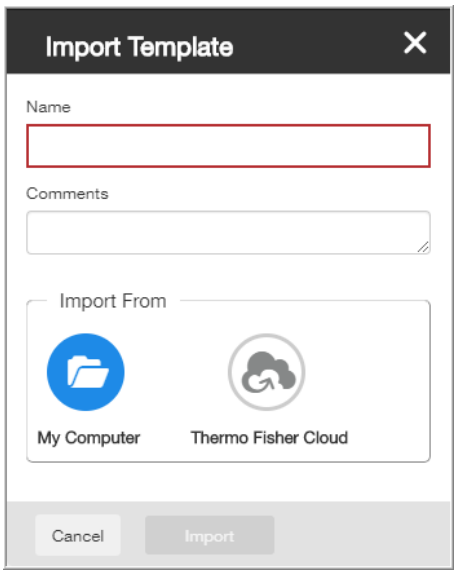
2. At the top right of the screen, click **Template Manager**.



3. In the **Template Manager** dialog box, click **Import**.

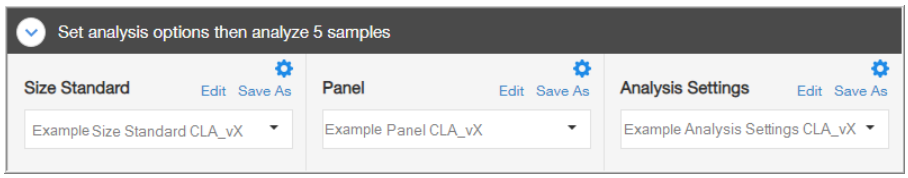


4. In the **Import Template** dialog box, enter a name for the template (include a "CLA_vX suffix), click **My Computer**, then click **Import**.



5. Navigate to, then select the ZIP file for your instrument that you previously downloaded (see "(If needed) Download newer versions of settings files" on page 35), then click **Open**.
The ZIP file is listed in the **Template Manager**.

6. Click **Apply**.
The settings are applied to the project.



Import individual settings (alternative method)

If you did not import an MSA settings template, or if you want to update specific settings only, you can import individual settings.

Import an MSA panel (ZIP file created in the Microsatellite Analysis Software)

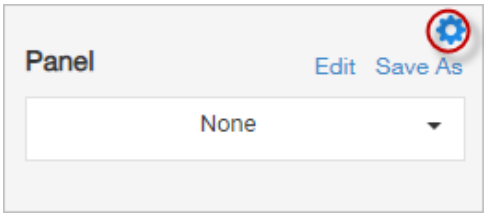
Note: This step is not required if you imported an MSA settings template.

A panel ZIP file is created by exporting a panel from the Microsatellite Analysis Software. For information on exporting a panel, click ?.

If your computer already contains the correct size standard and analysis method, and you only need to import panel and bins, follow this procedure to import the panel and bins.

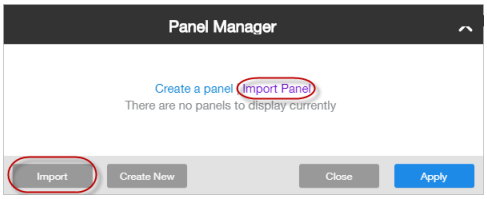
1. Access the Microsatellite Analysis Software as described in "Access the Microsatellite Analysis Software and import files" on page 29.

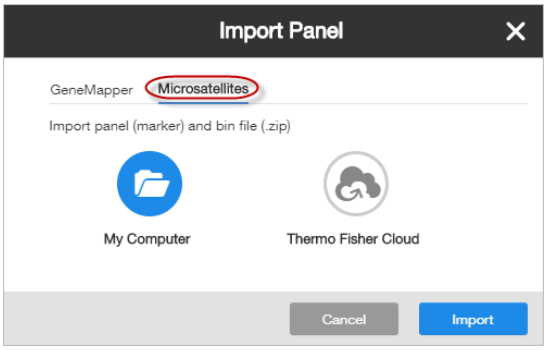
2. Click ⚙ in the **Panel** pane.



3. Click **Import** in the **Panel Manager**.

4. Click **Microsatellites** in the **Import Panel** dialog box.





5. Click the import location, then select the panel ZIP file.

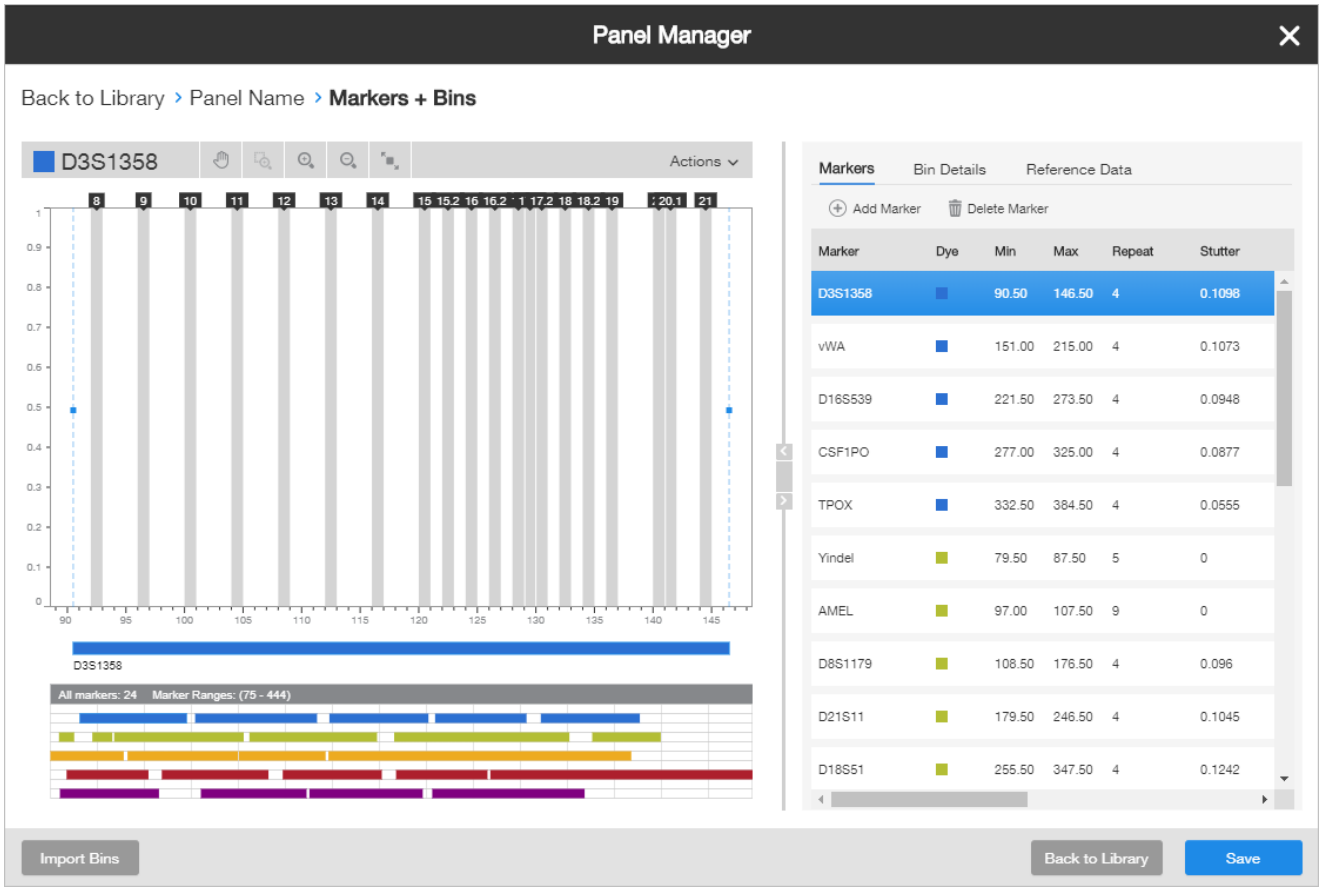
6. Click **Open**, then click **Import**.

7. Navigate to, then select the ZIP file that you previously downloaded (see "(If needed) Download newer versions of settings files" on page 35), then click **Open**.

8. Enter a panel name, then click **Next**.

Note: If a character limit warning is displayed, delete all characters starting with the first parenthesis in the name. For example if the name is "GF_3500_MSA_Panel_CLA_v1 (GF_3500_v2) Imported", delete "(GF_3500_v2) Imported".

The Panel Manager displays the panels and associated bins.



IMPORTANT! If the left pane does not show gray bins, click **Import Bins** at the bottom left of the screen, then import the associated bin file.

9. Click **Save**, then click **Back to Library**.

10. Click **Apply** to select the panel for the project.

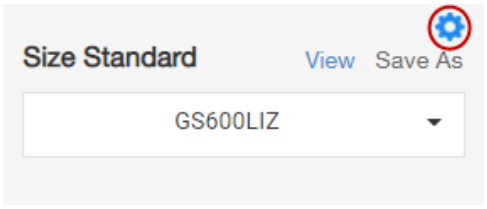
Import a CLA size standard (XML files provided by Thermo Fisher Scientific)

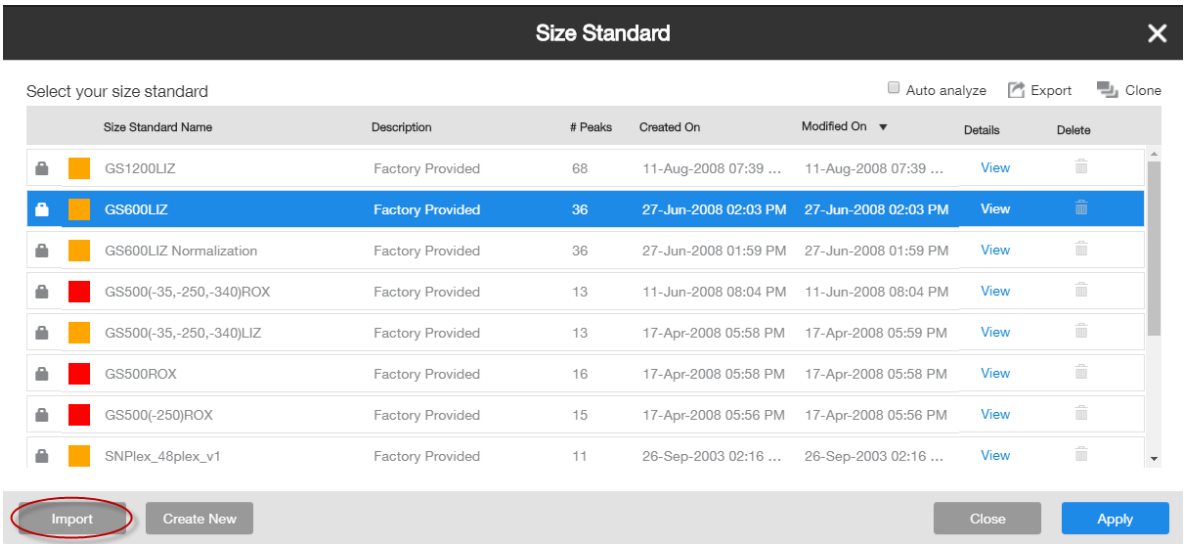
Note: This step is not required if you imported an MSA settings template.

1. Access the Microsatellite Analysis Software as described in "Access the Microsatellite Analysis Software and import files" on page 29.

2. Click ⚙ in the **Size Standard** pane.

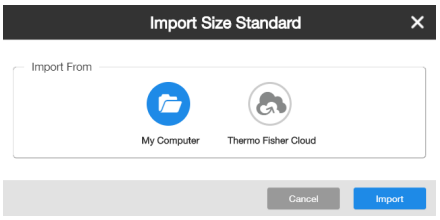
3. Click **Import** in the **Size Standard** library.



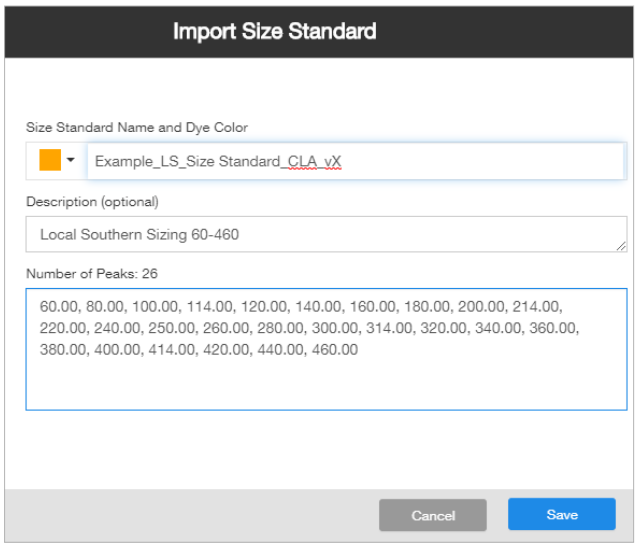


4. In the **Import Size Standard** dialog box, click the location of the files, then click **Import**.

5. Navigate to and select the size standard XML file, then click **Open**.



6. In the **Import Size Standard** dialog box, edit the size standard information if needed, then click **Save**.



Note: If a character limit warning is displayed, delete all characters starting with the first parenthesis in the name. For example if the name is "GF_MSA_SizeStd_CLA_v1 (GF_3500_v2) Imported", delete "(GF_3500_v2) Imported".

The size standard is added to the **Size Standard** library.

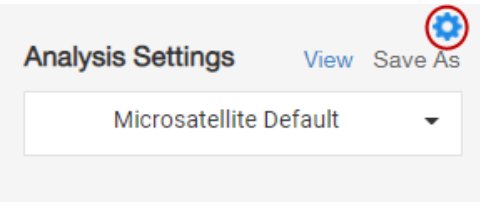
7. In the **Size Standard** library, click **Apply** to select the size standard for the project.

Import a CLA analysis settings (XML file provided by Thermo Fisher Scientific)

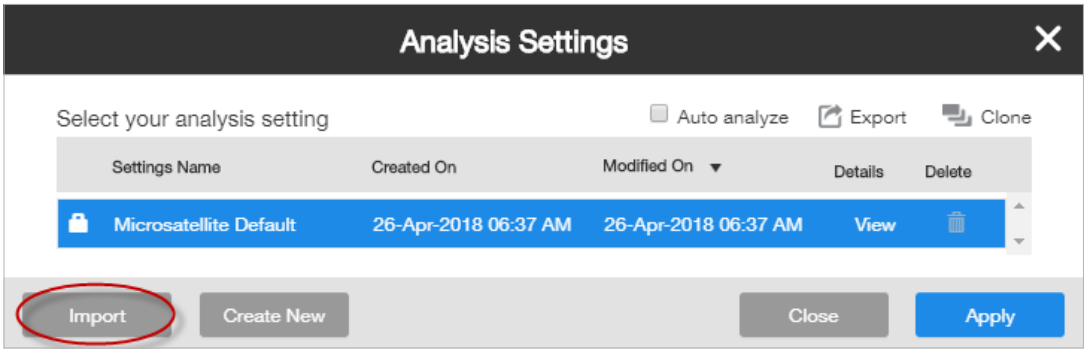
Note: This step is not required if you imported an MSA settings template.

1. Access the Microsatellite Analysis Software as described in “Access the Microsatellite Analysis Software and import files” on page 29.

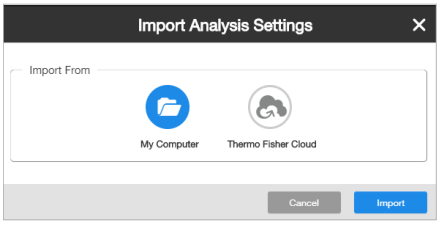
2. Click ⚙ in the **Analysis Settings** pane.



3. Click **Import** in the **Analysis Settings** library.



4. In the **Import Analysis Settings** dialog box, click the location of the files, then click **Import**.



5. Navigate to and select the analysis settings XML file for your instrument, then click **Open**.

6. Click **Save**.

Note: If a character limit warning is displayed, delete all characters starting with the first parenthesis in the name. For example if the name is "GF_3500_MSA_AM_CLA_v1 (GF_3500_v2) Imported", delete "(GF_3500_v2) Imported".

The analysis settings file is added to the **Analysis Settings** library.

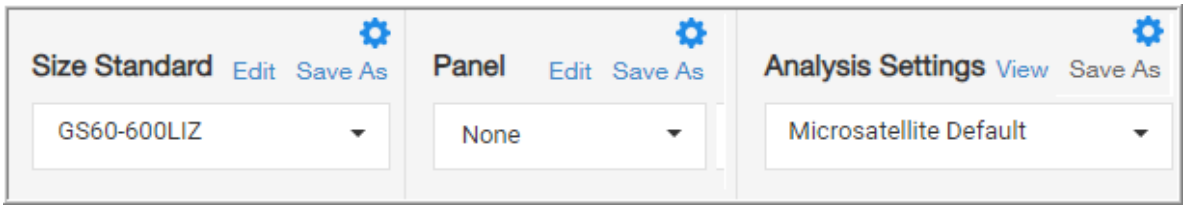
7. In the **Analysis Settings** library, click **Apply** to select the analysis settings for the project.

Plot and table settings

Plot and table settings are not stored in separate files that you can import and export. However, the current plot and table settings are retained when you export a template from the Microsatellite Analysis Software. For information on exporting a template, click ?.

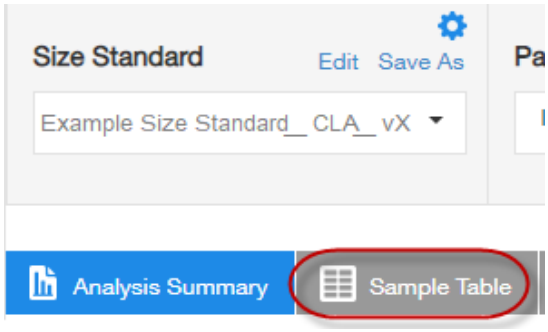
Select settings and analyze samples in the Microsatellite Analysis Software

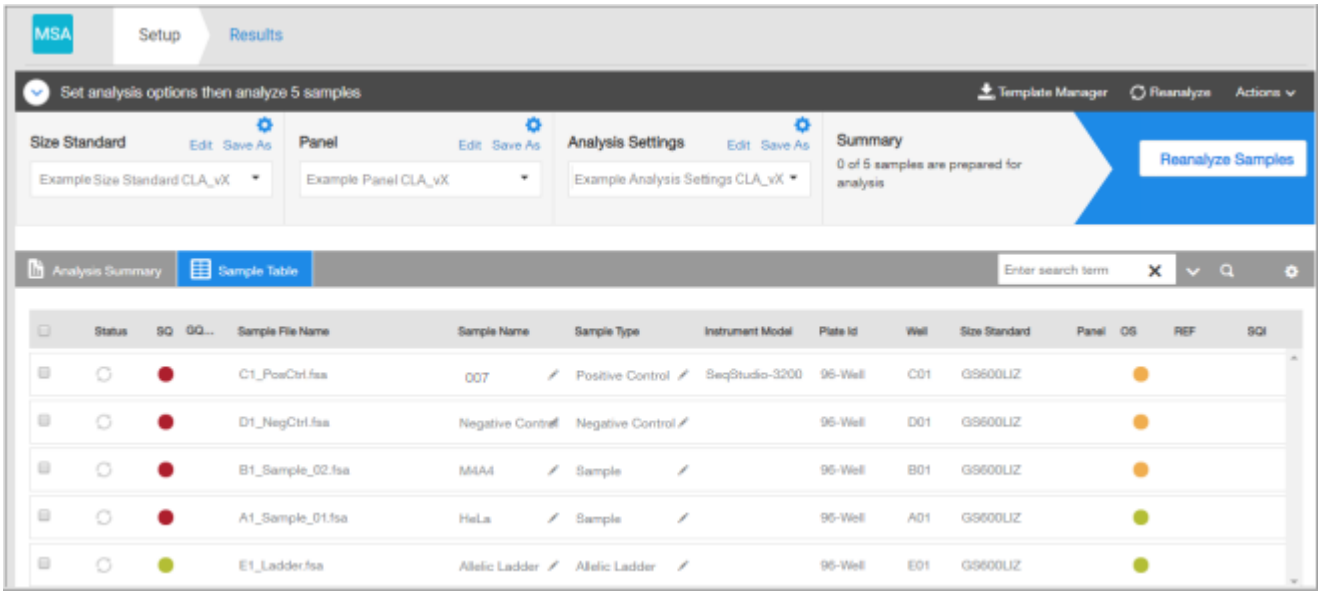
1. If CLA settings are not selected, click ⚙ for **Size Standard**, **Panel**, and **Analysis Settings**, then select the appropriate CLA size standard, CLA panel, and CLA analysis settings.



IMPORTANT! Analysis settings are instrument-dependent. Size standard may also be instrument-dependent. Select an analysis settings file that corresponds to the instrument on which the imported data was collected.

2. Click the **Sample Table** tab.





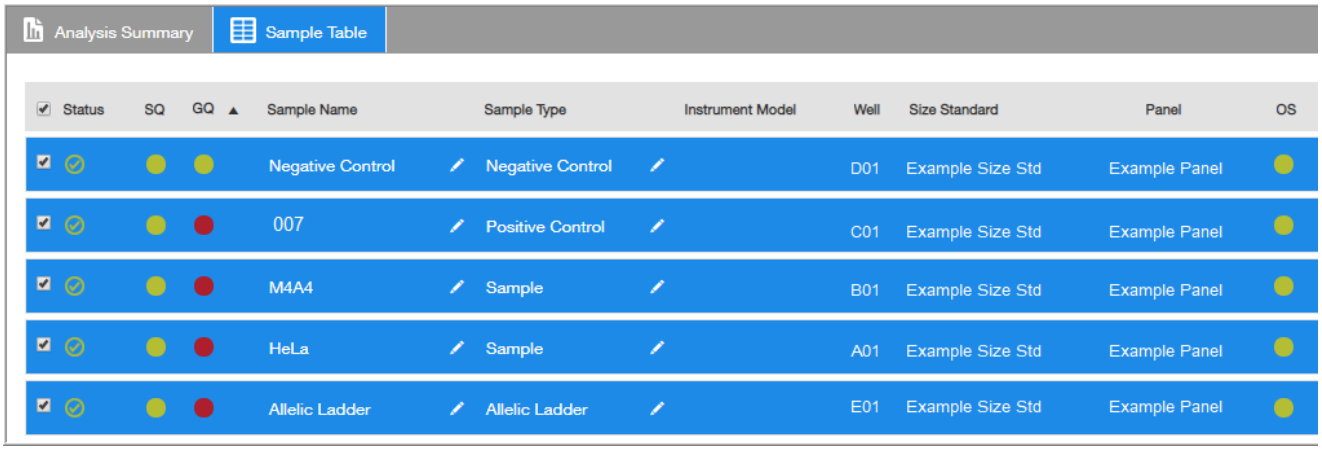
In the figure on the previous page, the **SQ** result for the samples is 🔴 because the samples are automatically analyzed with the size standard from the data collection software.

Note: For more information about the columns in the sample table, click ?.

3. If needed, click a **Sample Type** field, then select the sample type for the sample.
   At least 1 allelic ladder sample type is required.

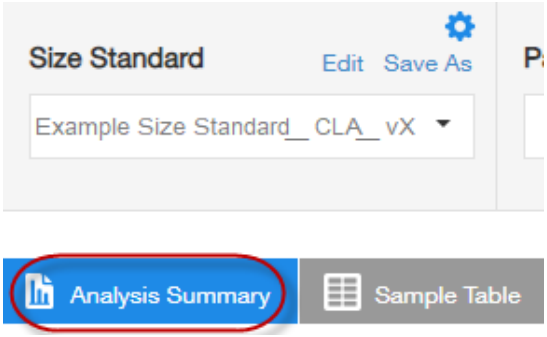
4. Select the checkboxes for the samples to analyze. You can click the checkbox in the header to select all samples.

5. Click **Reanalyze Samples**.

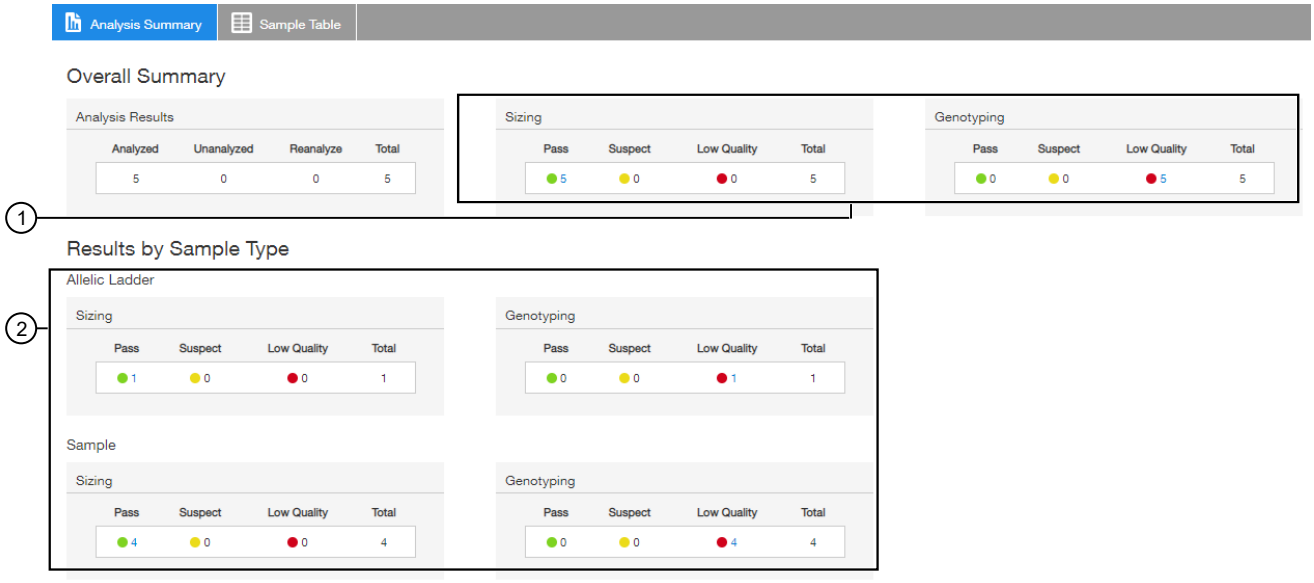


Review the analysis summary

1. Click the **Analysis Summary** tab.



Note: For more information about the columns in the sample table, click ?.



① Sizing and genotyping results for all samples in the project.
② Sizing and genotyping results for all samples in the project, organized by sample type.

2. Click the number next to any ●, ●, or ● indicator to display the results for the sample or allelic ladder.

Examine low-quality sizing results

Before proceeding, ensure that you have selected the correct size standard and analysis method (which may be instrument-specific) for the data you are analyzing.

View data collection settings

1. In the **Setup** screen, open the **Sample Details** window using one of the following methods:
   - Right-click on the sample file in the Sample Table, then click **View Sample Details**.
   - Click **Actions** ▸ **View Sample Details**.
2. Click **Information** to review the data collection settings.
3. Click **Close**.

Examine EPT and raw data

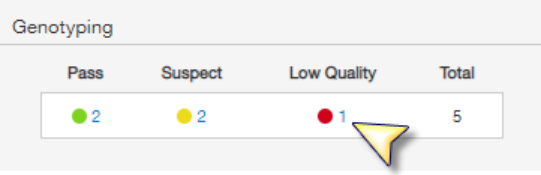
Review the EPT (ElectroPherogram Telemetry) plot to identify instrument performance issues that can affect size quality.

Review the raw data to evaluate anomalies, the causes of poor size-calling, and to determine the start and stop points for analysis. The start point for data analysis occurs after the primer peak and before the first sizing peak. The stop point for analysis occurs after the last sizing peak.

1. Open the sample details window using one of the following methods:
   - Right-click on the sample file in the Sample Table, then click **View Sample Details**.
   - Click **Actions** ▸ **View Sample Details**.
2. Click **EPT** to view the EPT plot.
3. Click **Raw Data** to view the raw data plot.
4. Click **Close**.

Review size matches

1. In the **Setup** screen, click the **Analysis summary** tab.
2. In the **Sizing** analysis results pane, click on the blue number next to 🟡 or 🔴 to review size matches for these samples in the **Size Matching** tab of the **Results** screen.



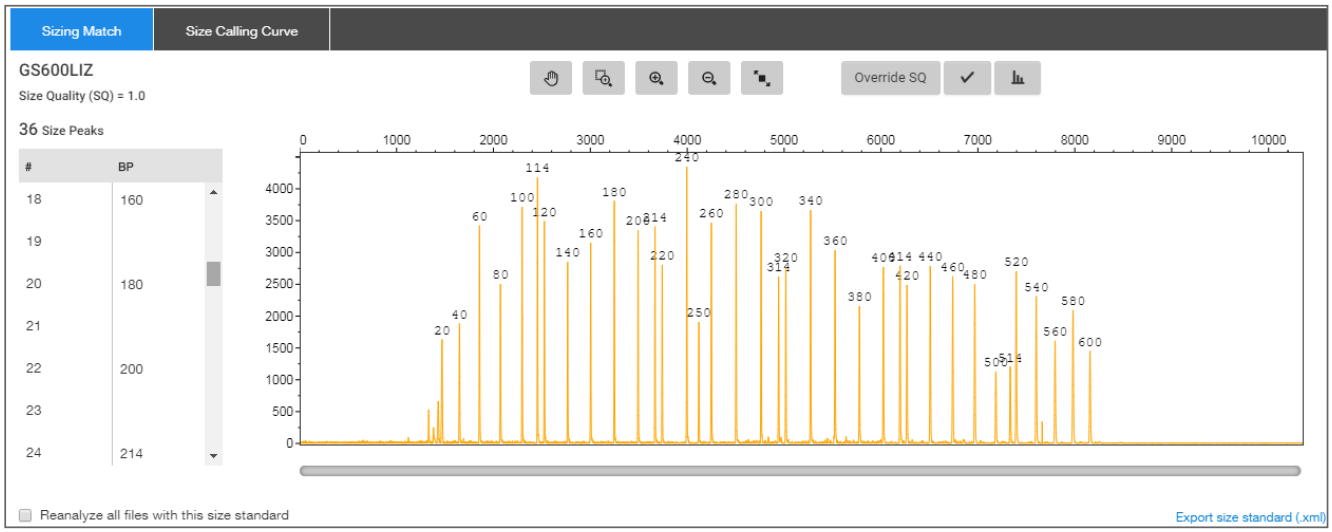
3. Modify the size matches as needed.

Modify size matches

If the software does not correctly detect size standard peaks, you can modify the size matches, then apply the modified size standard definition to all selected samples in the project.

1. Select an option to view the peak assignments for the size standard peaks in the selected sample:

Option	Action
View in Size Matching tab	1. In the **Results** screen, click the **Size Matching** tab. 2. Select a sample from the sample list to view the peak assignments in the Sizing Match pane at the bottom of the screen.
View in Size Match Editor	1. In the **Results** screen, click the **Sizing** tab. 2. Select one or more samples from the sample list. 3. Click **Actions** ▸ **View Size Match Editor**.



2. Examine all size standard peaks to ensure that all peaks are present, peaks are labeled correctly, and the sizes match the fragment sizes in the size standard definition file that is used for the sample.

3. Change size values or delete peaks as needed, then auto-adjust size matches. This function is useful if the software incorrectly labels a peak.

Task	Action
Change a size value	1. Click the size value in the list, or click a peak or peak size label in the plot, then select a different value from the dropdown list. 2. Select the same peak, then click (auto adjust). For example, changing the 110 peak to 100, then clicking sequentially shifts the 110 size label to the current 100 peak, the 120 size label to the current 110 peak, and so on.
Delete a peak	1. Click the size value in the list, or click a peak or peak size label in the plot, then select **Delete** in the dropdown list. 2. Select the same peak, then click . For example, deleting the 110 peak, then clicking sequentially shifts the 110 size label to the current 120 peak, the 120 size label to the current 130 peak, and so on.

4. Click to recalculate the SQ using the new sizes.

5. Click **Override SQ**.

   The SQI (Sizing Quality Invalid) indicator is displayed:

   - In the top right of the **Results** screen, in the **Sizing** tab, any sample that is analyzed with the size standard—Displays **SQI: Y**.

     Note: If **SQI** does not display in the **Sizing** tab, click , then search for **Customize the plot view**.

   - In the **Setup** screen Sample Table —Displays in the SQI field.

6. *(Optional)* To create a new size standard definition for the project using the modified size matches, click **Reanalyze all files with this size standard**.

7. Click **Done**.

Modify the analysis settings

For a description of analysis settings, click ?, then search for **Analysis Settings**.

1. In the **Setup** screen, in the **Analysis Settings** pane, click ⚙.

2. Select the analysis setting from the list, then select **Edit**.
   The following settings are recommended for CLA data analysis. Modify other settings as needed.

Analysis parameter	CLA GlobalFiler™ kit	
	SeqStudio™ Flex, SeqStudio™, 3500	3730
Baseline Window Size	33	33
Peak Window Size	13	9
Smoothing	Light (default)	Light (default)
Size Calling	Local Southern (60–460)	Local Southern (60–460)

3. Select **Auto analyze**, then click **Apply**.

Adjust Size Quality Flag ranges

1. In the **Setup** screen, in the **Analysis Settings** pane, click ⚙.

2. Select an analysis setting, then click **Edit**.

3. Click the **Sizing Settings** tab.

4. Click **Quality Flags**.

5. Adjust the low, medium and high ranges as appropriate.

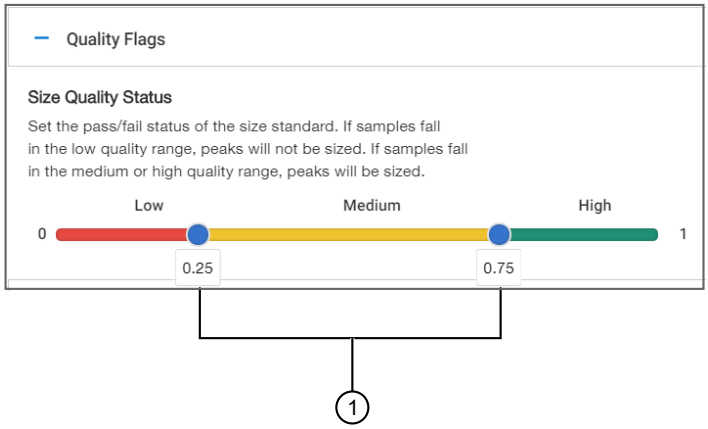


Figure 10 Quality Flag ranges

① Drag to adjust the quality flag ranges.

6. Click **Save**, select the **Auto analyze** checkbox, then click **Apply**.

Examine low-quality genotyping results

Before proceeding, ensure that you have selected the correct panel and analysis method (which may be instrument-specific) for the data you are analyzing.

About PQVs (Process Quality Value symbols ● ■ ● ▲ ●)

PQVs are flags that reflect the quality of results. ● ● are displayed for GQ (genotyping quality) if any peak in the sample does not meet expected thresholds. ▲ is displayed for individual PQVs in the genotypes table.

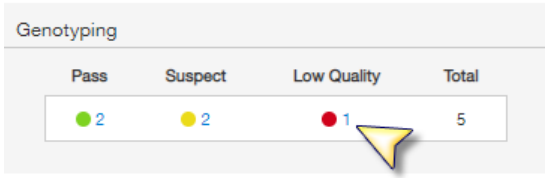
Thresholds are set in the **Peak Quality** and **Quality flags** tabs in the analysis method.

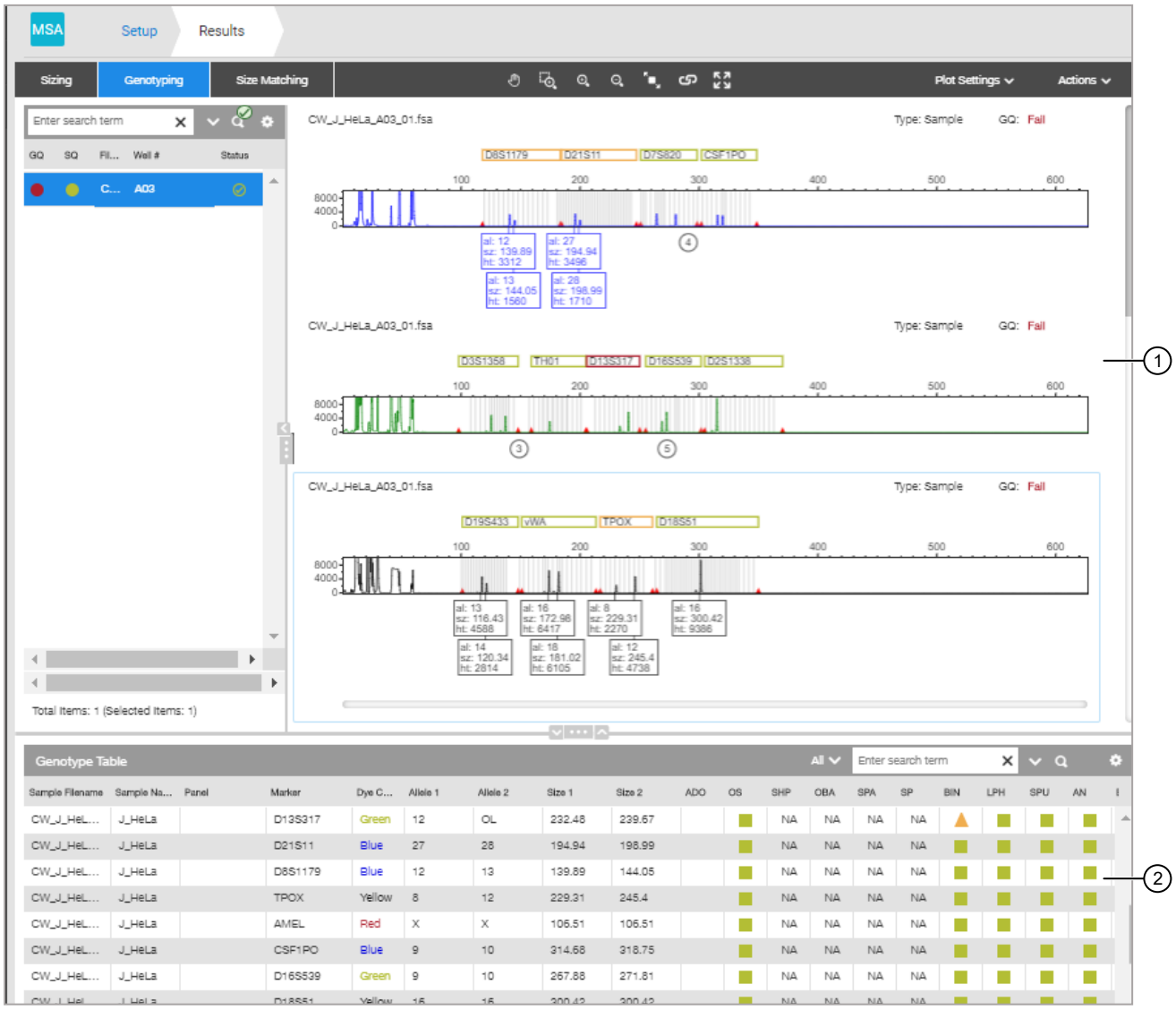
For definitions of PQVs, click ?, then search for **Genotyping Quality (GQ) status and Process Quality Values (PQVs)**.

Review samples with Suspect and Low Quality GQ values

1. In the **Setup** screen, click the **Analysis summary** tab.

2. In the **Genotyping** analysis results pane, click on the blue number next to ● or ● to display the **Genotyping** tab of the **Results** screen.

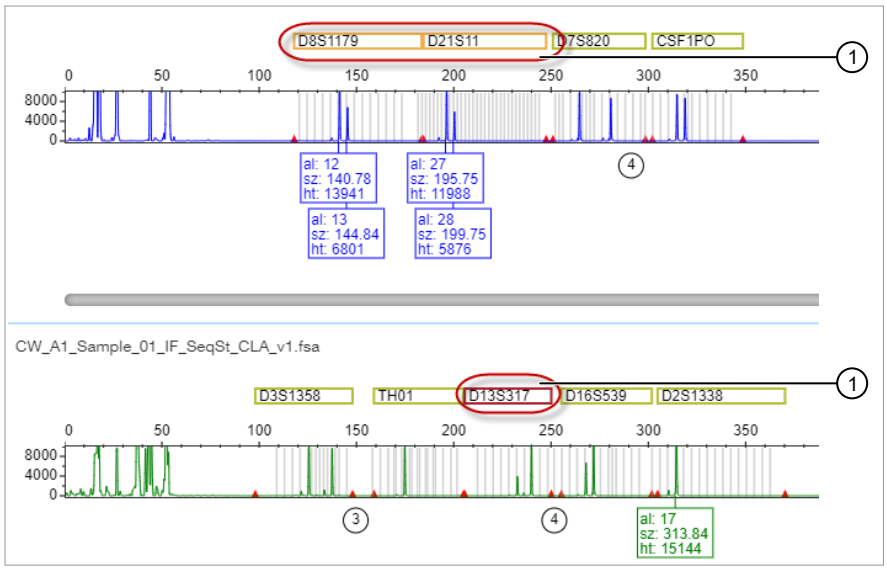




① Genotypes plot
② Genotypes table

Note: You may have to scroll down to view the genotypes table.

3. Examine the plots for yellow or red marker headers, which indicate that one or more PQVs have been triggered. You can double-click on a marker header to zoom on the marker range.



① Yellow marker header
② Red marker header

4. Proceed to "Review PQVs of samples with Suspect and Low Quality GQ or PQV values" on page 51.

Review PQVs of samples with Suspect and Low Quality GQ or PQV values

1. Display the genotypes results screen (see "Review samples with Suspect and Low Quality GQ values" on page 49).

2. Scroll down to view the genotypes table, then double-click a row with a 🟡 🔴 GQ or PQV result. The associated marker is selected in the plot.

Note: You may have to scroll to view the selected marker. Double-click the marker header to zoom.

The following figures are examples of 🟡 and 🔴 GQ results:

- Figure 11 shows a 🔴 GQ result that is caused by an off-ladder allele (OL peak label and BIN PQV).
- Figure 12 shows a 🟡 GQ result is caused by a peak height ratio that does not meet the threshold (PHR PQV).

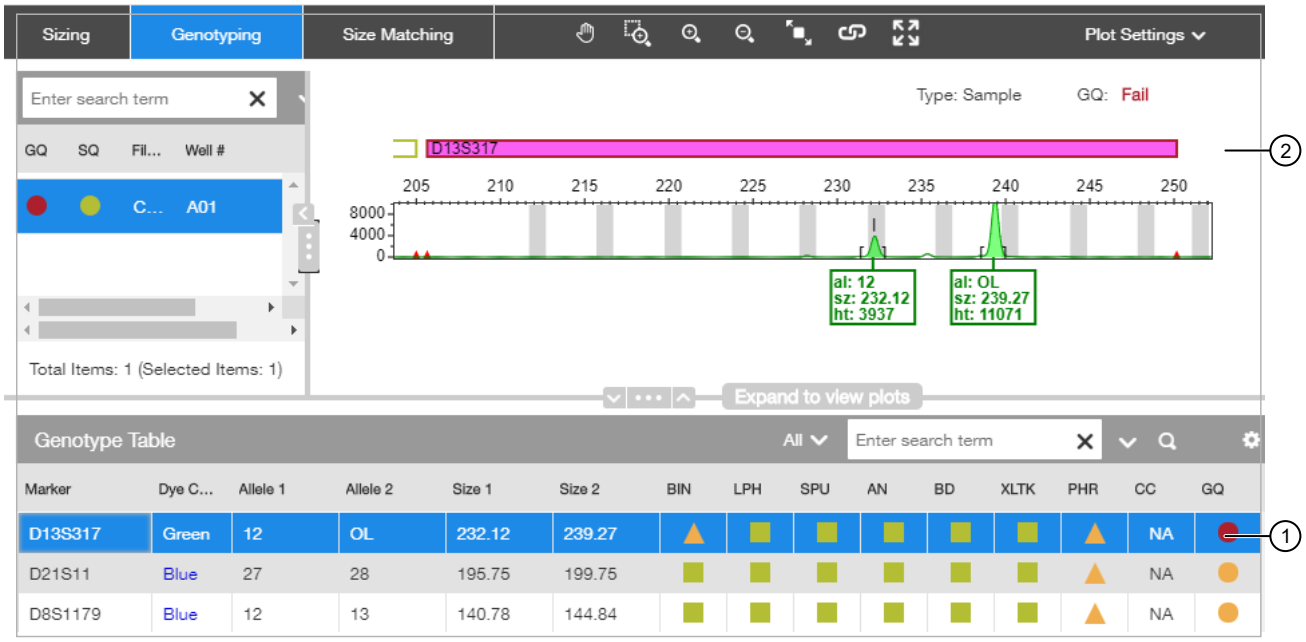


Figure 11 Example 1

① Double-click a row
② Associated marker is selected in the plot

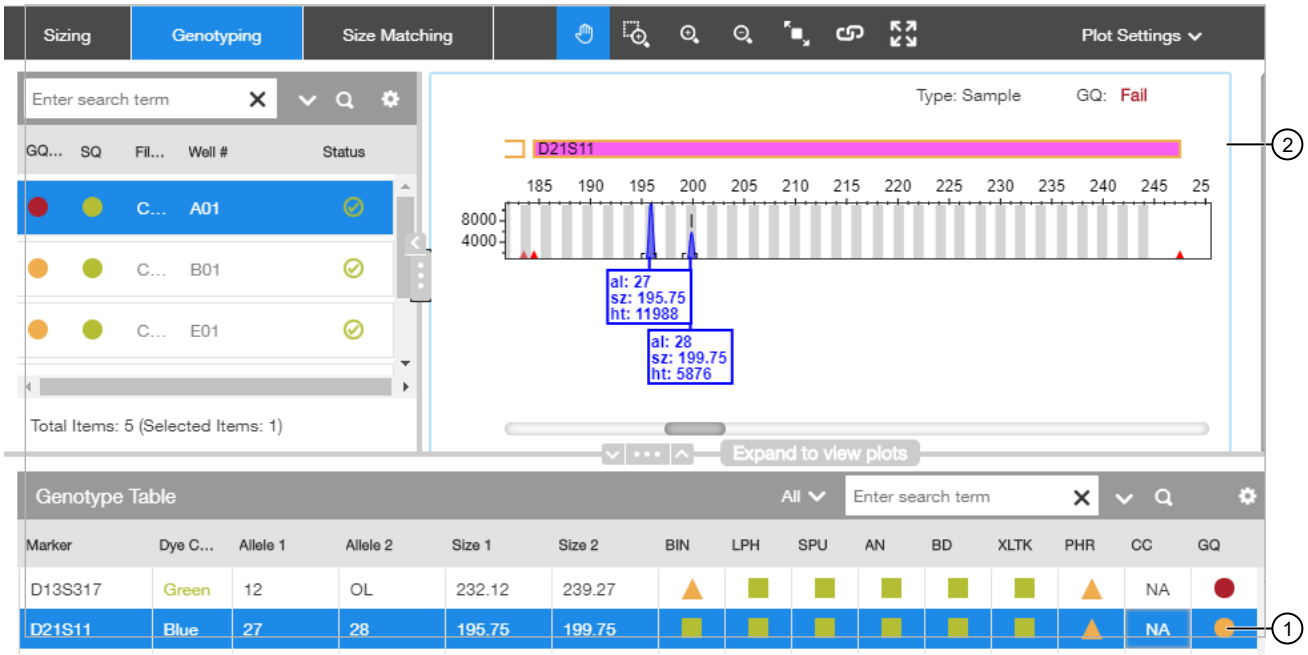


Figure 12 Example 2

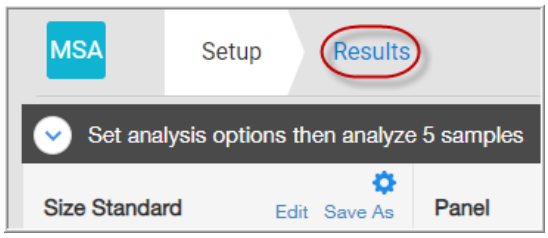
① Double-click a row
② Associated marker is selected in the plot

3. As needed, edit alleles. For information, click ?, the search for **Review, edit, and add alleles**.

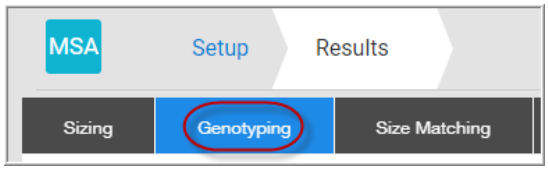
Export the genotypes table

You can export the genotypes table for reference when you compare the genotypes to the ATCC database.

1. Open the project that contains the samples of interest.
2. Click the **Results** tab.



3. Click the **Genotyping** tab.



4. Scroll down to view the genotypes table, then display the columns that you want to export.
5. Scroll to the top of the screen, then select **Actions** ▸ **Export Genotypes Table**.
6. Select the location to save the file, then click **Export**.

   If the web browser is configured to prompt before saving, navigate to the location to save, then click **Save**.

   If the web browser is not configured to prompt before saving, the file is saved to the **Download** folder.

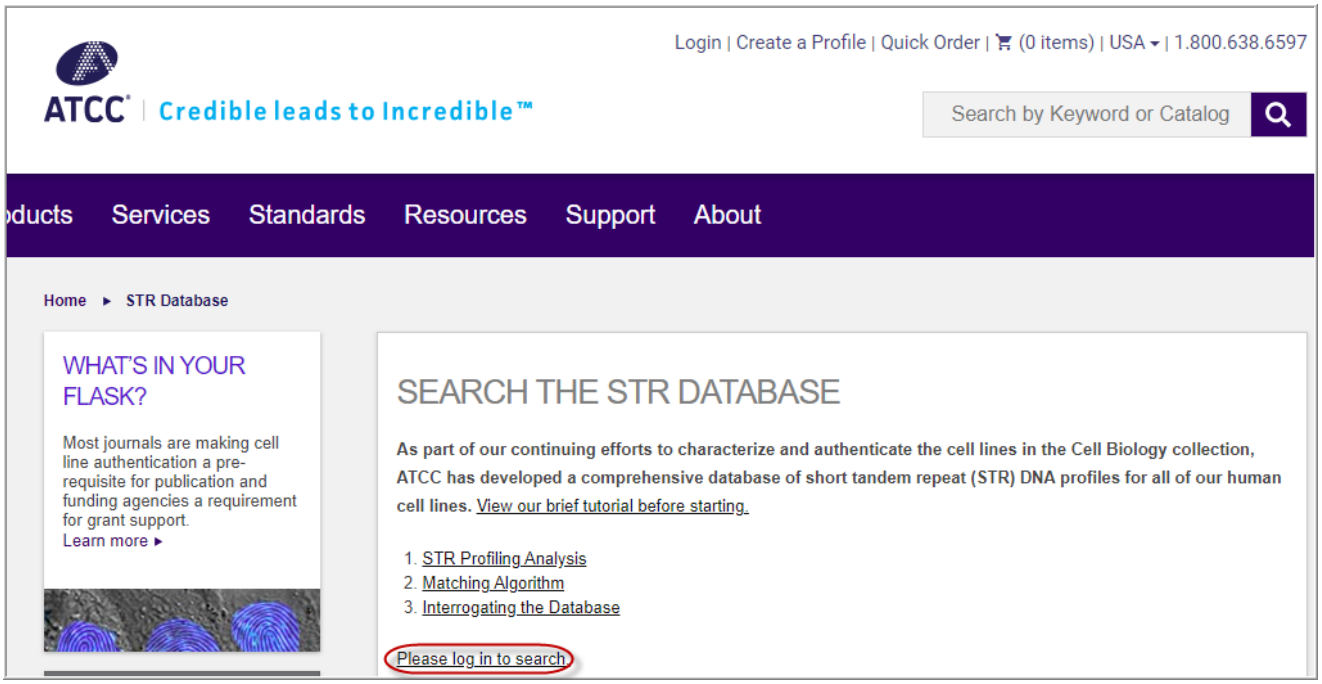
Verify cell line identity using the ATCC database

Cell line identity can be verified by uploading genotypes to the ATCC cell line STR database. The ATCC database requires you to enter genotypes for 8 specific loci, all of which are present in the Applied Biosystems™ CLA STR kits.

1. In a web browser (for example, Google™ Chrome™), navigate to **www.atcc.org/STR_Database**.

   Note: Click **View our brief tutorial before starting** if you are not familiar with this website.

2. Click **Please log in to search**, then log in or create a user profile.



3. Refer to the genotypes from the file you exported, then enter the genotypes in the ATCC screen.

4. Click **Matches >= 80%**.

   After a few minutes, a list of cell lines in the ATCC database that match the STRs present in the test sample is displayed.

Note: The DSMZ at the Leibniz Institute maintains a database of 8-17 STR alleles for common cell lines. To use the database, see the DSMZ instructions; go to:

https://celldive.dsmz.de/str

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5 Analyze data with GeneMapper™ Software

This chapter contains brief procedures. For more information on using the software, click **(Help)** or select the **Help** menu.

For information on known artifacts in STR kits, see “Application notes” on page 115.

Overview of GeneMapper™ Software

GeneMapper™ Software is a flexible genotyping software package that provides DNA sizing and quality allele calls for all Applied Biosystems™ electrophoresis-based genotyping systems. GeneMapper™ Software helps increase data processing efficiency with a multiuser, client-server deployment. The software uses Process Quality Values (PQVs) for automated identification that reduces data review time for high throughput genotyping. In addition, the security and audit features help users meet 21 CFR 11 requirements.

For information on obtaining the GeneMapper™ Software, contact your local field application specialist.

Note: You may observe minor differences in results if you analyze the same data in the Microsatellite Analysis Software.

Allelic ladder requirements for CLA data analysis

- CLA analysis requires at least one allelic ladder sample per run folder. Perform the appropriate internal validation studies before you use multiple allelic ladder samples in an analysis.

  For multiple allelic ladder samples, the GeneMapper™ Software calculates allelic bin offsets by using an average of all allelic ladders that use the same panel in 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 ladders in 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. Analysis will fail if the **Allelic Ladder Sample Type** is not specified.
- 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 allelic ladders do exist. Off-ladder (OL) alleles can contain full and/or partial repeat units. An off-ladder allele is an allele that occurs outside the 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 protocol.

Names and versions of settings files used in this section

The names and version numbers of the settings files that are shown in this section may differ from the names and version numbers that you see when you download or import files.

Set up the GeneMapper™ Software for CLA analysis (before first use of the kit)

About importing files into the GeneMapper™ Software database

Before you use GeneMapper™ Software to analyze CLA data for the first time, you must import the latest settings files (see "(If needed) Download newer versions of panel and bin files" on page 59).

IMPORTANT! Settings files are instrument-specific. Ensure that you import files for the instrument that corresponds to the data you will analyze.

Note: The names and version numbers of the files that are shown in this section may differ from the file names and version numbers that you see when you download or import files.
If you need help to determine the correct files to use, contact your local field applications scientist or technical support.

File to import	File type	Description	See
Panel	TXT	Defines the markers (loci) that are being interrogated. **IMPORTANT!** Use only panel and bin TXT files that are provided by Thermo Fisher Scientific. If you use panel and bin files that you create in the GeneMapper™ Software, you may see off-ladder alleles.	60
Bins	TXT	Defines bins (location of expected alleles) for each marker.	
Analysis Method	XML	Defines the settings used for peak detection, allele calling, and peak quality flags.	63
Size Standard	XML	Defines the sizes of the fragments present in the size standard.	62
Table Settings	XML	Hide or show columns, filter results, sort order.	64
Plot Settings	XML	Defines settings for electropherogram plot display including Sample Header Settings, Genotypes Header settings, Sizing Table settings, peak labels settings, and display settings for Sample and Genotype plots.	

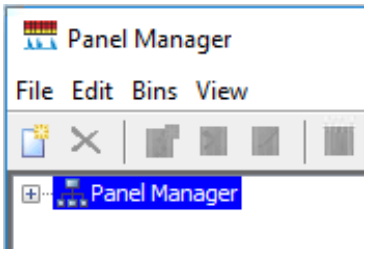
Workflow: Set up GeneMapper™ Software

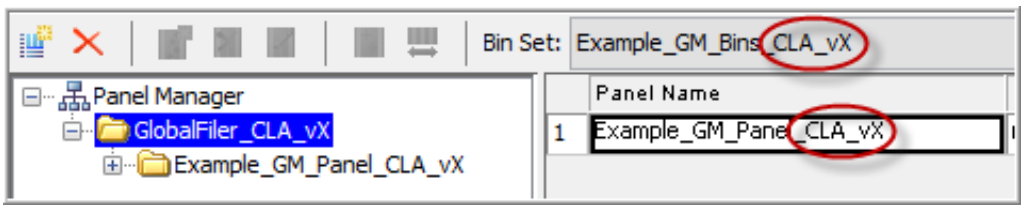
Set up GeneMapper™ Software

- **Check panel and bin file versions on your computer**
- **(If needed) Download newer versions of panel and bin files**
- **Import panel and bins**
- **Import analysis method**
- **Import the size standard**
- **Import table settings and plot settings**

Check panel and bin file versions on your computer

1. Start the GeneMapper™ Software, then log in with the appropriate user name and password.
2. Select **Tools** ▸ **Panel Manager**.
3. Check the version of files that are currently available in the **Panel Manager**:
   a. Select **Panel Manager** in the navigation pane.
   b. Expand the **Panel Manager folder** and any subfolders to identify the analysis file version that is already installed for your kit choice.

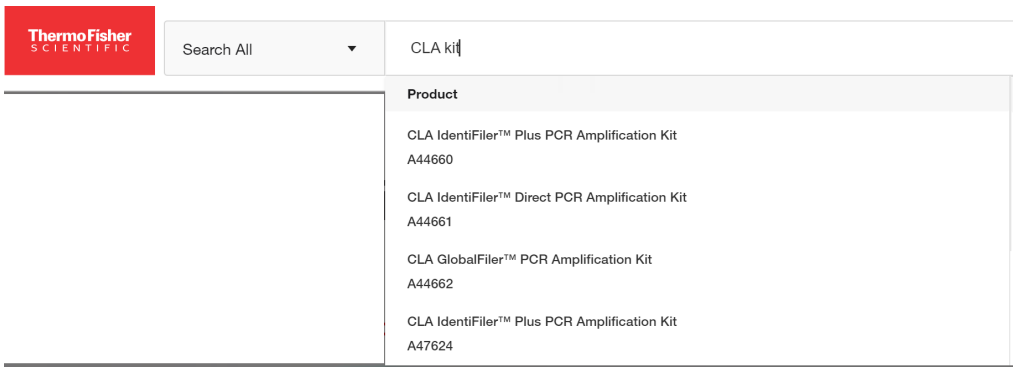




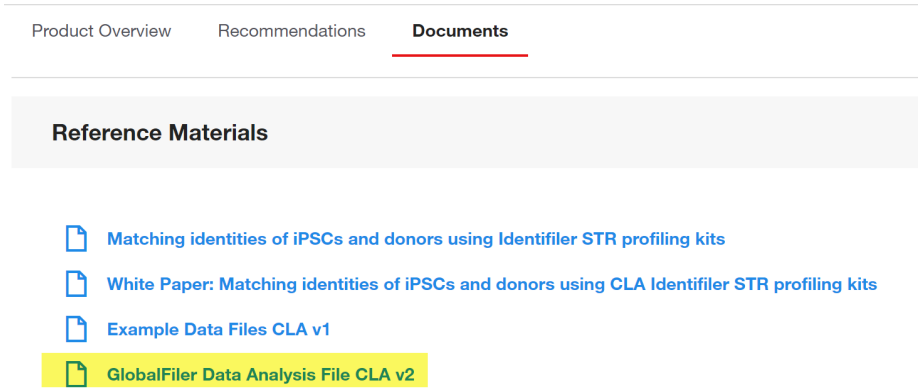
4. Check the version of files available for import into the **Panel Manager**:
   a. Select **Panel Manager**, then select **File** ▸ **Import Panels** to open the **Import Panels** dialog box.
   b. Navigate to, then open the **Panels** folder, then check the version of panel, bin, and stutter files installed.
5. Check for newer versions of the files on the website as described in the next procedure.

(If needed) Download newer versions of panel and bin files

1. Go to **www.thermofisher.com**.
2. In the search field, type **CLA kit**, then select the kit of interest.



3. Select the **Documents** tab, then scroll down to the **Reference Materials** section.



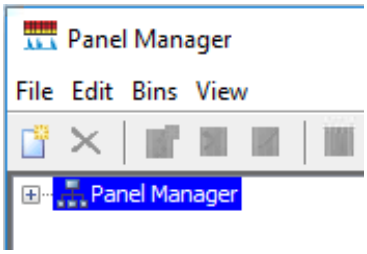
4. If this is the first time you are using the GeneMapper™ Software, or if a newer version of the download file is available, click the file name to download the file.
5. Unzip the file.
   The ZIP file contains a separate folder for each instrument type. Each instrument folder contains a folder with Microsatellite Analysis Software settings (ignore this folder) and GeneMapper™ Software settings.
   The GeneMapper™ Software folder contains GF_Analysis_Settings_CLA_vX folder, which contains the settings needed to analyze data in the GeneMapper™ Software.

Import panel and bins

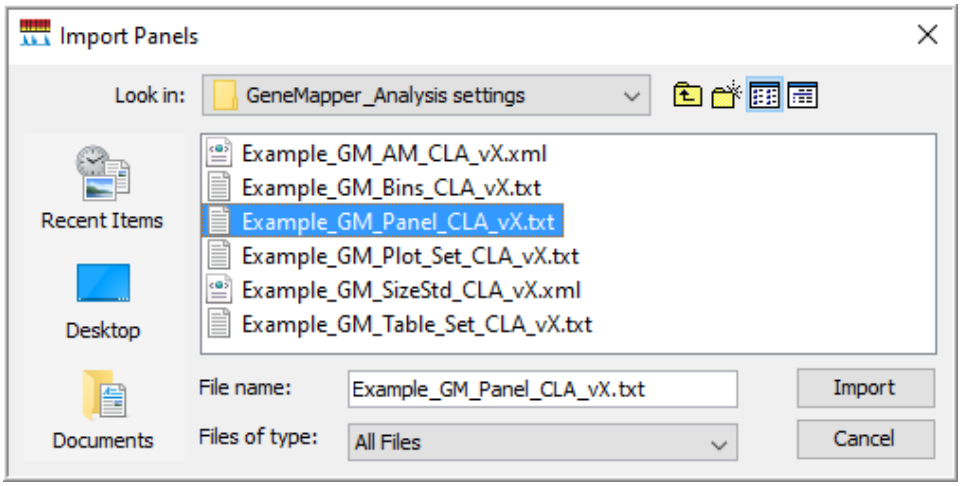
IMPORTANT! Use only panel and bin TXT files that are provided by Thermo Fisher Scientific. If you use panel and bin files that you create in the GeneMapper™ Software, you may see off-ladder alleles.

1. Start the GeneMapper™ Software, then log in with the appropriate user name and password.
2. Select **Tools** ▸ **Panel Manager**.
3. In the left pane, click **Panel Manager**.

   Note: If you do not click on **Panel Manager**, the import commands are not available.

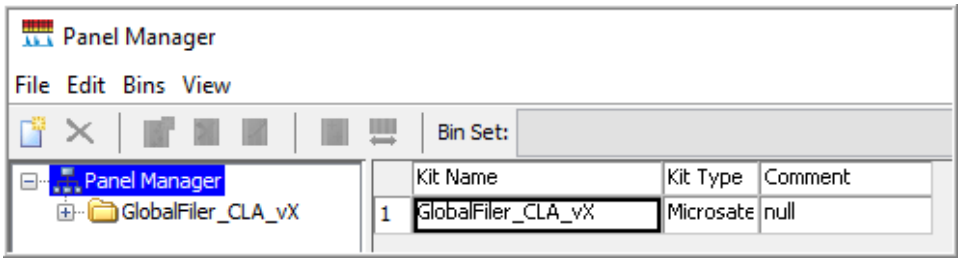


4. Import the panel file:
   a. Select **File** ▸ **Import Panels** to open the **Import Panels** dialog box.
   b. Navigate to, then select, the appropriate GM_Panel_CLA_vX.txt you obtained in "(If needed) Download newer versions of panel and bin files" on page 59.

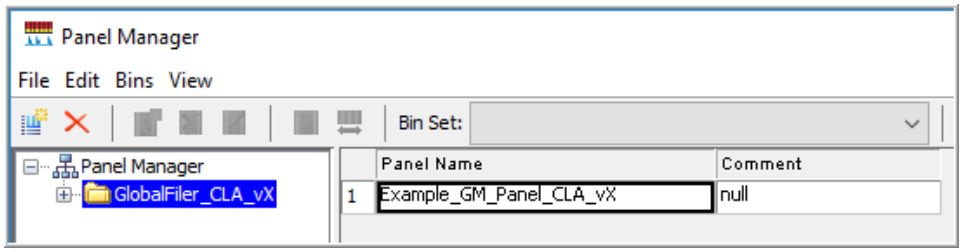


   c. Click **Import**.

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

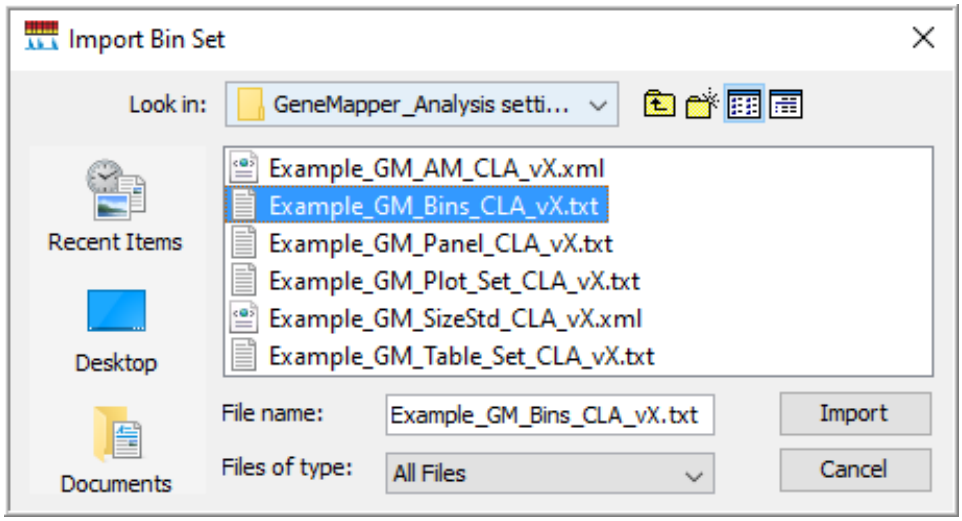


5. Import the bins file:
   a. Select the Identifiler folder in the navigation pane.



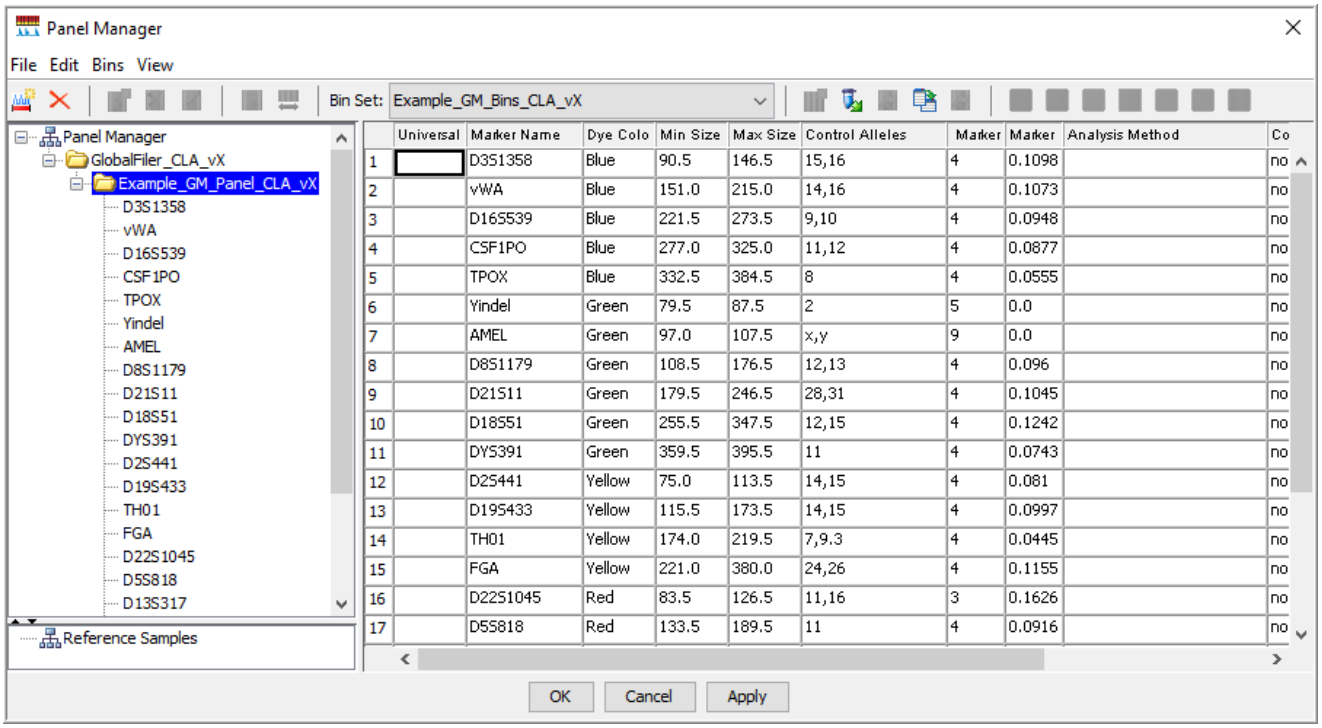
   b. Select **File ▸ Import Bin Set** to open the **Import Bin Set** dialog box.

   c. Navigate to, then select, the appropriate GM_Bins_CLA_vX.txt file.



6. *(Optional)* View the imported panels and bins in the navigation pane: Double-click the Identifiler folders in the left pane.

   The panel information is displayed in the right pane and the markers are displayed below it.



	Universal	Marker Name	Dye Colo	Min Size	Max Size	Control Alleles	Marker	Marker	Analysis Method	Co
1		D3S1358	Blue	90.5	146.5	15,16	4	0.1098		no
2		vWA	Blue	151.0	215.0	14,16	4	0.1073		no
3		D16S539	Blue	221.5	273.5	9,10	4	0.0948		no
4		CSF1PO	Blue	277.0	325.0	11,12	4	0.0877		no
5		TPOX	Blue	332.5	384.5	8	4	0.0555		no
6		Yindel	Green	79.5	87.5	2	5	0.0		no
7		AMEL	Green	97.0	107.5	x,y	9	0.0		no
8		D8S1179	Green	108.5	176.5	12,13	4	0.096		no
9		D21S11	Green	179.5	246.5	28,31	4	0.1045		no
10		D18S51	Green	255.5	347.5	12,15	4	0.1242		no
11		DYS391	Green	359.5	395.5	11	4	0.0743		no
12		D2S441	Yellow	75.0	113.5	14,15	4	0.081		no
13		D19S433	Yellow	115.5	173.5	14,15	4	0.0997		no
14		TH01	Yellow	174.0	219.5	7,9.3	4	0.0445		no
15		FGA	Yellow	221.0	380.0	24,26	4	0.1155		no
16		D22S1045	Red	83.5	126.5	11,16	3	0.1626		no
17		D5S818	Red	133.5	189.5	11	4	0.0916		no

7. Click **Apply**, then click **OK** to add the panel and bins to the GeneMapper™ Software database.

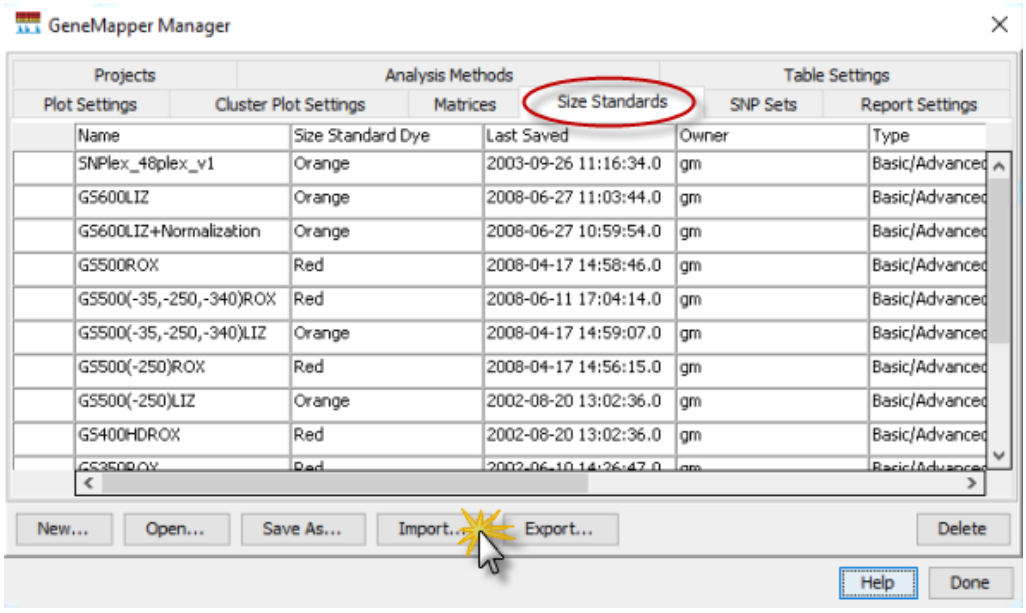
   IMPORTANT! If you close the **Panel Manager** without clicking **Apply**, the panels, bin sets, and marker stutter are not imported into the software database.

Import the size standard

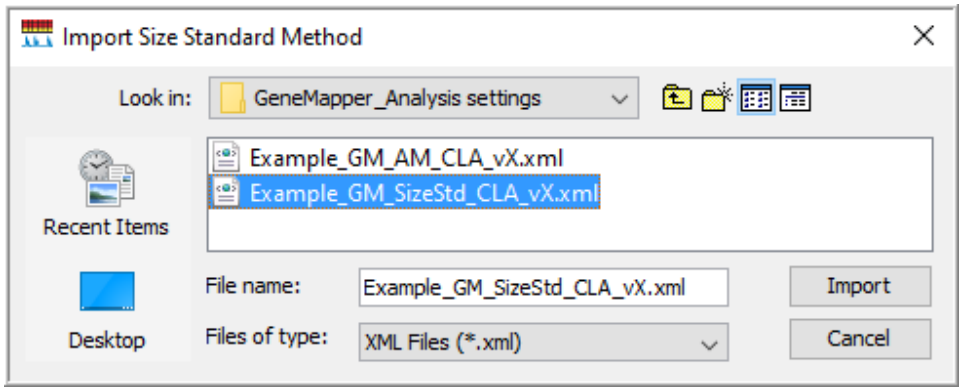
Import the latest size standard from the website into the GeneMapper™ Software database:

1. In the GeneMapper™ Software, select **Tools** ▸ **GeneMapper Manager**.
2. In the **GeneMapper Manager** window, select the **Size Standards** tab.

3. In the **Size Standards** tab, click **Import.**



4. Navigate to, then select, the appropriate GM_SizeStd_CLA_vX.xml file you obtained in "(If needed) Download newer versions of panel and bin files" on page 59.



5. Click **Import**.

6. Click **Done**.

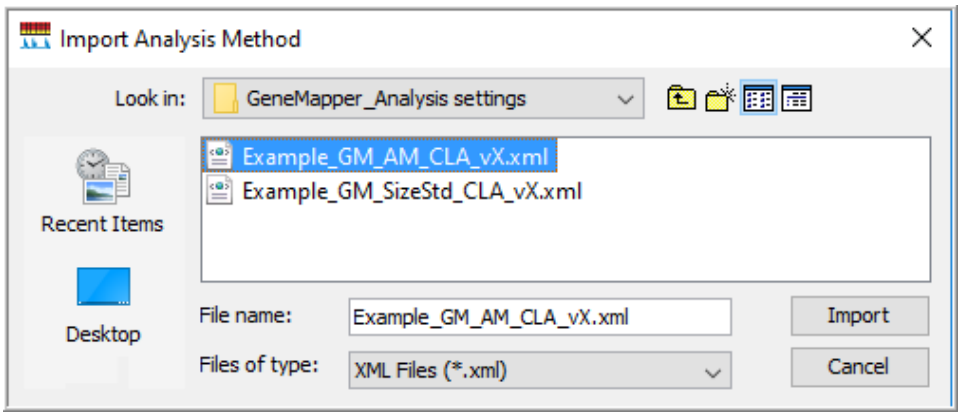
Import analysis method

1. In the GeneMapper™ Software, select **Tools ▸ GeneMapper Manager**.

2. In the **GeneMapper Manager** window, select the **Analysis Methods** tab.

3. In the **Analysis Methods** tab, click **Import**.

4. Navigate to, then select, the GM_AM_CLA_vX.xml file for your instrument type that you obtained in "(If needed) Download newer versions of panel and bin files" on page 59.



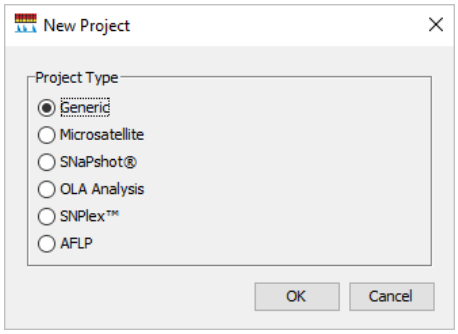
5. Click **Done**.

Import table settings and plot settings

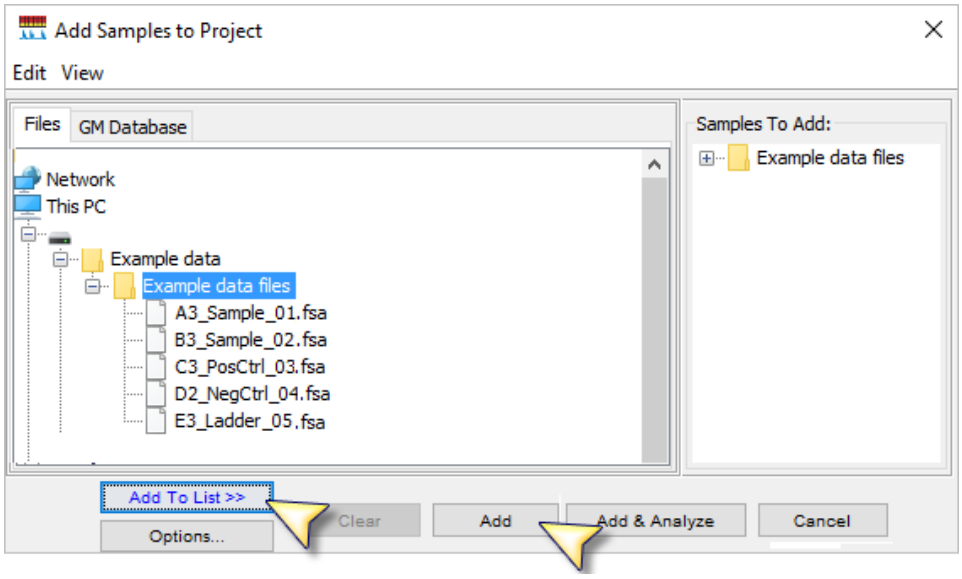
1. Start the GeneMapper™ Software, then log in with the appropriate user name and password.
2. Select **Tools** ▸ **GeneMapper Manager**.
3. In the **Table Settings** tab, click **Import**.
4. Navigate to, then select, the appropriate GM_Table_Set_CLA_vX.xml file you obtained in "(If needed) Download newer versions of panel and bin files" on page 59.
5. Click **Import**.
6. Repeat for plot settings.
7. Click **Done**.

Create a project and analyze sample files with GeneMapper™ Software

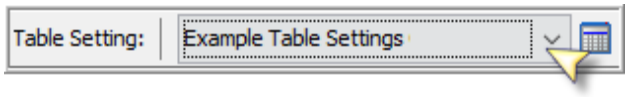
1. If a project is currently open, select **File ▸ New Project**, then select **Generic**.



2. In the **Project** window, select **File ▸ Add Samples to Project**, select the folder that contains the sample files, click **Add to List**, then click **Add**.



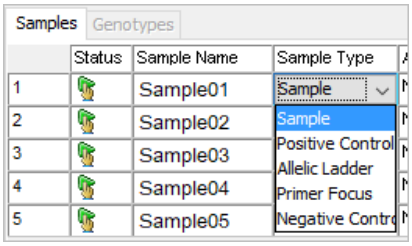
3. Select the table setting that determines the columns that are displayed in samples table and genotypes table: Click the arrow next to the **Table Setting** list, then select the setting.



4. Apply analysis settings to the samples in the project:

   a. Click the **Sample Type** field in the first row, then select the appropriate sample type.

   Repeat for each sample.

   IMPORTANT! You need at least 1 allelic ladder in the project.

   b. Repeat for the **Analysis Method**, **Panel**, and **Size Standard** fields. Select the objects you imported in "Set up the GeneMapper™ Software for CLA analysis (before first use of the kit)" on page 57.

   IMPORTANT! Analysis method is instrument-specific. Ensure that you select the appropriate analysis method for the sample files in the project.

   c. *If this is the first time the analysis method is used*: Double-click the analysis method in the first row, click the **Allele** tab. Select the bin set you imported in "Set up the GeneMapper™ Software for CLA analysis (before first use of the kit)" on page 57.

   d. Fill in remaining rows.

   Note: You can fill in the first row, click the column header to select all rows, then use **Ctrl+D** to fill down remaining rows.

5. 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.
   - The table displays the row of the sample currently being analyzed in green (or red if analysis failed for the sample).
   - When analysis is complete, the **SQ** field displays a PQV result, and the **Genotypes** tab is available.

Samples | Genotypes

	Status	Sample	Sample Type	Analysis Method	Panel	Size Standarc	SQ	Well
1		A1	Sample	Example CLA Ar	Example_Cl	Example CLA		A01
2		B1	Sample	Example CLA Ar	Example_Cl	Example CLA		B01
3		C1	Sample	Example CLA Ar	Example_Cl	Example CLA		C01
4		D1	Sample	Example CLA Ar	Example_Cl	Example CLA		D01
5		E1	Sample	Example CLA Ar	Example_Cl	Example CLA		E01
6		F1	Sample	Example CLA Ar	Example_Cl	Example CLA		F01

Review analyzed results in the sample and genotypes table

1. If you have not already done so, select the table setting that determines the headers that are displayed in sample and genotype plot: Click the arrow next to the **Table Setting** list at the top of the screen, then select the setting.

2. In the samples table, examine the SQ (sizing quality) result.

Samples Genotypes

	Status	Sample File	Sample	Sample Type	Analysis Method	Panel	Size Standarc	Run Name	Run Date & Time	SQ
1		A1_A1_Sample_2	A1	Sample	Example CLA Ar	Example_Cl	Example CLA	GF SeqStudio	2018-06-11 15:38:51	
2		B1_B1_Sample_2	B1	Sample	Example CLA Ar	Example_Cl	Example CLA	GF SeqStudio	2018-06-11 15:38:51	
3		C1_C1_Sample_2	C1	Sample	Example CLA Ar	Example_Cl	Example CLA	GF SeqStudio	2018-06-11 15:38:51	
4		D1_D1_Sample_2	D1	Sample	Example CLA Ar	Example_Cl	Example CLA	GF SeqStudio	2018-06-11 15:38:51	
5		E1_E1_Sample_2	E1	Sample	Example CLA Ar	Example_Cl	Example CLA	GF SeqStudio	2018-06-11 17:37:52	

3. Click the **Genotypes** tab, then scroll to the right to examine the PQV flags (see "About PQVs (Process Quality Value symbols)" on page 73.

Note: To change the sort order or to display additional PQVs, click **(Table Settings)**, click the **Sample** or **Genotypes** tab, then select additional PQVs.

Samples Genotypes

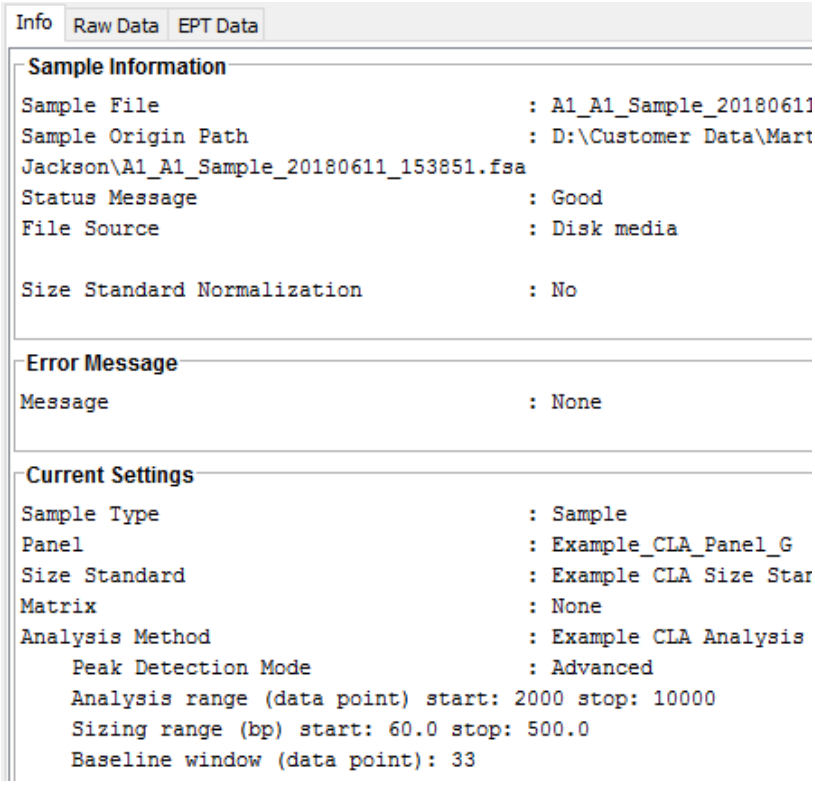
	Sample File	Samp	Panel	Marker	Allele 1	Allele 2	Size 1	Size 2	Height 1	Height 2	PHR	AN	GQ
1	A1_A1_Sample_2	A1	Example_CLA_Pa	AMEL							NA	NA	
2	H6_H6_Sample_2	H6	Example_CLA_Pa	AMEL							NA		
3	A1_A1_Sample_2	A1	Example_CLA_Pa	CSF1PO							NA	NA	
4	C4_C4_Sample_2	C4	Example_CLA_Pa	CSF1PO	9	11	295.06	303.08	2309	5290			
5	C5_C5_Sample_2	C5	Example_CLA_Pa	CSF1PO	9	11	295.12	303.07	3036	6126			

Examine low-quality sizing results

Before proceeding, ensure that you have selected the correct size standard and analysis method (which may be instrument-specific) for the data you are analyzing.

View data collection settings

1. In the **Project** window, select a sample in the sample table, then select **View ▸ Sample Info**.
   Scroll down to view all settings.



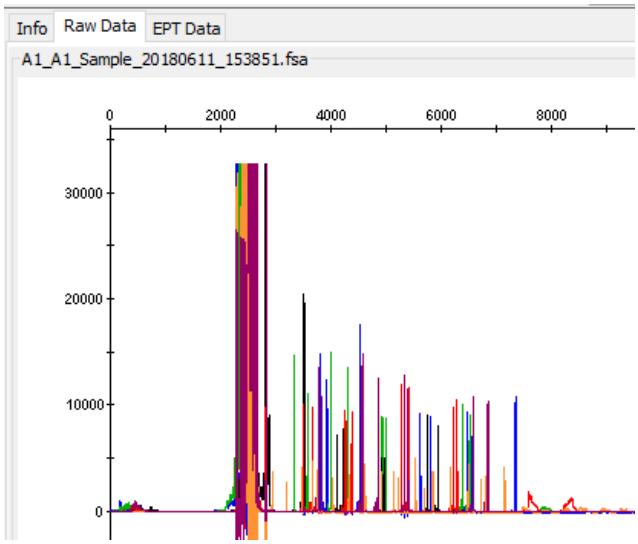
2. To return to the **Project** window, click **Project** in the left pane.

Examine EPT and raw data

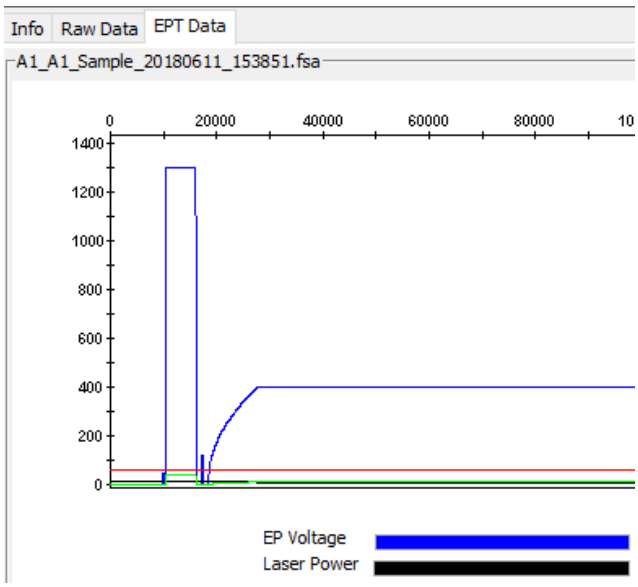
Review the EPT (ElectroPherogram Telemetry) plot to identify instrument performance issues that can affect size quality.

Review the raw data to evaluate anomalies, the causes of poor size-calling, and to determine the start and stop points for analysis. The start point for data analysis occurs after the primer peak and before the first sizing peak. The stop point for analysis occurs after the last sizing peak.

1. In the **Project** window, select a sample in the sample table, then select **View ▸ Sample Info**.
2. Click **Raw Data** to view the raw data plot.



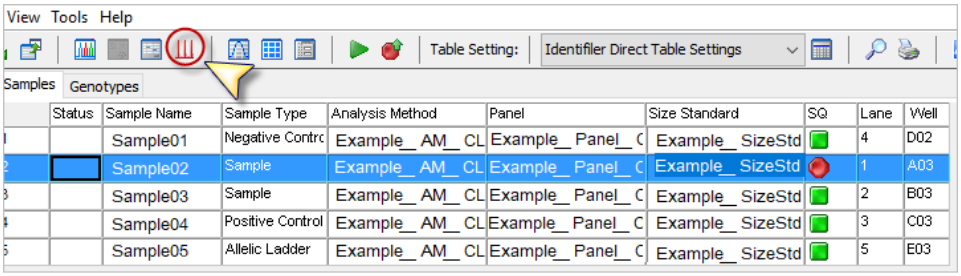
3. Click the **EPT** tab to view the EPT plot.



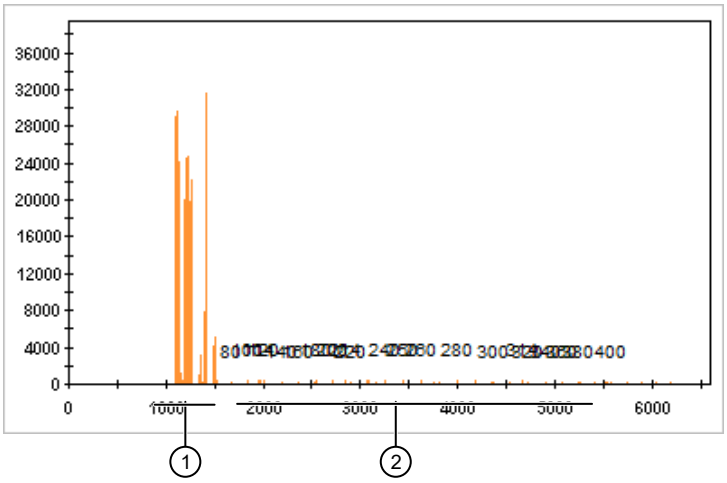
4. To return to the **Project** window, click **Project** in the left pane.

Review size matches

1. In the sample table, select a sample with **SQ**, then click **(Size Match Editor)**.

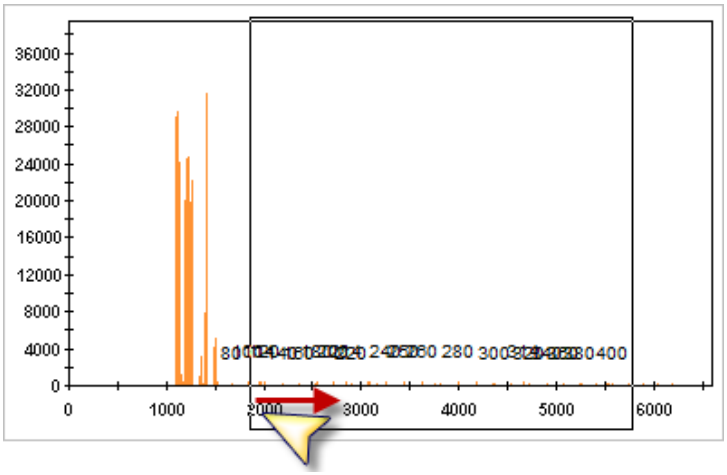


The **Size Match Editor** is displayed.

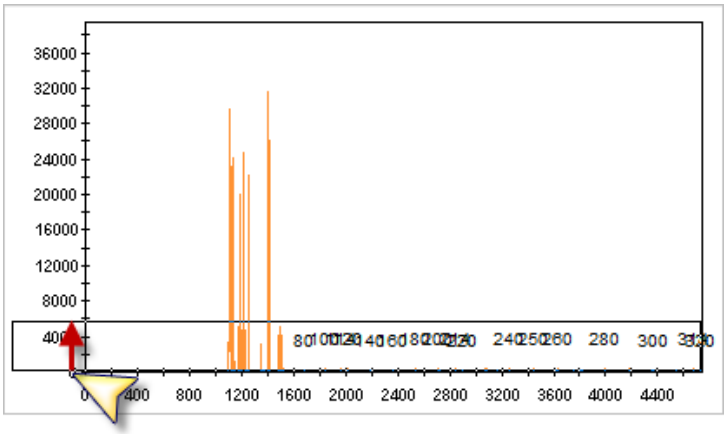


① Primer peaks
② Size standard peaks

2. Zoom in on the size standard peaks:
   a. Place the cursor on a number in the y-axis, then click-drag to the right.



b. Place the cursor on a number in the x-axis, then click-drag up.

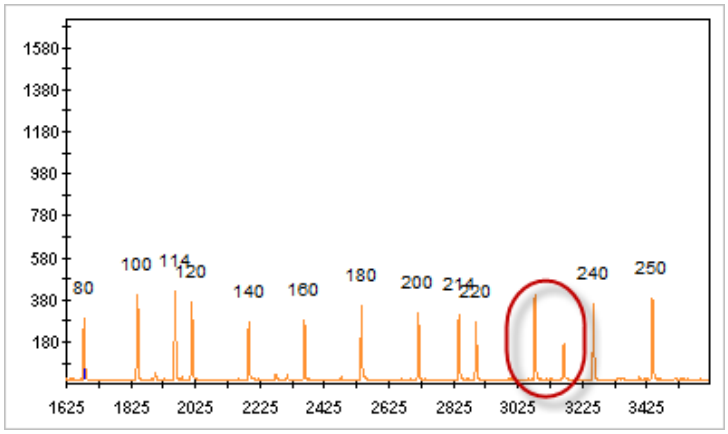


You may have to repeat these steps a few times to zoom on the size standard peaks.

To unzoom to full range, double-click a number in the x-axis.

3. When the trace is zoomed and you can distinguish peaks, compare the profile you observe to the profile for the GeneScan™ 600 LIZ™ Size Standard v2.0 (Figure 3 on page 13). Determine if all size standard peaks are correctly labeled.

   In the example below, 2 peaks were unlabeled, and all remaining peaks are incorrectly labeled.



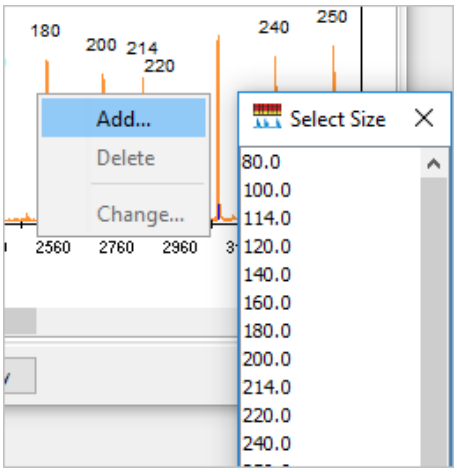
4. Add or delete peaks, or change values, as needed (see “Modify size matches” on page 72).

Modify size matches

If the software does not correctly detect size standard peaks, you can modify the size matches, then apply the modified size standard definition to all selected samples in the project.

1. Display and zoom on the trace in the **Size Match Editor** (see “Review size matches” on page 70.

2. Use Figure 3 on page 13 as a reference to assign the correct sizes to the peaks: Right-click an unlabeled peak, select **Add**, then double-click the correct size (in this example, 240).

Option	Action
Change a size value	Double-click the size value in the trace, then select a different value.
Add a size value to a peak	Right-click an unlabeled peak, select **Add**, then select a size value. **Note:** If you cannot select a peak, it indicates the peak is below the **Peak Amplitude Threshold** in the analysis method and is not detected. 
Delete a size value from a peak	Right-click a labeled peak, then select **Delete**.
Override the SQ	After you correct all size assignments, click **Override SQ**.

3. Click **Apply**.

4. Reanalyze samples to apply the new settings.

Modify the analysis method

For a description of analysis settings, click ?, then search for **Analysis Method Editor**. View the help topics for Microsatellite settings.

1. In the **Project** window, double-click the analysis method for the sample of interest.
2. Modify the settings.

   The following settings are recommended for CLA data analysis. Modify other settings as needed.

Analysis parameter	CLA GlobalFiler™ kit	
	SeqStudio™ Flex, SeqStudio™, 3500	3730
Baseline Window Size	33	33
Peak Window Size	13	9
Smoothing	Light (default)	Light (default)
Size Calling	Local Southern (60–460)	Local Southern (60–460)

3. Click **OK**.
4. Reanalyze samples to apply the new settings.

Adjust Size Quality Flag ranges

1. In the **Project** window, double-click the analysis method for the sample of interest.
2. Click the **Quality Flags** tab.
3. Adjust the settings as needed.

   For definitions of PQVs, click ?, then click the **Search** tab, type the PQV name, then click **List Topics**.
4. Click **OK**.
5. Reanalyze samples to apply the new settings.

Examine low-quality genotyping results

About PQVs (Process Quality Value symbols)

PQVs are flags that reflect the quality of results. and are triggered if a peak does not meet expected thresholds.

Thresholds are set in the **Peak Quality** and **Quality flags** tabs in the analysis method.

For definitions of PQVs, click ?, then click the **Search** tab, type the PQV name, then click **List Topics**.

Review PQVs of samples with Suspect and Low Quality GQ or PQV values

1. Click the **Genotypes** tab, then scroll to the right to examine the PQV flags (see "About PQVs (Process Quality Value symbols)" on page 73.

   Note: To change the sort order or to display additional PQVs, click **(Table Settings)**, click the **Sample** or **Genotypes** tab, then select additional PQVs.

Samples | Genotypes

	Sample File	Samp	Panel	Marker	Allele 1	Allele 2	Size 1	Size 2	Height 1	Height 2	PHR	AN	GQ
1	A1_A1_Sample_2	A1	Example_CLA_Pa	AMEL							NA	NA	
2	H6_H6_Sample_2	H6	Example_CLA_Pa	AMEL							NA		
3	A1_A1_Sample_2	A1	Example_CLA_Pa	CSF1PO							NA	NA	
4	C4_C4_Sample_2	C4	Example_CLA_Pa	CSF1PO	9	11	295.06	303.08	2309	5290			
5	C5_C5_Sample_2	C5	Example_CLA_Pa	CSF1PO	9	11	295.12	303.07	3036	6126			

2. To view the electropherogram for a marker, select the rows in the table that correspond to the marker, then click **(Display Plots)**.

   Figure 13 shows a GQ result that is caused by:

   - A peak height ratio that does not meet the threshold (PHR PQV).
   - The detection of 3 peaks in the marker (AN PQV).

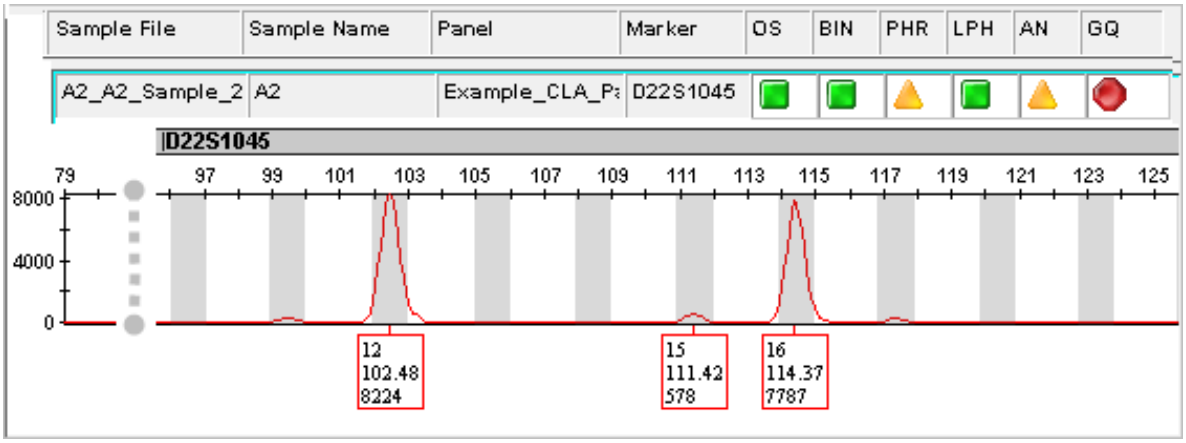
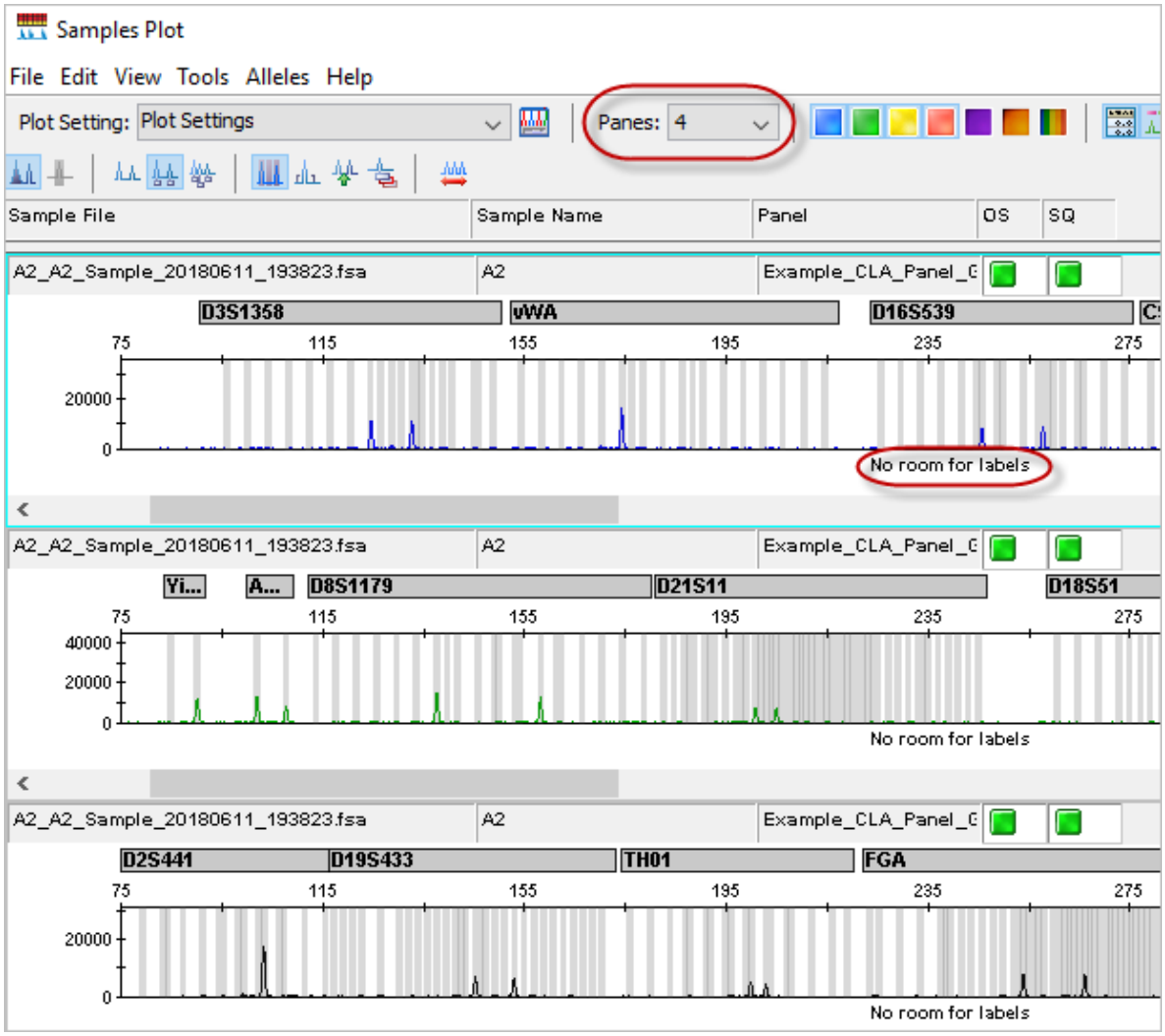


Figure 13 Example 1

3. As needed, edit alleles. For information, click , search for **Sample/Genotypes Plot**, then click **List Topics**. Double-click the **Sample/Genotypes Plot** topic, then click **Modifying Alleles**.

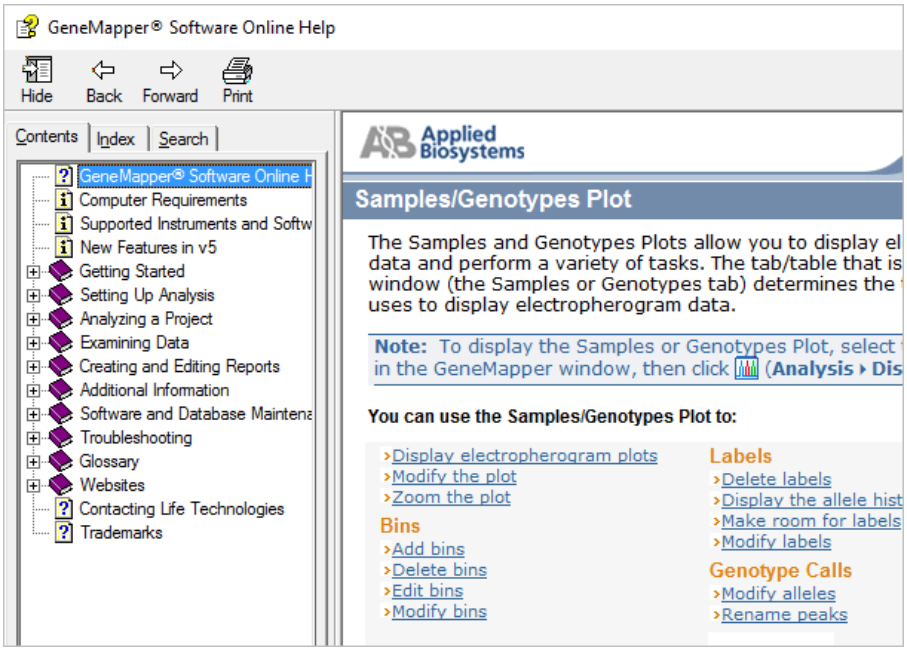
Display and zoom on sample plots

1. To view the electropherogram for a sample, click the sample in the sample table, then click **(Display Plots)**.



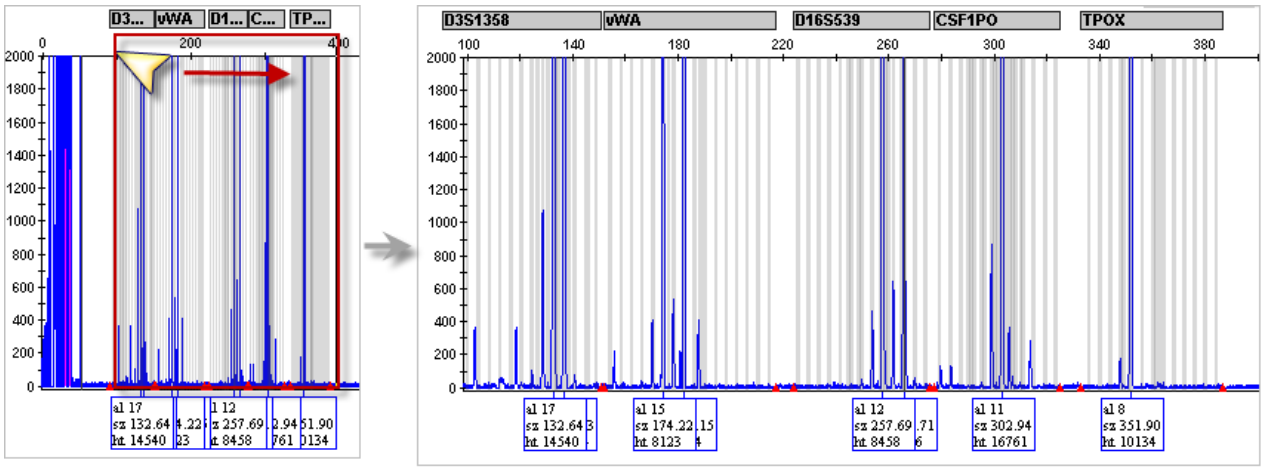
Note: To show fewer traces on a screen and make room for peak labels, change the **Panes** setting to **2**.

For more information on the samples plot, select **Help** ▸ **Contents and Index**.

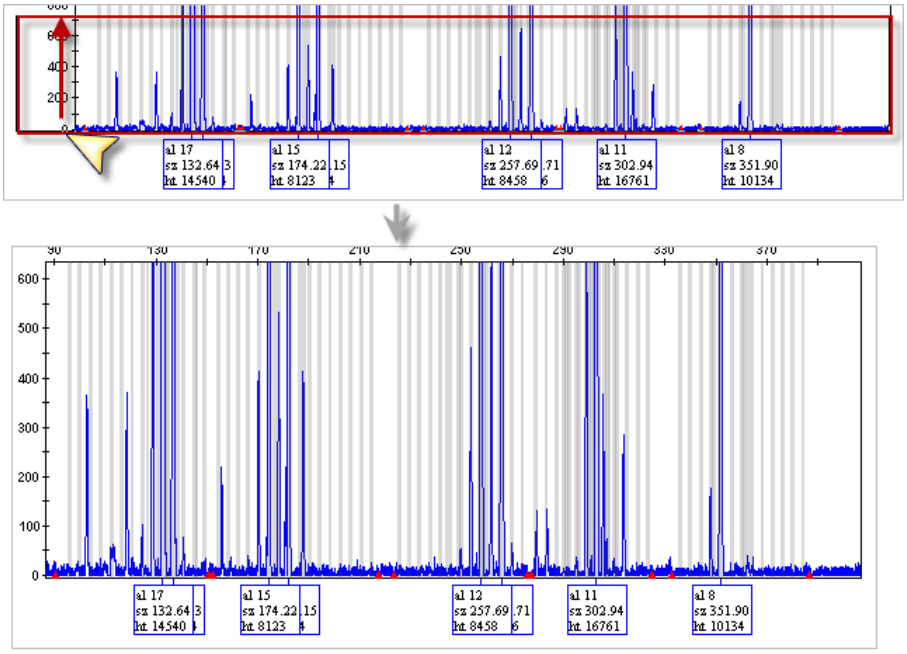


2. Select the plot setting that determines the headers that are displayed in sample and genotype plot: Click the arrow next to the **Plot Setting** list at the top of the screen, then select the setting.

3. To zoom on the plot, select **View ▸ Zoom**, or:
   - To zoom on the x- axis, place the cursor on the x-axis *at the top of the plot*, then click-drag to the right.



- To zoom on the y- axis, place the cursor on the 0 y-axis, then click-drag up.

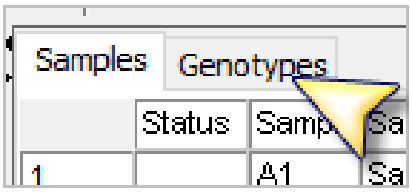


- To unzoom double-click the x-axis.

4. To close the sample plot, click the X in top-right corner of the screen.

Display and zoom genotype plots

1. In the project window, click the **Genotypes** tab.

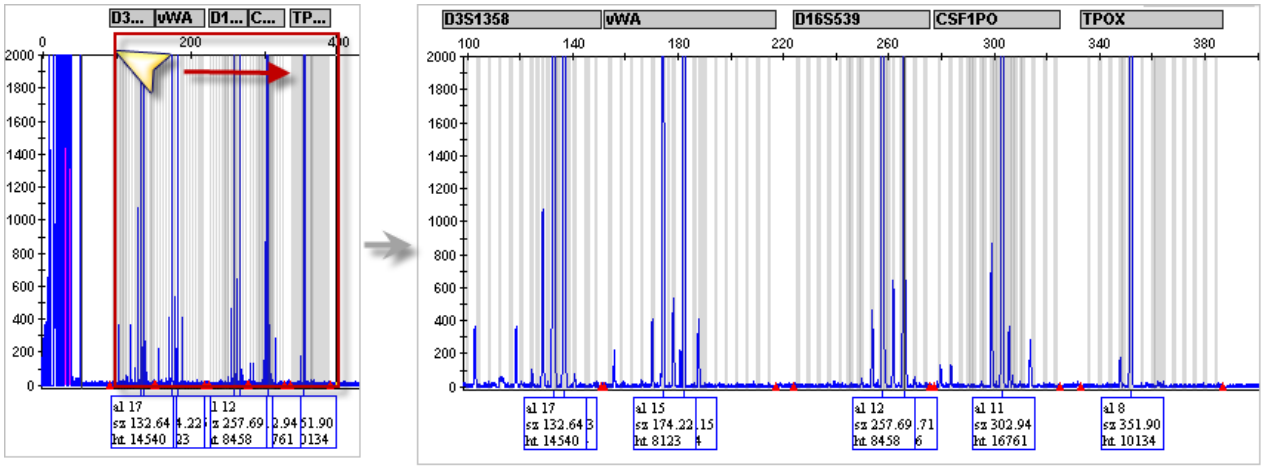


2. If you have not already done so, select the plot setting that determines the headers that are displayed in sample and genotype plot: Click the arrow next to the **Plot Setting** list at the top of the screen, then select the setting.

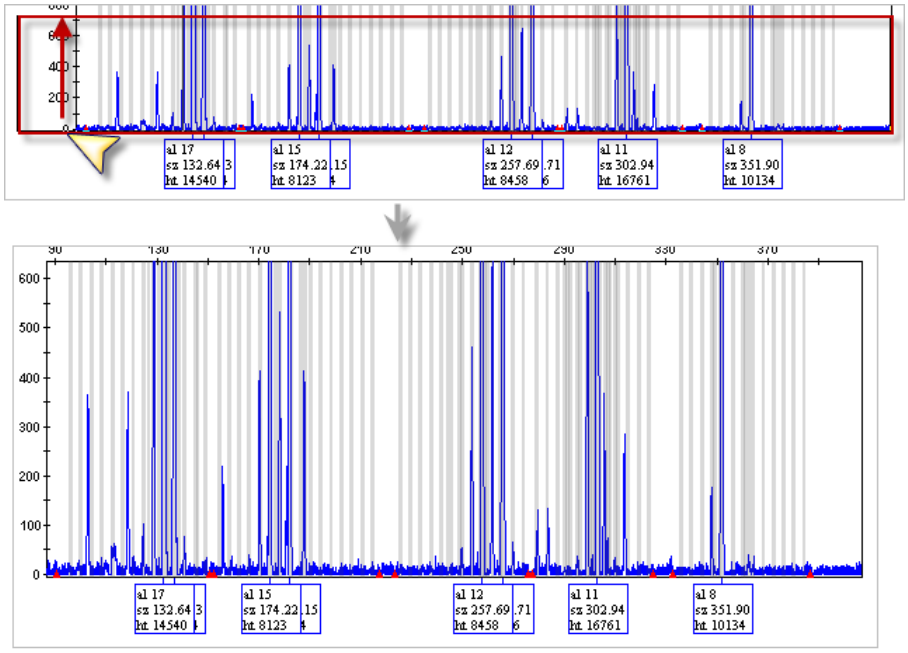
3. To view the electropherogram for a marker, click a rows in the table that correspond to the marker, then click **(Display Plots)**.

4. To zoom on the plot, select **View ▸ Zoom**, or:

   - To zoom on the x- axis, place the cursor on the x-axis *at the top of the plot*, then click-drag to the right.



   - To zoom on the y- axis, place the cursor on the 0 y-axis, then click-drag up.

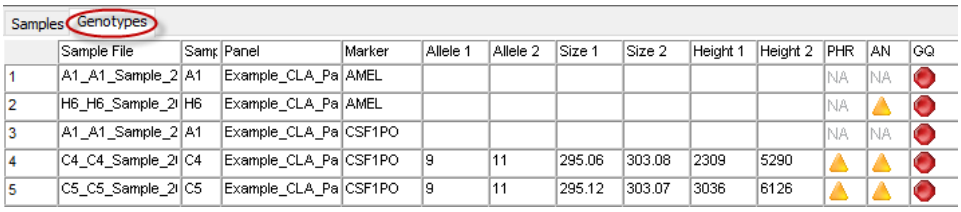


   - To unzoom double-click the x-axis.

Export the genotypes table

You can export the genotypes table for reference when you compare the genotypes to the ATCC database.

1. Open the project that contains the samples of interest.
2. Click the **Genotypes** tab.



	Sample File	Samp	Panel	Marker	Allele 1	Allele 2	Size 1	Size 2	Height 1	Height 2	PHR	AN	GQ
1	A1_A1_Sample_2	A1	Example_CLA_Pa	AMEL							NA	NA	
2	H6_H6_Sample_2(	H6	Example_CLA_Pa	AMEL							NA		
3	A1_A1_Sample_2	A1	Example_CLA_Pa	CSF1PO							NA	NA	
4	C4_C4_Sample_2(	C4	Example_CLA_Pa	CSF1PO	9	11	295.06	303.08	2309	5290			
5	C5_C5_Sample_2(	C5	Example_CLA_Pa	CSF1PO	9	11	295.12	303.07	3036	6126			

3. Display the columns that you want to export.
4. Select **File** ▸ **Export Table**.
5. Select the export file type (the default is TXT).
6. Click **Export**.

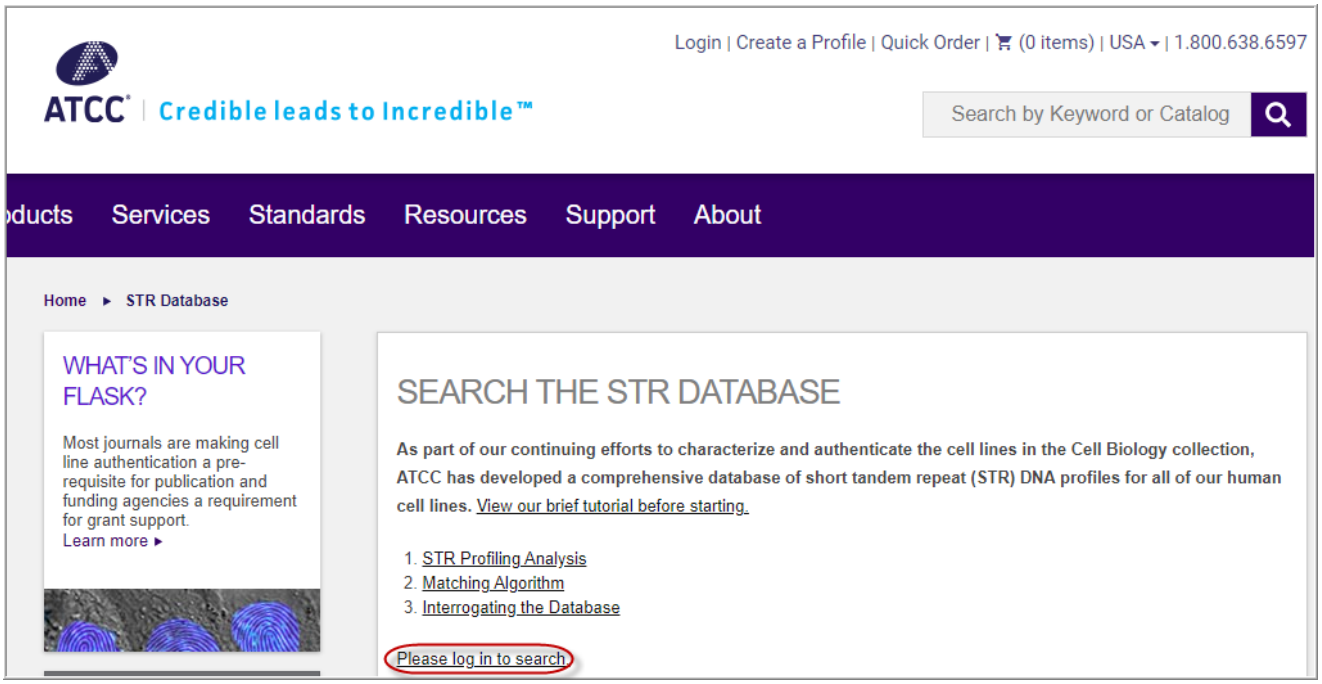
Verify cell line identity using the ATCC database

Cell line identity can be verified by uploading genotypes to the ATCC cell line STR database. The ATCC database requires you to enter genotypes for 8 specific loci, all of which are present in the Applied Biosystems™ CLA STR kits.

1. In a web browser (for example, Google™ Chrome™), navigate to **www.atcc.org/STR_Database**.

Note: Click **View our brief tutorial before starting** if you are not familiar with this website.

2. Click **Please log in to search**, then log in or create a user profile.



3. Refer to the genotypes from the file you exported, then enter the genotypes in the ATCC screen.

4. Click **Matches >= 80%**.

   After a few minutes, a list of cell lines in the ATCC database that match the STRs present in the test sample is displayed.

Note: The DSMZ at the Leibniz Institute maintains a database of 8-17 STR alleles for common cell lines. To use the database, see the DSMZ instructions; go to:

https://celldive.dsmz.de/str

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6 Troubleshooting

Causes of extra peaks

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

Stutter peak definition

Stutter is a well-characterized PCR artifact that refers to the appearance of a minor peak that is typically one repeat unit smaller or larger than the target STR allele product (Butler, 2005; Mulero *et al*., 2006). Sequence analysis of stutter products at tetranucleotide STR loci has revealed that the minus stutter product is missing a single tetranucleotide core repeat unit relative to the main allele (Walsh *et al.*, 1996). Stutter peaks are caused by slippage in the DNA strand.

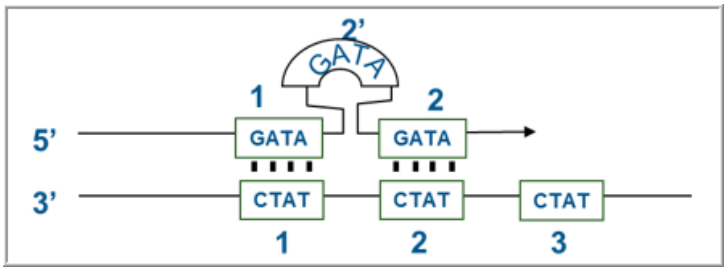


Figure 14 Plus stutter is caused by slippage in the top DNA strand. Example of tetranucleotide stutter (one unit = 4 bp).

The CLA GlobalFiler™ kit includes tetranucleotide markers and one trinucleotide marker (DS22S1045). The kit also uses faster thermal cycling parameters than other tetranucleotide repeat STR kits, which can produce higher stutter and more atypical stutter events.

The proportion of the stutter product relative to the main allele (percent stutter) is measured by dividing the height of the stutter peak by the height of the main allele peak.

3′ A nucleotide addition peak definition

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

Artifact peak definition

Artifacts and anomalies are seen in all molecular biological systems. Artifacts are typically reproducible. Anomalies are non-reproducible, intermittent occurrences that are not observed consistently in a system (for example, spikes and baseline noise).

Pull-up peak definition

Pull-up peaks are observed if simultaneous fluorescence occurs in multiple dye color channels. Pull-up is caused by the overlapping emission spectrum of fluorescent dyes and usually occurs when the fluorescence signals approach the dynamic range of the instrument.

Pull-up peaks may not appear at the exact location of an allele peak. Evaluate any peak that is ±0.5 bp from an allele peak as a possible pull-up peak.

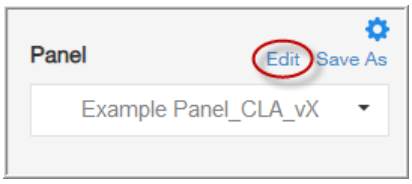
Stutter peak analysis settings

The parameters are used to identify and label stutter peaks for a marker in an electropherogram.

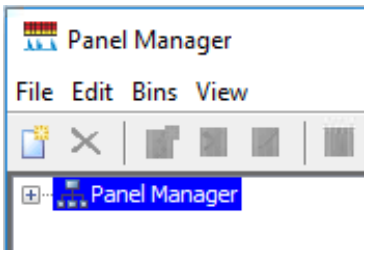
Parameter	Description	Location
Repeat unit	Number of bases in the repeat unit for the designated marker. For example, a tetra repeat unit contains 4 bases. Can be used to determine the number of basepairs from an allele peak at which stutter may be observed.	**Panel Manager**
Stutter ratio	Ratio of secondary peak to allele peak below which a peak is considered to be stutter and is filtered (is not labeled). The GeneMapper™ Software provides settings for minus and plus stutter peaks. If **Use marker-specific stutter ratio if available** is selected, the minus stutter ratio in the **Panel Manager** is used instead of the minus stutter ratio in the analysis method. **Note:** The CLA analysis method (**Allele** tab) specifies **Use marker-specific stutter ratio**.	Analysis method **Allele** tab
Stutter distance	Size window around an expected stutter peak (because a stutter peak may not migrate at an exact size). Example: A stutter distance of 3.25 to 4.75 specifies a ± 0.75 bp window around the possible –4 bp stutter peak. The GeneMapper™ Software provides settings for minus and plus stutter peaks.	Analysis method **Allele** tab
Stutter filter (not a setting)	If a peak is within the stutter distance, and the stutter-to-allele peak ratio is below the stutter ratio in the analysis method or **Panel Manager**, the peak is filtered (not labeled in the electropherogram). **Note:** To be analyzed, a peak must exceed the **Peak Amplitude Threshold** in the analysis method **Peak Detector** tab.	NA

Determine the repeat units, stutter ratio, and stutter distance for a marker

1. Open the **Panel Manager** to display the repeat unit and the stutter ratio:
   - Microsatellite Analysis Software—Click **Edit** in the **Panel** pane to display the **Panel Manager** (Figure 15 on page 85).



- GeneMapper™ Software—
  a. Select **Tools** ▸ **Panel Manager**.
  b. In the left pane, click **Panel Manager**.



  c. Double-click the panel folder, then double-click the panel in the folder to display the **Panel Manager** (Figure 16 on page 86).

All STR markers in the CLA GlobalFiler™ kit are tetranucleotide repeats (4 base pairs in a repeat unit) except for D22S1045, which is a trinucleotide repeat (3 base pairs in a repeat unit)

Note: AMEL and Yindel markers are not STR markers. Disregard the values listed for these markers.

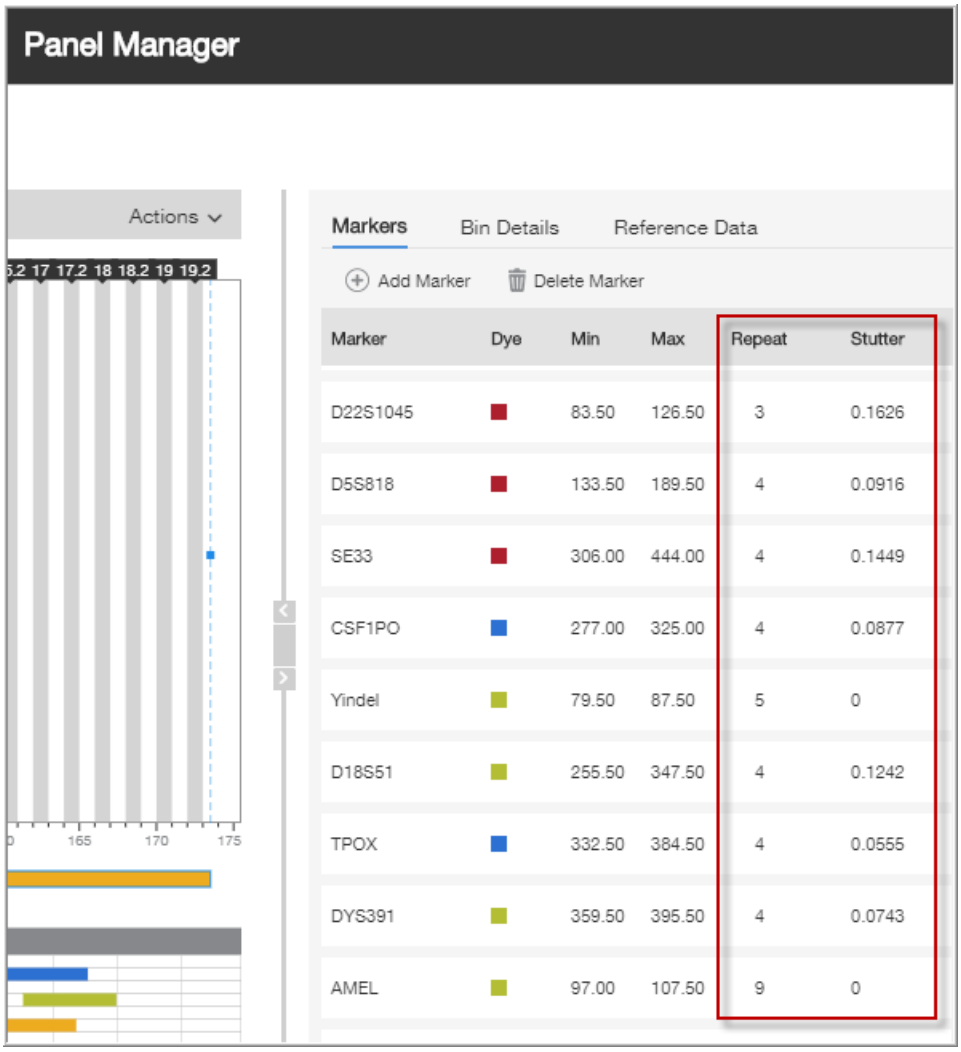


Figure 15 Panel Manager Microsatellite Analysis Software: marker repeat unit and stutter ratio for minus stutter peaks

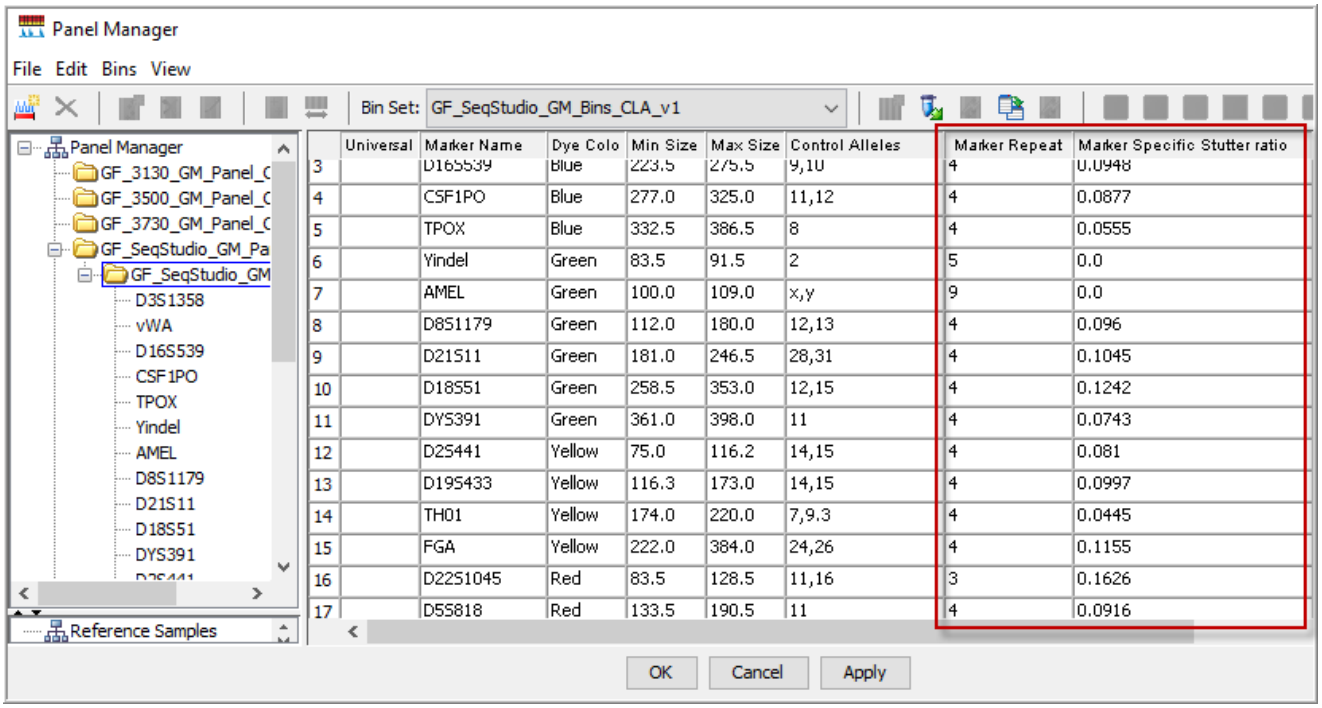
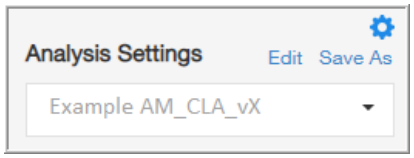


Figure 16 Panel Manager GeneMapper™ Software: marker repeat unit and stutter ratio for minus stutter peaks

2. Open the analysis settings or analysis method **Allele** tab to display the stutter distance:

   - Microsatellite Analysis Software—Click **Edit** in the **Analysis Settings** pane to display the analysis method, click the **Genotype Settings** tab, then click the **Allele** pane (Figure 17 on page 87).



- GeneMapper™ Software—In the samples tab, double-click the analysis method field, then click the **Allele** tab (Figure 18 on page 88).

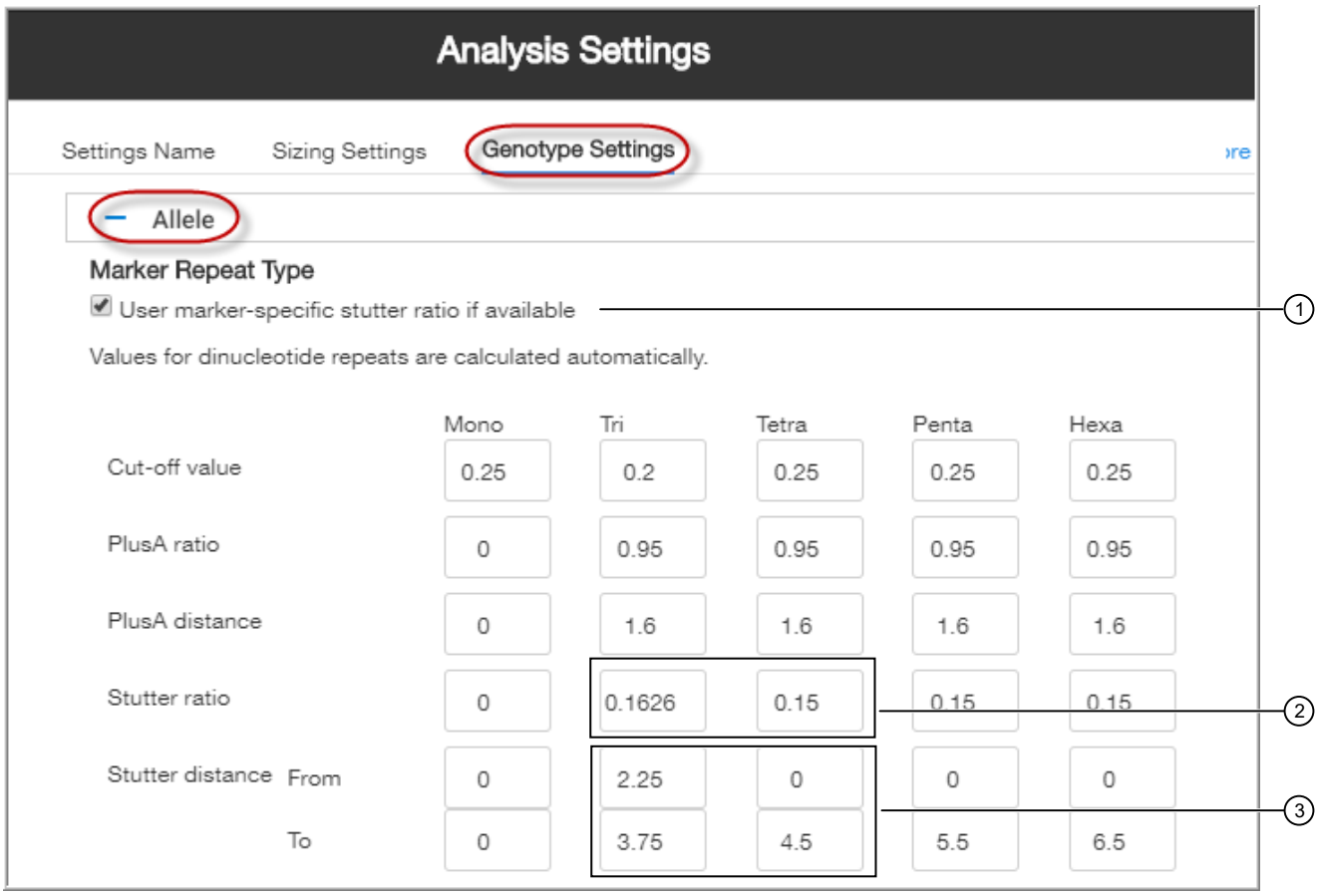


Figure 17 Analysis Settings Allele tab Microsatellite Analysis Software: stutter distance and ratio

① If selected, the minus stutter ratios listed in the panel are used instead of the minus stutter ratios listed on the screen.
② Stutter ratios in the analysis method are not used if **Use marker-specific stutter ratio if available** is selected.
③ Stutter distances for minus stutter peaks

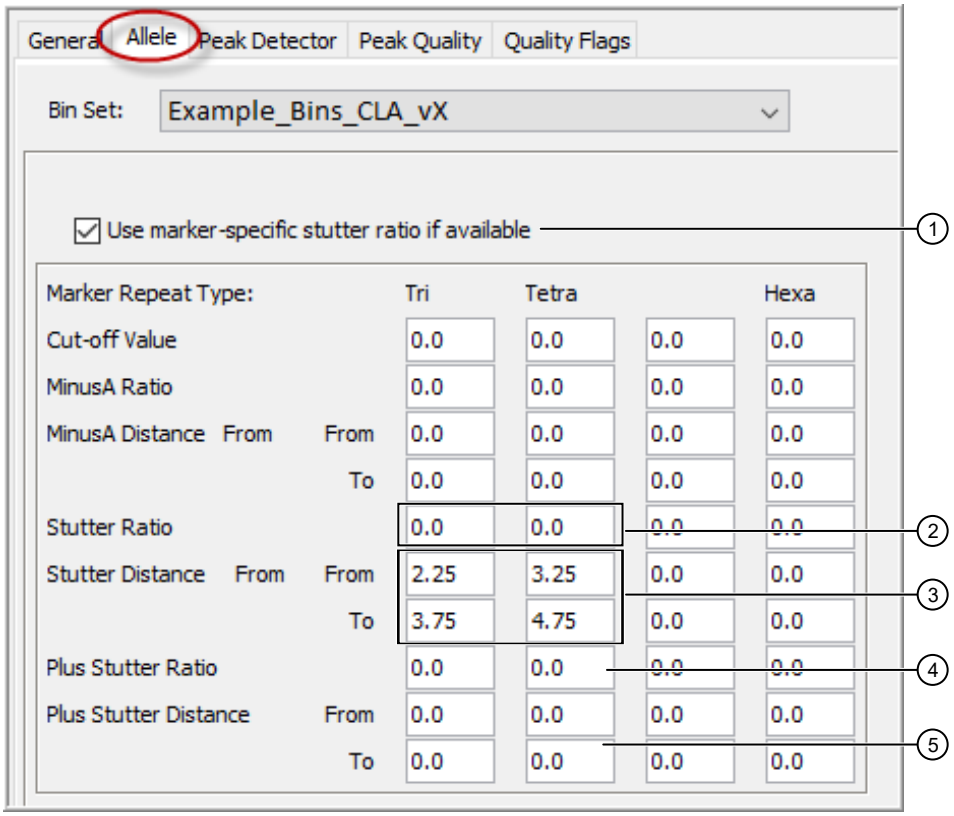


Figure 18 Analysis method Allele tab GeneMapper™ Software: stutter distance and ratio

① If selected, the minus stutter ratios listed in the panel are used instead of the minus stutter ratios listed on the screen.

② Stutter ratios are not used if **Use marker-specific stutter ratio if available** is selected.

③ Stutter distances for minus stutter peaks

④ Plus stutter ratios

⑤ Plus stutter distances for plus stutter peaks

Guidelines for troubleshooting and data interpretation CLA

- To investigate an SQ 🔴 (sizing fails), perform the procedures in:
  - Microsatellite Analysis Software—"Examine low-quality sizing results" on page 45.
  - GeneMapper™ Software—"Examine low-quality sizing results" on page 67.

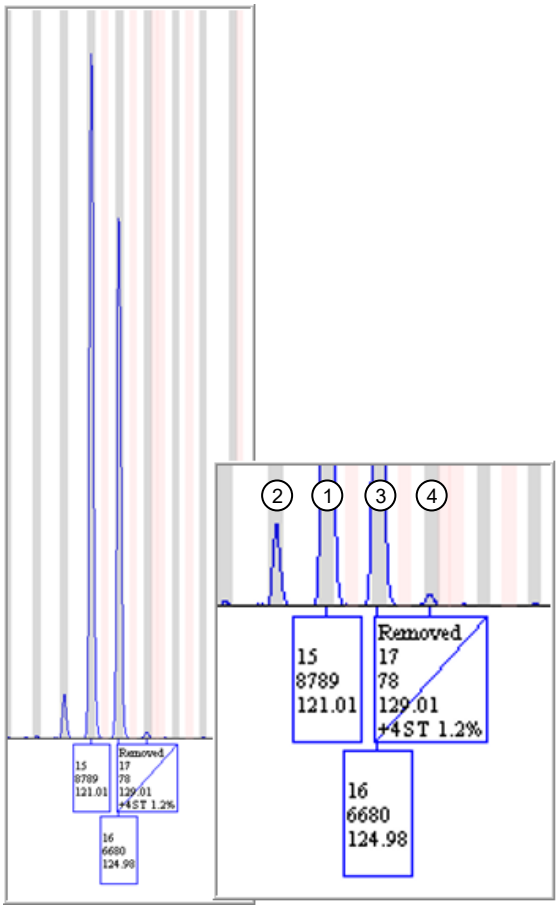
  For more information, see the following sections in this chapter.
- To investigate an GQ 🔴 (genotyping fails), perform the procedures in:
  - Microsatellite Analysis Software—"Examine low-quality genotyping results" on page 49.
  - GeneMapper™ Software—"Examine low-quality genotyping results" on page 49.

  For more information, see the following sections in this chapter.

- For information on the PQV symbols that are displayed in the samples or genotypes table ( ), see:
  - Microsatellite Analysis Software—"About PQVs (Process Quality Value symbols)" on page 49.
  - GeneMapper™ Software—"About PQVs (Process Quality Value symbols)" on page 73.

  For more information, see the following sections in this chapter.
- To be detected and labeled, a stutter peak must exceed the **Peak Amplitude Threshold** in the analysis method and the stutter ratio in the panel. The default stutter ratio filter values that are provided in the panel were determined by extensive studies. However, it is not unusual to observe stutter peaks that are above the stutter ratio filter values. If you consistently observe a stutter peak for a marker, you can create a filter to prevent the peak from being labeled (as determined by your standard operating protocol).
- Stutter peak amplitude is additive. Example: If an N+2 stutter peak from allele peak A migrates at the same location as an N–2 stutter peak from allele peak B, the stutter peak height is increased.
- If you run multiple allelic ladders, the allele calls are averaged for all allelic ladders in a project (Microsatellite Analysis Software) or a folder (GeneMapper™ Software). You can re-analyze a sample using the allelic ladder that is in the closest well position to the sample for possibly improved genotyping results. (To do so, either remove all but the allelic ladder of interest from the project and re-analyze, or create a new project with the sample and the allelic ladder.)
- To determine if a sample peak is expected at a specific size in a marker, display the electropherograms for the sample, size standard, and allelic ladder.
- Before you conclude that a peak is a possible microvariant (after ruling out that it is a stutter peak or artifact), confirm that the peak reproducibly migrates at the observed bp (by running samples in duplicate or by repeating the electrophoresis run).
- If you observe unexpected stutter peaks across all profiles, repeat amplification (ensure correct buffer pH, thermal cycling settings).

D3S1358 marker stutter peak examples (N–4 and N+4)



① Allele peak 15

② Filtered minus stutter peak (–4 bp from allele peak 15).

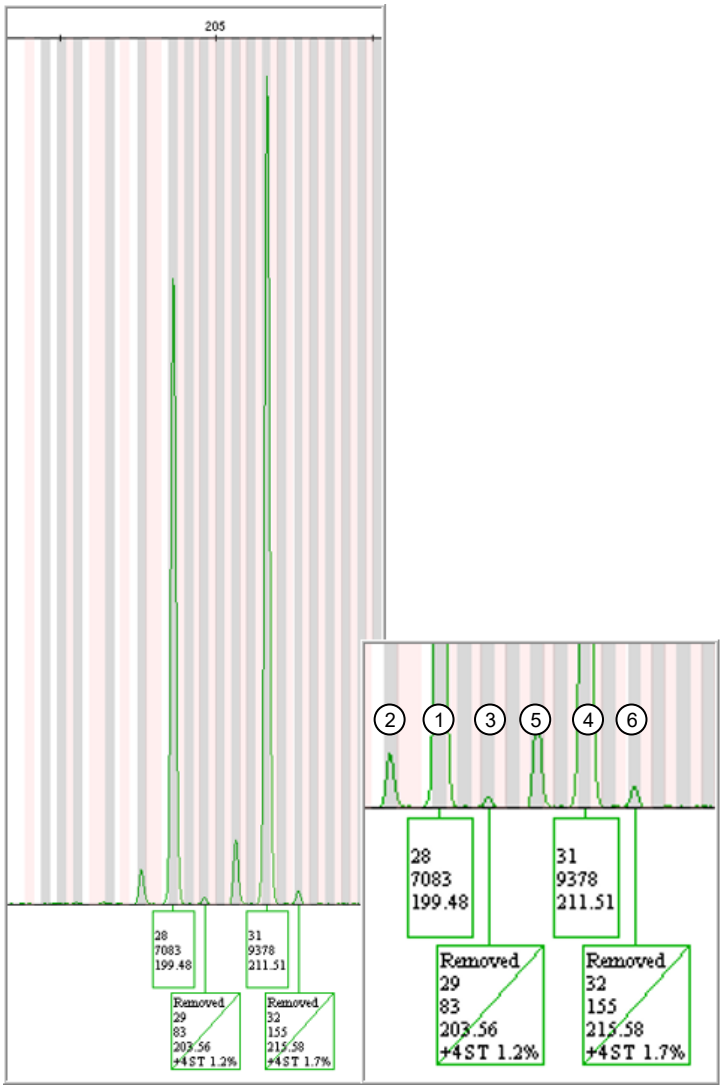
③ Allele peak 16

④ Labeled plus stutter peak (+4 bp from allele peak 16). It is not filtered (and is therefore labeled) because no plus stutter distance was set in the GeneMapper™ Software analysis method that was used to analyze this data.

Note: The Microsatellite Analysis Software does not provide settings for plus stutter.

Note: A stutter peak is filtered (not labeled) if it is 1) above the **Peak Amplitude Threshold** in the analysis method **Peak Detector** tab; 2) within the **Stutter Distance** range in the analysis method **Allele** tab; 3) above the stutter ratio (% of allele peak) in the panel. If a peak label is crossed out, it indicates that the allele label was manually deleted/removed.

D21S11 marker stutter peak examples (N–4 and N+4)

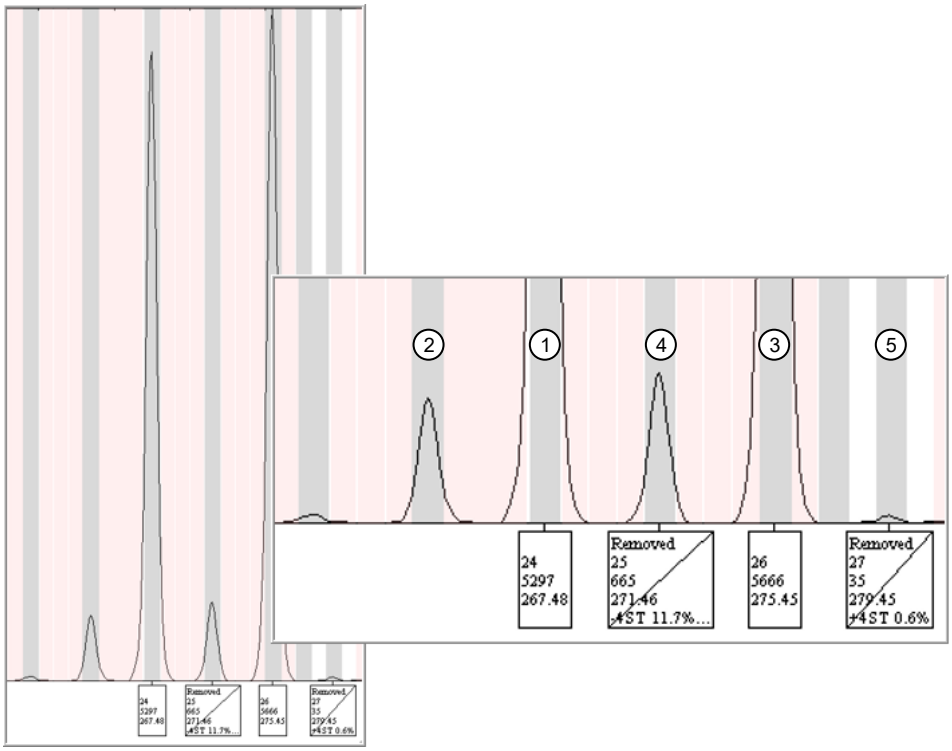


1. Allele peak 28
2. Filtered minus stutter peak (–4 bp from allele peak 28)
3. Labeled plus stutter peak (+4 bp from allele peak 28). It is not filtered (and is therefore labeled) because no plus stutter distance was set in the GeneMapper™ Software analysis method that was used to analyze this data.

   Note: The Microsatellite Analysis Software does not provide settings for plus stutter.
4. Allele peak 31
5. Filtered minus stutter peak (–4 bp from allele peak 31)
6. Labeled plus stutter peak (+4 bp from allele peak 31), see plus stutter description above.

Note: A stutter peak is filtered (not labeled) if it is 1) above the **Peak Amplitude Threshold** in the analysis method **Peak Detector** tab; 2) within the **Stutter Distance** range in the analysis method **Allele** tab; 3) above the stutter ratio (% of allele peak) in the panel. If a peak label is crossed out, it indicates that the allele label was manually deleted/removed.

FGA marker stutter peak examples (N–4 and N+4)



1. Allele peak 24
2. Filtered minus stutter peak (–4 bp from the allele peak 24)
3. Allele peak 26
4. Labeled stutter peak. This peak is a combined minus stutter and plus stutter peak. It is –4 bp from the allele peak 26 +4 bp from the allele peak 24. Peak height is elevated because the two stutter peak signals at the same size are additive.
5. Labeled plus stutter peak (+4 bp from allele peak 24). It is not filtered (and is therefore labeled) because no plus stutter distance was set in the GeneMapper™ Software analysis method that was used to analyze this data.

   Note: The Microsatellite Analysis Software does not provide settings for plus stutter.

Note: A stutter peak is filtered (not labeled) if it is 1) above the **Peak Amplitude Threshold** in the analysis method **Peak Detector** tab; 2) within the **Stutter Distance** range in the analysis method **Allele** tab; 3) above the stutter ratio (% of allele peak) in the panel. If a peak label is crossed out, it indicates that the allele label was manually deleted/removed.

SE33 and D1S1656 marker stutter peak examples (N–2 and N–4)

SE33 and D1S1656 markers are complex STRs with dinucleotide elements to their repeat structures.

N–2 stutter peaks are a normal observation in the SE33 and D1S1656 markers. N–4 stutter peaks are also often present. The default minus stutter settings filter out some stutter peaks, but elevated stutter peaks, or stutter peaks for which no stutter settings are set, may be labeled.

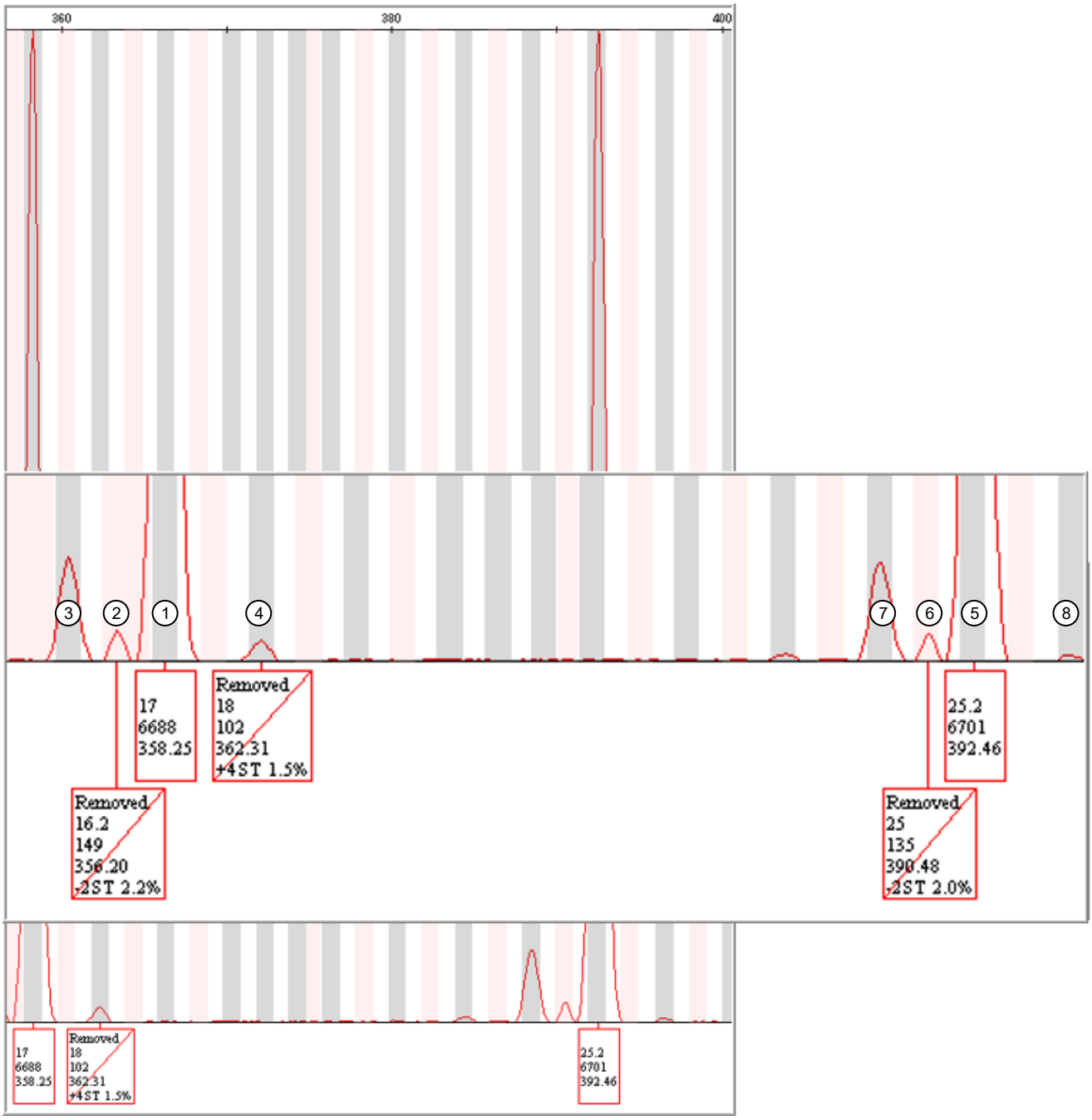


Figure 19 SE33 marker

① Allele peak 17

② Minus stutter peak (–2 bp from the allele peak 17). It is *not* filtered (therefore, it *is* labeled) because the software allows only one stutter filter per marker repeat unit. For tetranucleotide repeats, only –4 stutter peaks can be filtered.

③ Filtered minus stutter peak (–4 bp from the allele peak 17)

④ Plus stutter peak (+4 from the allele peak 17). It is not filtered (therefore, it is labeled) because no plus stutter distance was set in the GeneMapper™ Software analysis method that was used to analyze this data.

Note: The Microsatellite Analysis Software does not provide settings for plus stutter.

⑤ Allele peak 25.2

⑥ Minus stutter peak (–2 bp from the allele peak 25.2). It is not filtered (therefore, it is labeled), see the minus stutter (–2 bp) description above.

⑦ Filtered minus stutter peak (–4 bp from the allele peak 25.2)

⑧ Plus stutter peak (+4 bp from allele peak 25.2). It is not filtered (therefore, it is labeled), see the plus stutter (+4 bp) description above.

Note: A stutter peak is filtered (not labeled) if it is 1) above the **Peak Amplitude Threshold** in the analysis method **Peak Detector** tab; 2) within the **Stutter Distance** range in the analysis method **Allele** tab; 3) above the stutter ratio (% of allele peak) in the panel. If a peak label is crossed out, it indicates that the allele label was manually deleted/removed.

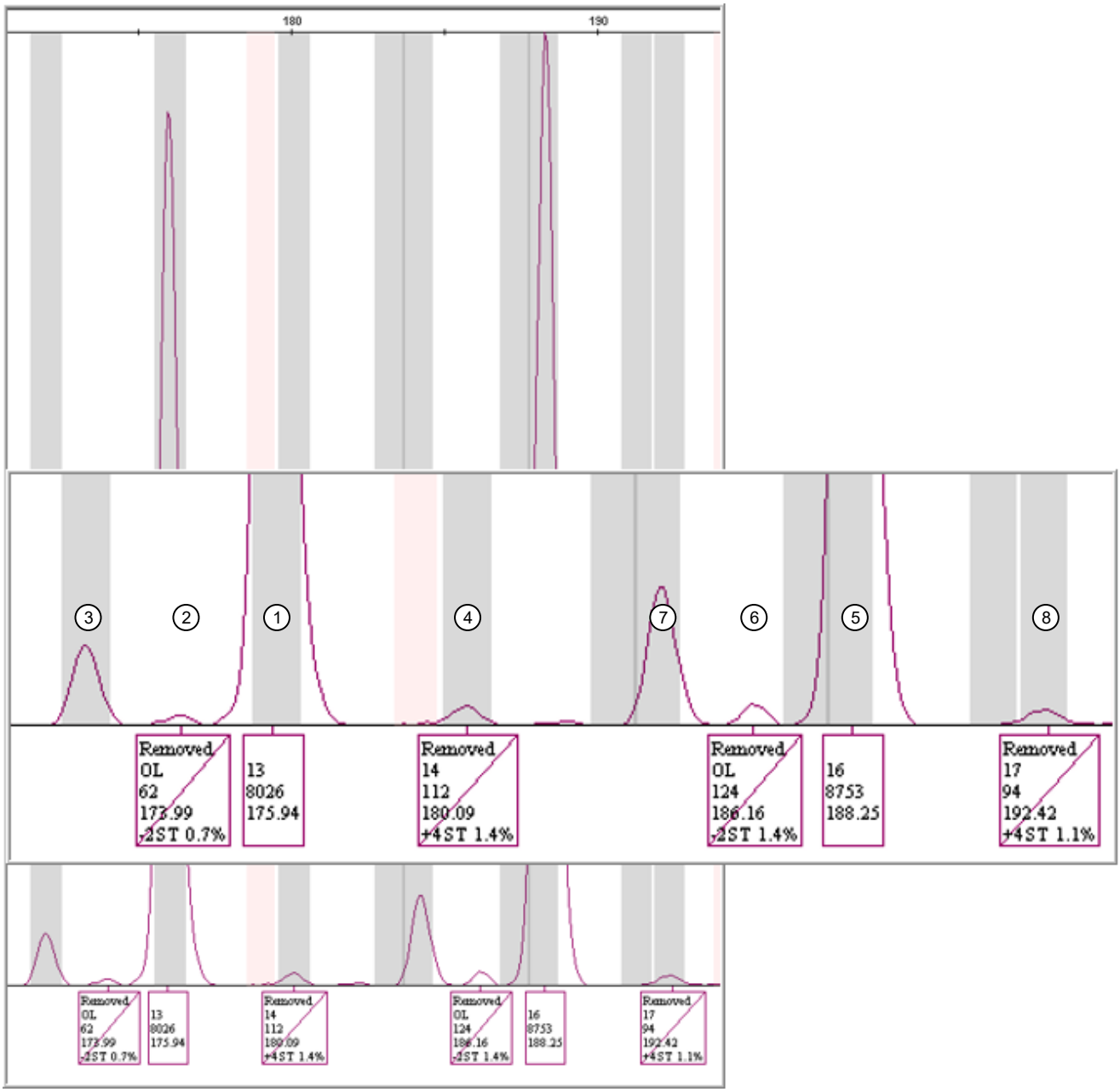


Figure 20 D1S1656 marker with expected N+2 stutter peaks

① Allele peak 13

② Minus stutter peak (–2 bp from allele peak 13)

③ Filtered minus stutter peak (–4 bp from allele peak 13)

④ Plus stutter peak (+4 bp from allele peak 28). It is not filtered (therefore, it is labeled), because no plus stutter distance was set in the GeneMapper™ Software analysis method that was used to analyze this data.

Note: The Microsatellite Analysis Software does not provide settings for plus stutter.

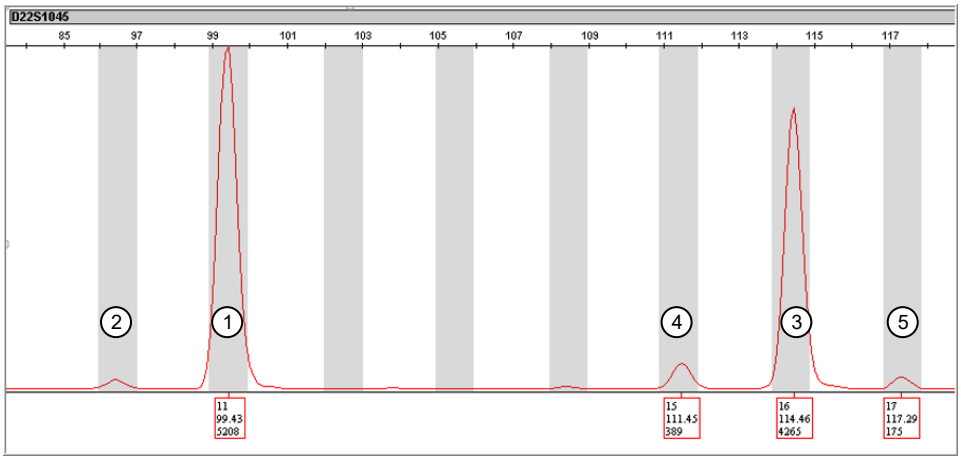
⑤ Allele peak 16

⑥ Minus stutter peak (–2 bp from allele peak 16)

⑦ Filtered minus stutter peak (–4 bp from allele peak 16)

⑧ Plus stutter peak (+4 bp from allele peak 16). It is not filtered (therefore, it is labeled), see plus stutter description above.

DS22S1045 marker stutter peak examples (N–3 and N+3)



① Allele peak 11

② Filtered minus stutter peak (–3 bp from the allele peak 11). It is not labeled because the peak signal is below the **Peak Amplitude Threshold** that is set in the analysis method **Peak Detector** tab.

③ Allele peak 16

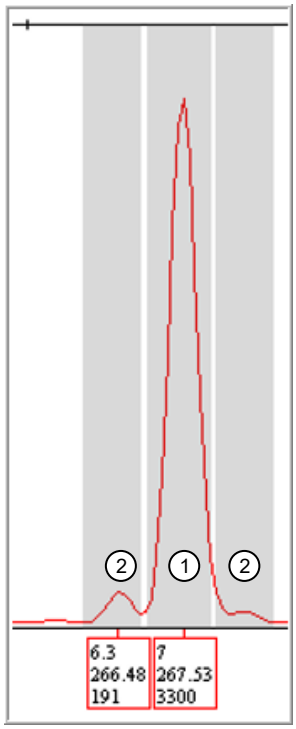
④ Labeled minus stutter peak (–3 bp from the allele peak 16)

⑤ Labeled plus stutter peak (+3 bp from the allele peak 16)

Note: A stutter peak is filtered (not labeled) if it is 1) above the **Peak Amplitude Threshold** in the analysis method **Peak Detector** tab; 2) within the **Stutter Distance** range in the analysis method **Allele** tab; 3) above the stutter ratio (% of allele peak) in the panel. If a peak label is crossed out, it indicates that the allele label was manually deleted/removed.

POP™ polymer-specific artifact examples (N–1 and N+1 shoulder peaks)

When using POP-7™ Polymer or when using the SeqStudio™ Genetic Analyzer Cartridge, N–1 and N+1 shoulder peaks are can be observed in any marker.



① Allele peak
② Shoulder peak

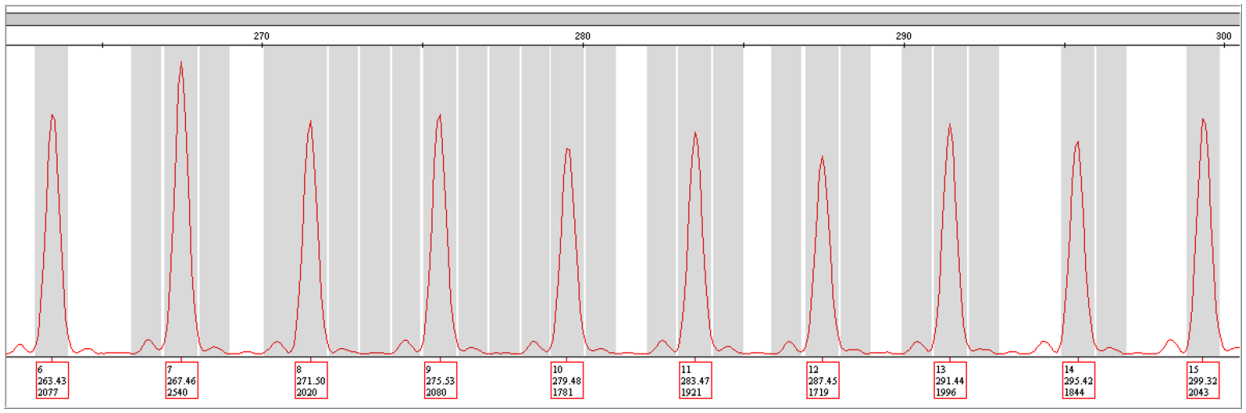


Figure 21 N–1 and N+1 shoulder peaks in SeqStudio™ Genetic Analyzer data D7S820 marker

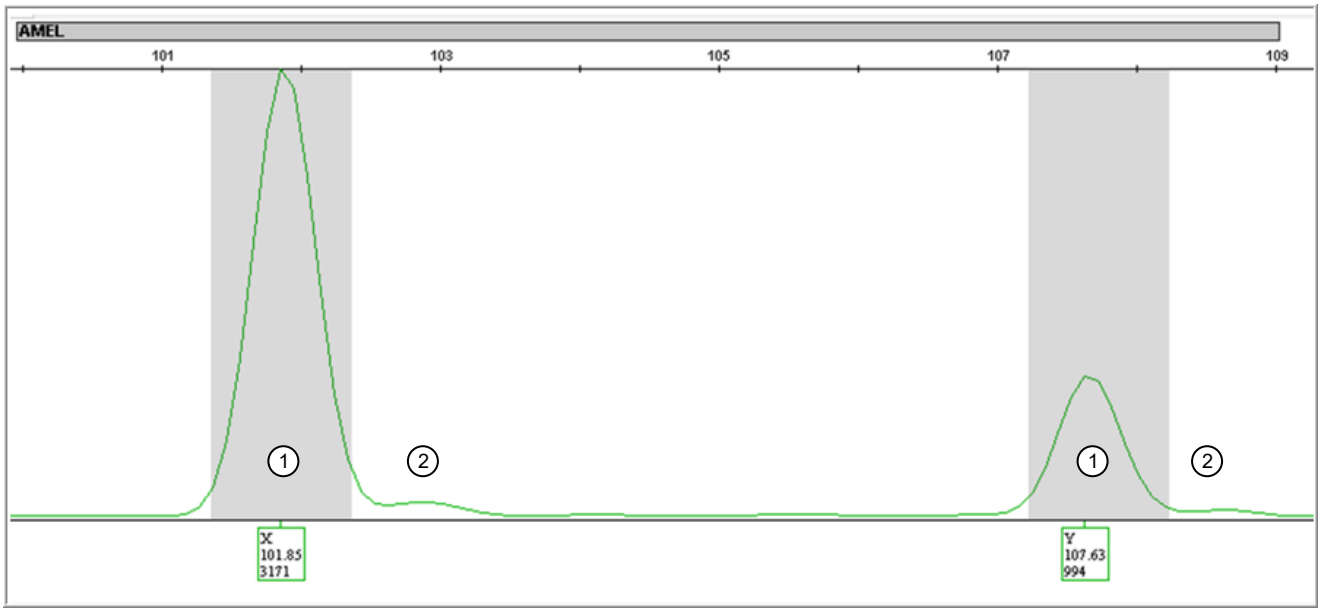


Figure 22 N+1 shoulder peaks in SeqStudio™ Genetic Analyzer data AMEL marker

① Allele peak
② N+1 shoulder peak

SE33 and D21S11 markers: off-ladder alleles caused by dye pull-up

If you observe an unexpected or off-ladder allele in the genotypes plot, note the bp/height values for the peak, then evaluate the peak in the samples plot with all dyes displayed. If the peak migrates at the same size as an allele peak (±0.5 bp), it may be a pull-up peak.

In Figure 23 on page 100, the OL peaks in the red dye migrate at the same size as the purple dye peaks, and are therefore possibly pull-up peaks instead of true DNA peaks.

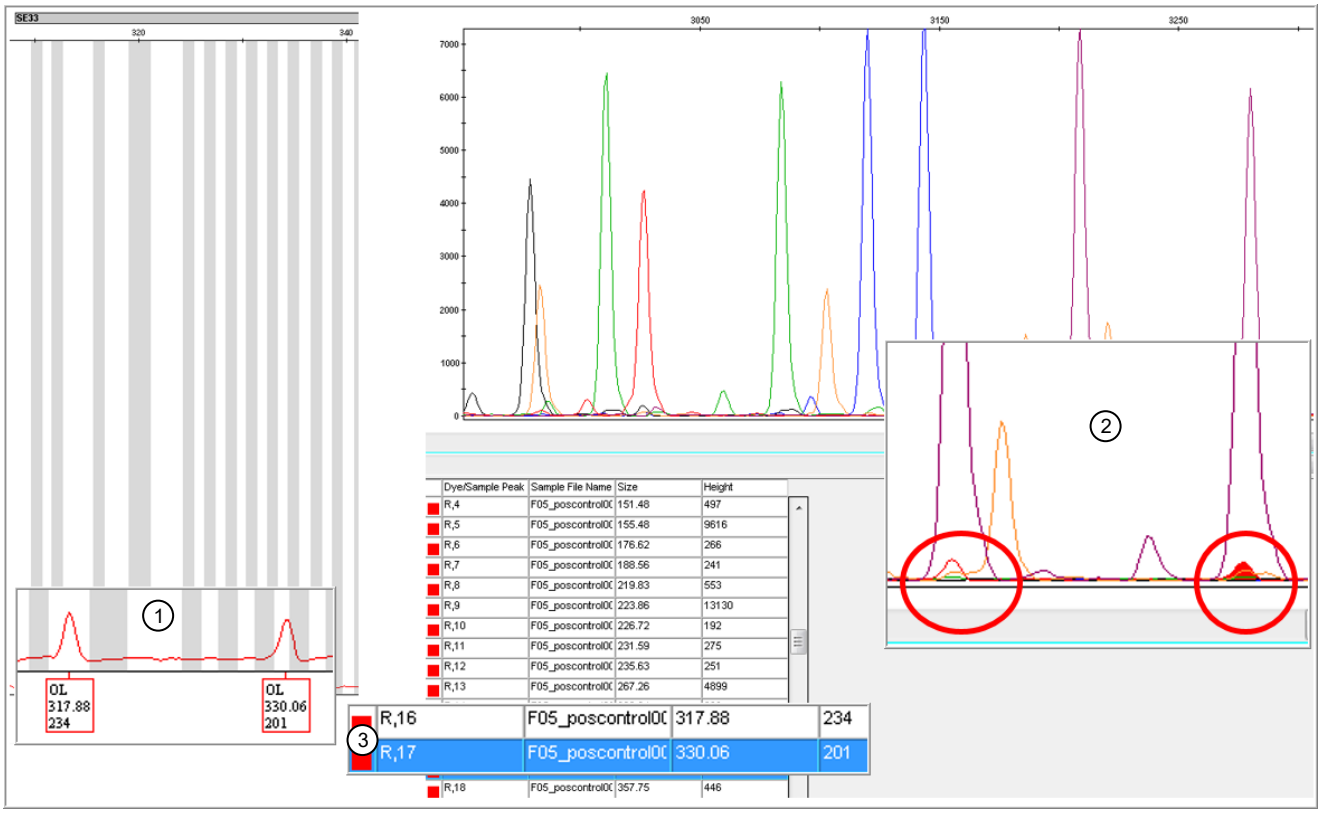


Figure 23 OL peak in SE33 marker

① Genotypes plot

② Samples plot with all dye channels displayed showing that the OL peaks in the red dye may be caused by dye pull-up from the purple dye channel.

③ Sizing table (with samples plot open, select **View ▸ Tables ▸ Sizing Table**)

In Figure 24 on page 101, the OL peak in the green dye migrates at the same size as the blue dye peak, and is therefore possibly a pull-up peak instead of true DNA peaks.

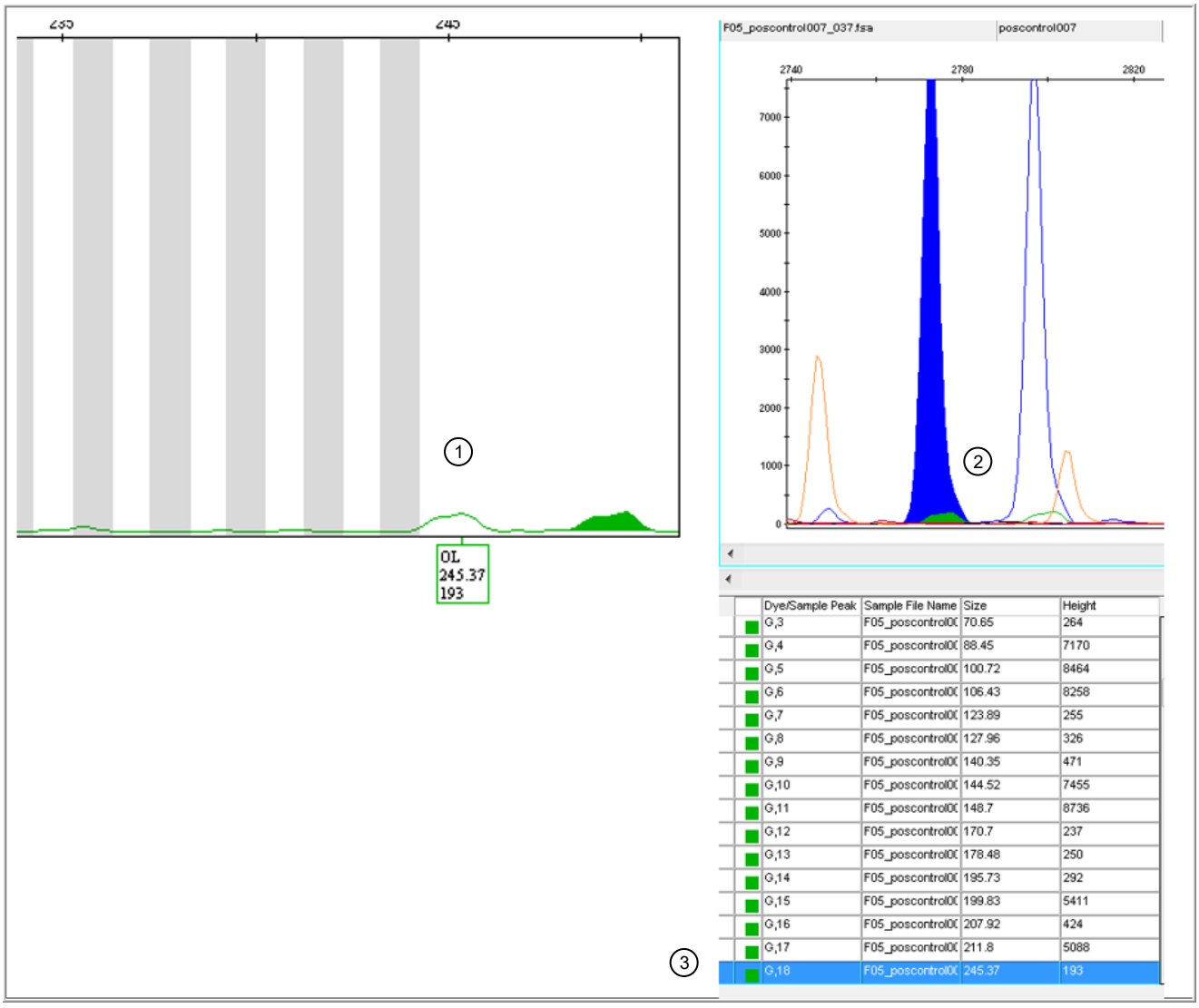


Figure 24 OL peak in D21S11 marker

① Genotypes plot

② Sample plot with all dye channels displayed showing that the OL peak in the green dye may be caused by dye pull-up from the blue dye channel.

③ Sizing table (with samples plot open, select **View** ▸ **Tables** ▸ **Sizing Table** ▸)

General troubleshooting

The Microsatellite Analysis Software uses colored circles 🟡 🔴 as quality indicators. The GeneMapper™ Software uses colored shapes 🔺 🛑 as quality indicators.

The troubleshooting information in the following section refers to colored circles 🟡 🔴, but the information applies to both the Microsatellite Analysis Software and the GeneMapper™ Software.

Observation	Possible cause	Recommended action
SQ 🔴 (sizing fails)	The correct size standard is not selected.	If SQ is 🔴 for all samples, ensure that the correct size standard is selected in the setup tab (Microsatellite Analysis Software) or sample table (GeneMapper™ Software). See: • Microsatellite Analysis Software—page 46 and page 46 • GeneMapper™ Software—page 70 and "Modify size matches" on page 72
	The peaks in the size standard are not correctly labeled. See:	If SQ is 🔴 for individual samples, examine the size standard for the samples and update if needed. • Microsatellite Analysis Software—page 46 and page 46 • GeneMapper™ Software—page 70 and "Modify size matches" on page 72
	Size standard was not added to the samples.	Prepare samples again, make sure to add size standard.
GQ 🔴 (genotyping fails)	The incorrect panel and bins are specified.	Select the correct panel and bins. See: • Microsatellite Analysis Software—page 42 • GeneMapper™ Software—page 65
	No allelic ladder is specified for the project.	Change at least one allelic ladder in the project to **Sample Type Allelic Ladder**. See: • Microsatellite Analysis Software—page 42 • GeneMapper™ Software—page 65
	You manually created the analysis method, panel, and bins in the GeneMapper™ Software. **IMPORTANT!** Use only panel and bin TXT files that are provided by Thermo Fisher Scientific. If you use panel and bin files that you create in the GeneMapper™ Software, you may see off-ladder alleles.	Change at least one allelic ladder in the project to **Sample Type Allelic Ladder**. See: • Microsatellite Analysis Software—page 42 • GeneMapper™ Software—page 65

Observation	Possible cause	Recommended action
SQ PQV is 🟡 or 🔴 and peaks do not contain size labels	The fragment sizes of the size standard definition do not match the positions of the detected peaks.	Examine the size standard. See: • Microsatellite Analysis Software—page 46 • GeneMapper™ Software—page 70
SQ PQV is 🟡 or 🔴 and one or more peaks are miscalled	Peak detection threshold associated with the size standard is set too high or low.	Adjust the analysis method so that the **Peak Amplitude Threshold** associated with the size standard is greater than the height of the miscalled peak.
SQ PQV is 🟡 or 🔴, peaks are clear and distinguishable, but have low signal strength	Peak detection threshold associated with the size standard is set too high or low.	Adjust the analysis method so that the **Peak Amplitude Threshold** associated with the size standard is greater than the height of the miscalled peak.
	Electrophoresis or pipetting error	Adjust the analysis method so that the **Peak Amplitude Threshold** associated with the size standard is greater than the height of the miscalled peak.
SQ PQV is 🟡 or 🔴 because size standard peaks occur within a primer peak	The smallest peaks of the size standard can appear within the primer range.	Create and analyze the data using a custom size standard that does not include the undetectable peak.
SQ PQV is 🟡 or 🔴, size standard peaks are clear and distinguishable, but consistently have low signal strength	Incorrect concentration of size standard in sample loading reagent.	Increase the concentration of size standard added to subsequent runs.
	Incorrect injection settings.	Review the injection settings of the run module for errors.
SQ PQV is 🟡 or 🔴 and size calling errors occur for different samples on the same capillary over multiple runs	Defective capillaries/arrays.	Troubleshoot the defective capillaries/arrays.
SQ PQV is 🟡 or 🔴, size standard peaks are clear and distinguishable, but consistently have low signal strength, and sizing failures occur in a regular pattern (the same wells fail repeatedly)	Electrophoresis or pipetting error.	Reinject the sample. If needed, prepare fresh sample.
Elevated stutter peaks or spurious peaks are observed	DNA input is too high.	Prepare samples with lower DNA input, then re-run.
Allelic dropout or imbalance in samples and size standard	DNA input is too low.	Prepare samples with increased DNA input if possible, then re-run.
	Incorrect thermal cycling parameters.	Use the thermal cycling parameters recommended in “Perform PCR” on page 20.

Observation	Possible cause	Recommended action
Allelic dropout or imbalance in samples and size standard *(continued)*	Use of a thermal cycler that is not listed in "Instrument and software compatibility" on page 15. Other thermal cyclers may have different thermal properties/ramp rates.	Use only thermal cyclers listed in "Instrument and software compatibility" on page 15. Confirm proper use of thermal cycler accessories such as compression pad, tray and retainers.
Allelic dropout or imbalance in samples only	PCR inhibitors in samples.	Prepare samples with decreased DNA input to reduce inhibitor concentration, then re-run.
Incorrect allele calls	Incorrect peak amplitude thresholds in analysis settings.	Adjust the analysis method.
Spikes and other non-reproducible, anomalous peaks	Use of expired reagents on the instrument, or instrument maintenance required.	Perform routine instrument maintenance.
		Replace expired reagents.

A Materials required but not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Sample preparation required materials

Item	Source
TE Buffer [low-TE buffer; 10 mM Tris-HCl (pH 8.0) and 0.1 mM EDTA]	12090015 or see "(Optional) Prepare low-TE buffer" on page 18.
Hi-Di™ Formamide, 25-mL	4311320

Thermal cycler required materials

ProFlex™ PCR System

Item	Source
ProFlex™ 96-well PCR System	4484075
ProFlex™ 2 × 96-well PCR System	4484076
ProFlex™ 3 × 32-Well PCR System	4484073

VeritiPro™ Thermal Cycler

Item	Source
VeritiPro™ Thermal Cycler, 96-well	A48141
(*Optional*) Tabletop centrifuge with 96-Well Plate Adapters	MLS

Capillary electrophoresis instrument required materials

SeqStudio™ Flex Series Genetic Analyzer

Item	Source
SeqStudio™ 8 Flex Genetic Analyzer (includes SeqStudio™ Flex Series Instrument Software)	A53627, A53628, or A53629
SeqStudio™ 24 Flex Genetic Analyzer (includes SeqStudio™ Flex Series Instrument Software)	A53630, A53631, or A53632
Anode Buffer Container 3500/Flex Series	4393927
Cathode Buffer Container 3500/Flex Series	4408256
Septa Cathode Buffer Container 3500/Flex Series	4410715
Capillary array 36-cm SeqStudio™ 8 Flex	A49104
Capillary array 36-cm SeqStudio™ 24 Flex	A49105
96-Well Standard Retainer & Base Set SeqStudio™ Flex Series	A49316
8-Tube Standard Retainer & Base Set SeqStudio™ Flex Series	A49296
8-Strip Septa 3500/Flex Series	4410701
96-Well Septa 3500/Flex Series	4412614
Conditioning Reagent Kit 3500/Flex Series	4393718
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
POP-7™ (960) Performance Optimized Polymer	4393714
POP-7™ (384) Performance Optimized Polymer	4393708
POP-4™ (960) Performance Optimized Polymer	4393710
POP-4™ (384) Performance Optimized Polymer	4393715

SeqStudio™ Genetic Analyzer

Item	Source
SeqStudio™ Genetic Analyzer (includes the SeqStudio™ Data Collection Software)	A35644
(Optional) SAE Administrator Console v2.0 or v2.1 **Note:** SAE Administrator Console v2.0 is compatible with SeqStudio™ Data Collection Software v1.1 to v1.2.3. SAE Administrator Console v2.1 is compatible with SeqStudio™ Data Collection Software v1.2.4.	v2.0—A46170 v2.1—A53717
(Optional) SeqStudio™ Plate Manager v1.2 or v2.0	Available on **apps.thermofisher.com** or for download at **thermofisher.com**
SeqStudio™ Genetic Analyzer Cartridge v2	A41331
SeqStudio™ Genetic Analyzer Cathode Buffer Container	A33401
Reservoir Septa (for Cathode Buffer Container)	A35640
SeqStudio™ Integrated Capillary Protector	A31923
Septa for SeqStudio™ Genetic Analyzer, 96 well	A35641
Septa for SeqStudio™ Genetic Analyzer, 8 strip	A36543
DS-36 Matrix Standard Kit (Dye Set J6)	4425042

3500/3500xL Genetic Analyzer

Item	Source
3500 Genetic Analyzer (includes 3500 Series Data Collection Software)	4405673
3500xL Genetic Analyzer (includes 3500 Series Data Collection Software)	4405633
Anode Buffer Container 3500/Flex Series	4393927
Cathode Buffer Container 3500/Flex Series	4408256
Septa Cathode Buffer Container 3500/Flex Series	4410715
3500 Genetic Analyzer 8-Capillary Array, 36 cm	4404683
3500xL Genetic Analyzer 24-Capillary Array, 36 cm	4404687
3500 Genetic Analyzer 8-Capillary Array, 50 cm	4404685
3500xL Genetic Analyzer 24-Capillary Array, 50 cm	4404689
Retainer & Base Set (Standard) for3500/3500xL Genetic Analyzer, 96 well	4410228
Retainer & Base Set (Standard) for3500/3500xL Genetic Analyzer, 8 tube	4410231
8-Strip Septa 3500/Flex Series	4410701

(continued)

Item	Source
96-Well Septa 3500/Flex Series	4412614
Conditioning Reagent Kit 3500/Flex Series	4393718
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
POP-7™ (960) Performance Optimized Polymer	4393714
POP-7™ (384) Performance Optimized Polymer	4393708
Polymer, POP-7™ (96)	A26073

3730/3730*xl* DNA Analyzer

Item	Source
3730*xl* DNA Analyzer (includes 3730*xl* Data Collection Software) **Note:** The 3730 DNA Analyzer is no longer available for sale. However, you can still run the kit on a 3730 DNA Analyzer with 3730/3730*xl* Data Collection Software 4.0.	A41046
3730*xl* DNA Analyzer 96-Capillary Array, 36-cm	4331244
3730*xl* DNA Analyzer 96-Capillary Array, 50-cm	4331246
3730 DNA Analyzer 48-Capillary Array, 36-cm	4331247
3730 DNA Analyzer 48-Capillary Array, 50-cm	4331250
Polymer Block Cleaning Kit	4335860
3730 Running Buffer (10X) , 500 mL	4335613
Hi-Di™ Formamide	4311320
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
POP-7™ Polymer, 1 x 28 mL	4363929
POP-7™ Polymer, 10 x 28 mL	4363935
POP-6™ Polymer, 1 x 7 mL	4352757

Analysis software required materials

GeneMapper™ Software

Item	Source
GeneMapper™ Software v6, Full Installation, (Windows™ 10/Windows™ 7 SP1)	A38888
GeneMapper™ Software v6, Client Installation, (Windows™ 10/Windows™ 7 SP1)	4475074
GeneMapper™ Software v5, Full Installation, (Windows™ 7 SP1)	A31128
GeneMapper™ Software v5, Client Installation, (Windows™ 7 SP1)	A38892

Microsatellite Analysis Software

Item	Source
Microsatellite Analysis Software	apps.thermofisher.com

Miscellaneous required materials

Plates and tubes

Item	Source
MicroAmp™ 96-Well Tray	N8010541
MicroAmp™ Reaction Tube with Cap, 0.2 mL	N8010540
MicroAmp™ 8-Tube Strip, 0.2 mL	N8010580
MicroAmp™ Optical 8-Tube Strip, 0.2 mL	4316567
MicroAmp™ Optical 8-Cap Strips	4323032
MicroAmp™ 96-Well Tray/Retainer Set (Adapter for 8-Tube Strip)	403081
MicroAmp™ 96-Well Base	N8010531
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Adhesive Film	4311971
MicroAmp™ Optical 96-Well Reaction Plate	N8010560
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4326659

Laboratory supplies

Item	Source
Various procedures	
Aerosol resistant pipette tips	MLS[1]
Microcentrifuge tubes	MLS
Pipettors	MLS
Tape, labeling	MLS
Tube, 50-mL Falcon™	MLS
Tube decapper, autoclavable	MLS
Deionized water, PCR grade	MLS
Vortex	MLS

[1] Major laboratory supplier

B Safety

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

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

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

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

AVERTISSEMENT ! **PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES.** Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety

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. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Document	Publication number
Thermal cyclers	
VeritiPro™ Thermal Cycler User Guide	MAN0019157
ProFlex™ PCR System User Guide	MAN0007697
ProFlex™ PCR System Kit Validation User Bulletin	100031595
SeqStudio™ Flex Series Genetic Analyzer	
SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide	100104689
SeqStudio™ Flex Series Instrument Software v1.1.1 User Bulletin	MAN0029757
SeqStudio™ Genetic Analyzer	
SeqStudio™ Genetic Analyzer Instrument and Software User Guide (v1.2 and later)	MAN0018646
3500/3500xL Genetic Analyzer	
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v3.3 User Guide	100079380
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v 3.1 User Guide	100031809
3730/3730*xl* DNA Analyzer	
3730xl DNA Analyzer with 3730xl Data Collection Software 5 User Guide	100077621
Applied Biosystems™ 3730/3730xl DNA Analyzers Getting Started Guide	4478016
Analysis software	
Microsatellite Analysis Software User Guide	MAN0017825
GeneMapper™ Software 6 Installation and Administration User Guide	100079198
GeneMapper™ Software 5 Installation and Administration User Guide	4476603
Note: Version 4.1 user documentation applies to Version 5 and Version 6 software. No features have changed in the newer versions of software.	

(continued)

Document	Publication number
GeneMapper™ Software v4.1 Installation Options User Bulletin	4403675
GeneMapper™ Software v4.1 Autoanalysis User Bulletin	4403670
GeneMapper™ Software v4.1 Reference and Troubleshooting Guide	4403673
GeneMapper™ Software v4.1 Quick Reference Guide	4403615
GeneMapper™ Software v4.1 Microsatellite Analysis Getting Started Guide	4403672

Application notes

Document	url
Application note: Authenticating human cell lines using CLA IdentiFiler and CLA GlobalFiler kits on capillary electrophoresis platforms	Download from **www.thermofisher.com/CLA**
Case study: Matching identities of iPSCs and donors using CLA Identifiler STR profiling kits	
Application note: Confidently identify human samples using the CLA Identifiler and CLA GlobalFiler STR kits	