

# CLA Identifiler™ Plus and Identifiler™ Direct PCR Amplification Kits

## USER GUIDE

for Cell Line Authentication

for use with:

SeqStudio™ Genetic Analyzer

3500/3500xL Genetic Analyzer

3730/3730xl DNA Analyzer (48-capillary)

3130/3130xl Genetic Analyzer

for use with:

Microsatellite Analysis Software

GeneMapper™ Software

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**Manufacturer:**  
Life Technologies Holdings Pte Ltd |  
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#07-06, Singapore 739256

**Products:**  
SeqStudio™ Genetic Analyzer  
3500/3500xL Genetic Analyzer  
3730/3730xl DNA Analyzer  
3130/3130xl Genetic Analyzer



**Manufacturer:**  
Life Technologies Ltd |  
7 Kingsland Grange |  
Woolston, Warrington WA1 4SR |  
United Kingdom

**Products:**  
CLA Identifiler™ Plus PCR Amplification Kit  
CLA Identifiler™ Direct PCR Amplification Kit



**Manufacturer:**  
Thermo Fisher Scientific Baltics UAB |  
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Vilnius, Lithuania

GeneScan™ 600 LIZ™ Size Standard v2.0

For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](http://thermofisher.com/symbols-definition).

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

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## Product description

The CLA Identifiler™ Plus and Identifiler™ Direct PCR Amplification Kits are 5-dye, 16-locus short tandem repeat (STR) analysis kits with 1 sex-discriminating marker. Both kits amplify the same loci as the AmpFℓSTR™ Identifiler™ PCR Amplification Kit.

The CLA Identifiler™ Plus kit uses genomic DNA.

The CLA Identifiler™ Direct kit uses cells spotted on a NUCLEIC-CARD™ matrix. This kit does not require DNA extraction or purification.

Features of the kits include:

- Compatible with SeqStudio™, 3500/3500xL, and 3730/3730xl genetic analyzers.
- Compatible with all cell line databasing loci standards and global databasing loci standards.

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

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

## Related products

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. It provides reduced amplification time and increased discrimination power compared to the AmpFℓSTR™ Identifiler™ Plus and AmpFℓSTR™ Identifiler™ Direct PCR Amplification Kits.

## Dyes used in the kits

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

## Loci amplified by the kits

Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	DNA Control 9947A
D8S1179	8	8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	6-FAM™	13, 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		30, 30
D7S820	7q11.21-22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		10, 11
CSF1PO	5q33.3-34	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		10, 12
D3S1358	3p	12, 13, 14, 15, 16, 17, 18, 19		VIC™
TH01	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3	8, 9.3	
D13S317	13q22-31	8, 9, 10, 11, 12, 13, 14, 15	11, 11	
D16S539	16q24.qter	5, 8, 9, 10, 11, 12, 13, 14, 15	11, 12	
D2S1338	2q35-37.1	15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28	19, 23	
D19S433	19q12-13.1	9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2	NED™	14, 15
vWA	12p12-pter	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24		17, 18
TPOX	2p23-2per	5, 6, 7, 8, 9, 10, 11, 12, 13		8, 8
D18S51	18q21.3	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		15, 19
Amelogenin	X: p22.1-22.3 Y: p11.2	X, Y		PET™



Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	DNA Control 9947A
D5S818	5q21-31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16	PET™	11, 11
FGA	4q28	17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2		23, 24

## Standards and controls that are required

The CLA Identifiler™ Plus and Identifiler™ Direct PCR Amplification Kits require the following panel of standards needed for PCR amplification, PCR product sizing, and genotyping:

- **DNA Control 9947A**—A positive control for evaluating the efficiency of the amplification step and STR genotyping of the kit allelic ladder. DNA Control 9947A is included in the kit. See “DNA Control 9947A profile” on page 10.
- **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 11.
- **Allelic ladder**—Developed for accurate characterization of the alleles amplified in each kit. The allelic ladder is included in each kit and allows automatic genotyping of most of the reported alleles for the loci in the kit. See “Loci amplified by the kits” on page 8 and “Allelic ladder profile” on page 12.

## DNA Control 9947A profile

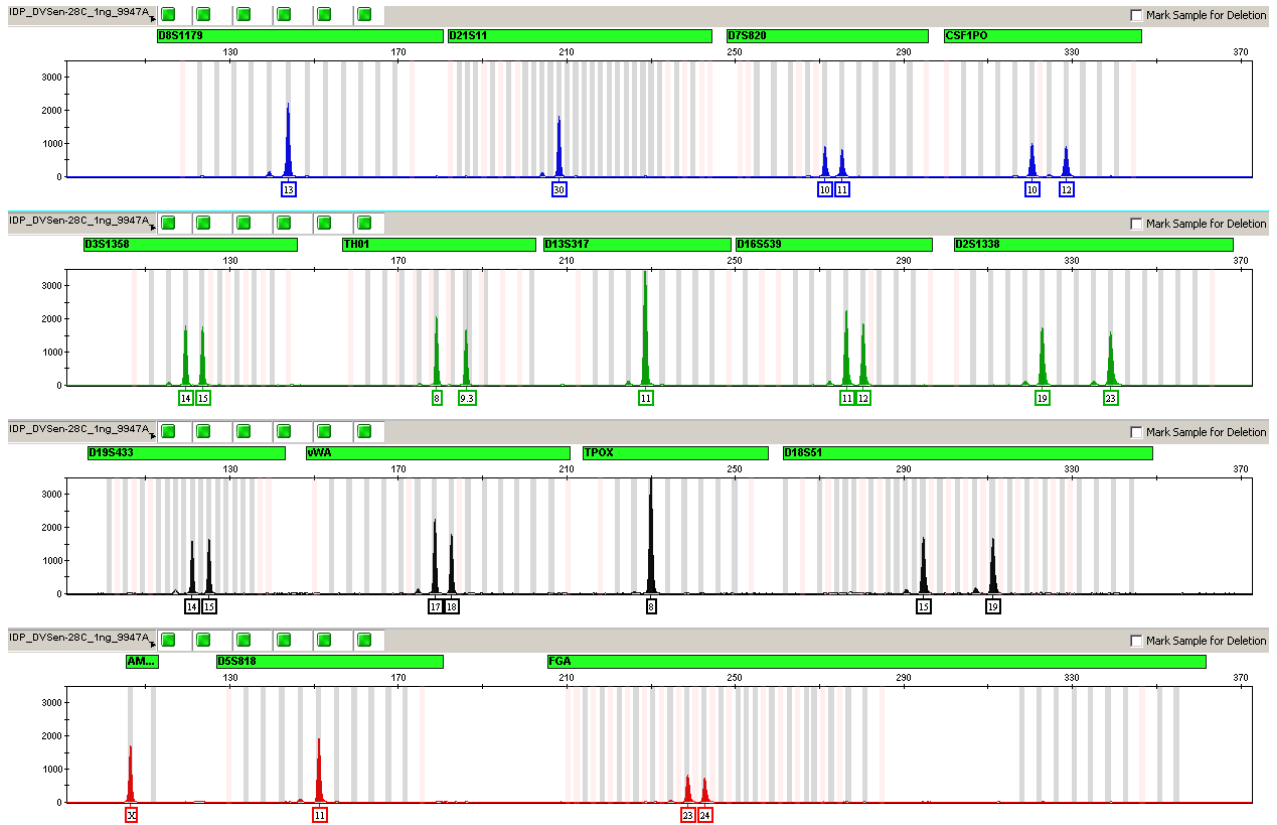


Figure 1 Control DNA 9947A (1 ng) amplified with the Identifiler™ Plus kit and analyzed on an Applied Biosystems™ 3130x/ Genetic Analyzer

## GeneScan™ 600 LIZ™ Size Standard v2.0

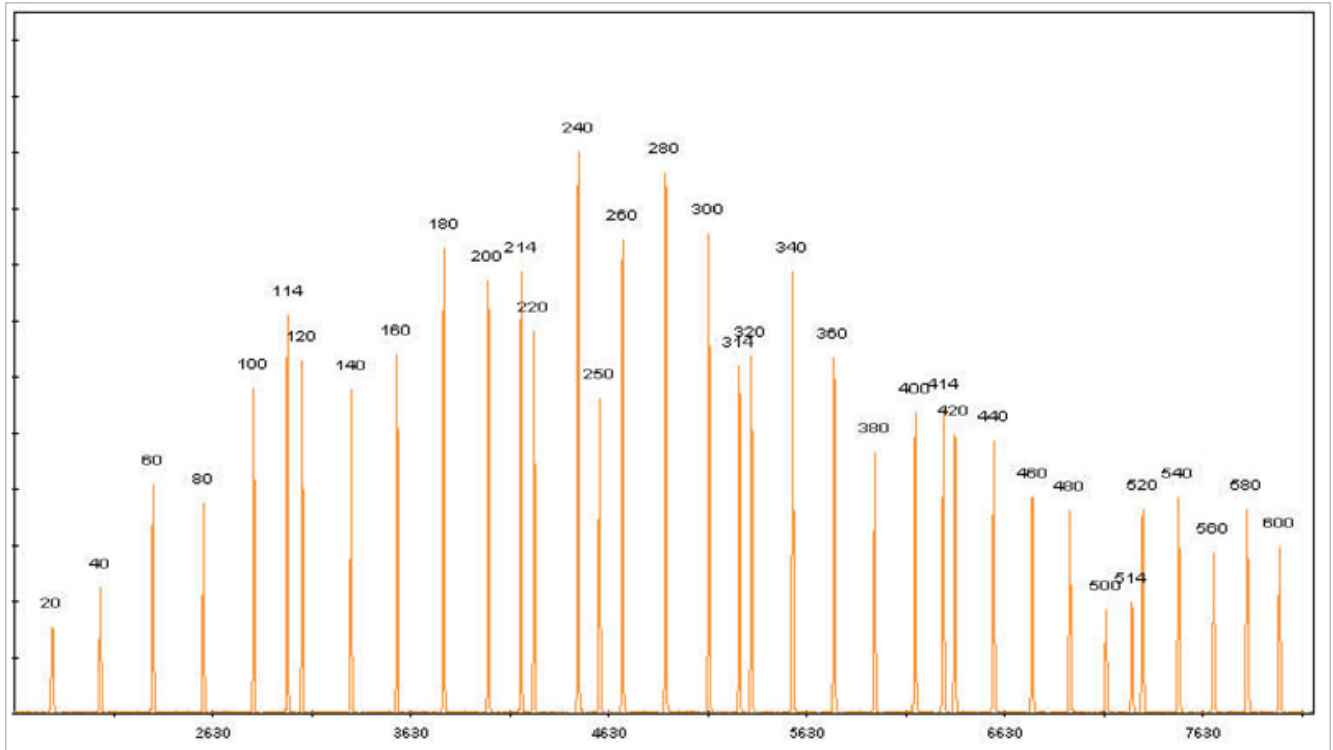


Figure 2 GeneMapper™ Software plot of the GeneScan™ 600 LIZ™ Size Standard v2.0

### Allelic ladder profile

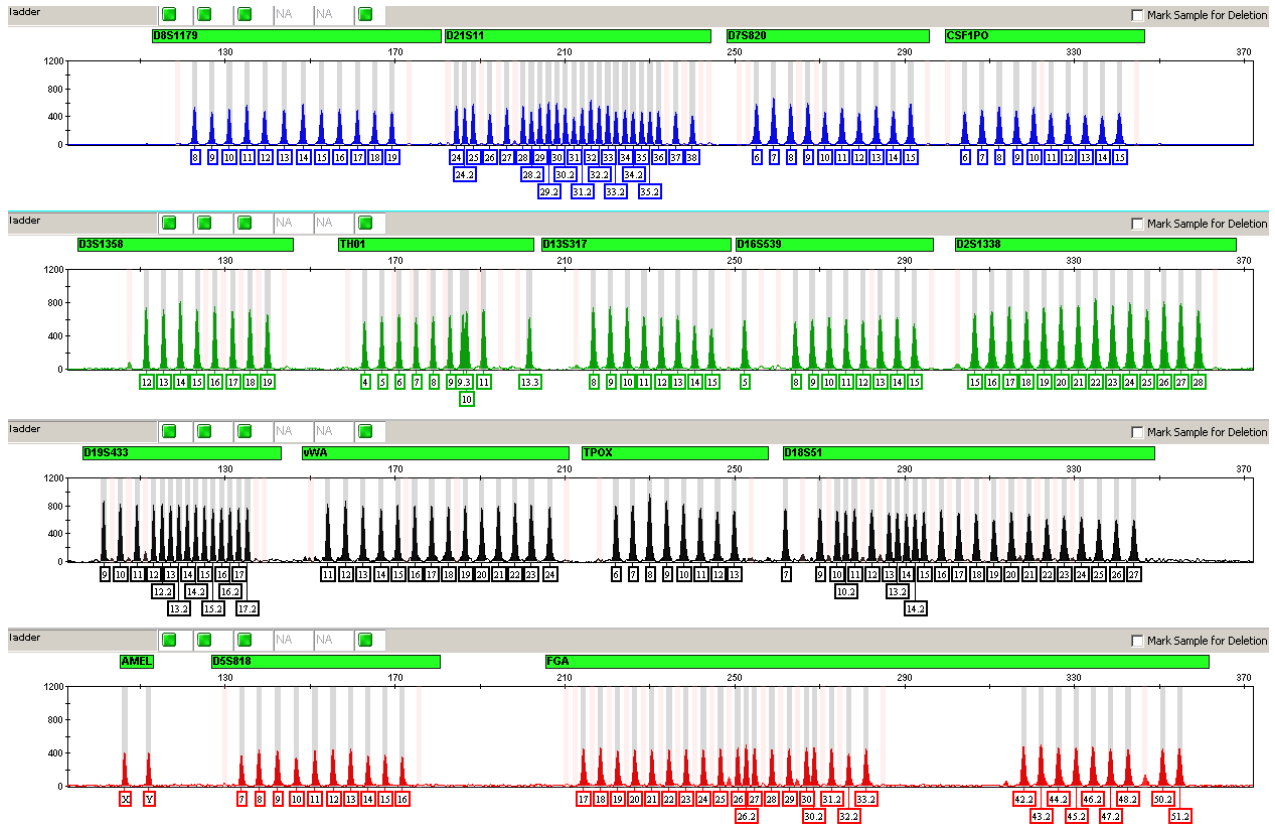


Figure 3 GeneMapper™ Software plot of the Identifier™ Allelic Ladder

## Contents and storage— Identifiler™ Plus kit

The Identifiler™ Plus kit contains sufficient quantities of the reagents for 200 reactions at 25 µL/amplification.

**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.

Contents	Description	200 reaction (Cat. No. 4427368)	Storage
Identifiler™ Plus Master Mix	Contains enzyme, salts, dNTPs, carrier protein, and 0.04% sodium azide.	2 × 1.0 mL	–25°C to –15°C on receipt. 2°C to 8°C after first use for up to 6 months or up to the expiration date stated on the kit (whichever comes first).
Identifiler™ Plus Primer Mix	Contains forward and reverse primers to amplify human DNA targets.	1 × 1.0 mL	–25°C to –15°C on receipt. 2°C to 8°C after first use for up to 6 months or up to the expiration date stated on the kit (whichever comes first). Store protected from light.
Identifiler™ Plus Allelic Ladder	Contains amplified alleles. See “Allelic ladder profile” on page 12 for information.	1 × 50.0 µL	–25°C to –15°C on receipt. 2°C to 8°C after first use up to the expiration date stated on the kit. Store protected from light.
AmpF $\ell$ STR™ Control DNA 9947A	Contains 0.1 ng/µL human female 9947A genomic DNA from cell line in 0.05% sodium azide and buffer. <sup>[1]</sup> See “DNA Control 9947A profile” on page 10 for information.	1 × 30.0 µL	–25°C to –15°C on receipt. 2°C to 8°C after first use up to the expiration date stated on the kit.

<sup>[1]</sup> DNA Control 9947A 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 9947A, the concentration may differ from the labeled concentration.

## Contents and storage—CLA Identifiler™ Direct kit

The Identifiler™ Direct kit contains sufficient quantities for 200 reactions at 25 µL/amplification.

**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.

Contents	Description	200 reaction (Cat. No. 4427368)	Storage
Identifiler™ Direct Master Mix	Contains enzyme, salts, dNTPs, carrier protein, and 0.04% sodium azide.	2 × 1.25 mL	–25°C to –15°C on receipt. 2°C to 8°C after first use for up to 6 months or up to the expiration date stated on the kit (whichever comes first).
Identifiler™ Direct Primer Mix	Contains forward and reverse primers to amplify human DNA targets.	2 × 1.25 mL	–25°C to –15°C on receipt. 2°C to 8°C after first use for up to 6 months or up to the expiration date stated on the kit (whichever comes first). Store protected from light.
Identifiler™ Direct Allelic Ladder	Contains amplified alleles. See “Allelic ladder profile” on page 12 for information.	1 × 50.0 µL	–25°C to –15°C on receipt. 2°C to 8°C after first use up to the expiration date stated on the kit. Store protected from light.
AmpF $\lambda$ STR™ Control DNA 9947A	Contains 0.1 ng/µL human female 9947A genomic DNA from cell line in 0.05% sodium azide and buffer <sup>[1]</sup> See “DNA Control 9947A profile” on page 10 for information.	1 × 50.0 µL	–25°C to –15°C on receipt. 2°C to 8°C after first use up to the expiration date stated on the kit.

<sup>[1]</sup> DNA Control 9947A 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 9947A, the concentration may differ from the labeled concentration.

## Required materials not supplied

See Appendix A, “Materials required but not supplied”.

## 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

The interpretation of aberrant peaks at a single locus can be challenging and ambiguous. However, with the analysis of 16 loci and 1 sex-discriminating marker in the CLA Identifiler™ Plus and Identifiler™ Direct PCR Amplification Kits, 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.

## Instrument and software compatibility

Instrument type	Compatible model
Thermal cyclers	<ul style="list-style-type: none"> <li>• ProFlex™ 96-well PCR System (Cat. No. 4484075)</li> <li>• ProFlex™ 2 × 96-well PCR System (Cat. No. 4484076)</li> <li>• ProFlex™ 3 × 32-Well PCR System (Cat. No. 4484073)</li> <li>• Veriti™ 96-Well Thermal Cycler (Cat. No. 4479071)</li> <li>• GeneAmp™ PCR System 9700, 96-Well Silver (Cat. No. N8050001)</li> <li>• GeneAmp™ PCR System 9700, 96-Well Gold-Plated (Cat. No. 4314878)</li> </ul> <p>The kit is not optimized for use with:</p> <ul style="list-style-type: none"> <li>• ProFlex™ 2 × Flat PCR System (Cat. No. 4484078)</li> <li>• ProFlex™ 2 × 384-well PCR System (Cat. No. 4484077)</li> <li>• Veriti™ Fast 96-Well Thermal Cycler (Cat. No. 4375305)</li> <li>• GeneAmp™ PCR System 9700 with the aluminium 96-well block (Cat. No. 4314879)</li> </ul>

Instrument type	Compatible model
Genetic analyzers	<ul style="list-style-type: none"> <li>• SeqStudio™ Genetic Analyzer v1.1.4 or later</li> <li>• 3500/3500xL Genetic Analyzer with any of the following:               <ul style="list-style-type: none"> <li>– 3500 Data Collection v3.3 Software (Windows™ 10 operating system)</li> <li>– 3500 Data Collection v3.1 Software (Windows™ 7 operating system)</li> </ul> </li> <li>• 3730/3730xL DNA Analyzer (48-capillary recommended) with any of the following:               <ul style="list-style-type: none"> <li>– Data Collection v5 Software (Windows™ 10 operating system)</li> <li>– Data Collection v4 Software (Windows™ 7 operating system)</li> </ul> </li> </ul> <p><b>Note:</b> The 48-capillary array is recommended for the 3730xl instrument. The close proximity of capillaries in the 96-capillary array can introduce cross-talk between capillaries and interfere with data interpretation.</p> <ul style="list-style-type: none"> <li>• 3130/3130xL DNA Analyzer with Data Collection Software v4</li> </ul>
Analysis software	<ul style="list-style-type: none"> <li>• Microsatellite Analysis Software (accessible from <a href="https://apps.thermofisher.com">apps.thermofisher.com</a>)</li> <li>• GeneMapper™ Software v6 Software (Windows™ 10 operating system)</li> <li>• GeneMapper™ Software v5 Software (Windows™ 7 operating system)</li> </ul>

## Workflow

### Perform PCR

If you are analyzing a cell suspension, see:  
Chapter 2, “Perform PCR: CLA Identifiler™ Plus kit”

If you are spotting a cell suspension on a  
NUCLEIC-CARD™ matrix, see:  
Chapter 3, “Perform PCR: CLA Identifiler™ Direct  
kit”



### Perform electrophoresis, see:

- “Electrophoresis software setup: SeqStudio™” on page 29
- “Electrophoresis software setup: 3500/3500xL” on page 29
- “Electrophoresis software setup: 3730/3730xl instruments” on page 30
- “Electrophoresis software setup: 3130/3130xl instruments” on page 30



“Prepare samples for electrophoresis” on page 33



### Analyze data, see:

- Chapter 5, “Analyze data with Microsatellite Analysis Software”
- Chapter 6, “Analyze data with GeneMapper™ Software”





# Perform PCR: CLA Identifiler™ Plus kit

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## 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. 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](http://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:

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

Off-scale data is a problem because:

- Quantification (peak height and area) for off-scale peaks is not accurate. For example, an allele peak that is off-scale can cause the corresponding stutter peak to appear higher in relative intensity, thus increasing the calculated percent stutter.
  - Multicomponent analysis of off-scale data is not accurate. This inaccuracy results in poor spectral separation (“pull-up”).
- Incomplete +A nucleotide addition.

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

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

## Before you begin

### Prepare low-TE buffer

For optimal results, we recommend using low-TE buffer for sample preparation. Prepare it as described in this procedure or buy it from Teknova (Cat. No. T0223).

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

**Note:** Adjust the volumes accordingly for specific needs.

2. Aliquot, then autoclave the solutions.
3. Store the aliquots at room temperature.

## Thaw reagents (before first use of the kit)

Thaw the master mix and primer mix.

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

**IMPORTANT!** Thawing is required only during first use of the kit. After first use, reagents are stored at 2°C to 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 set, 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 centrifuging the tubes briefly or tapping the bottles on the bench.
2. Pipette the required volumes of components into an appropriately sized, clear (non-colored), polypropylene tube:

Reaction component	Volume per reaction
Master mix	10.0 µL
Primer mix	5.0 µL

**Note:** The allelic ladder is not a sample. Do not include it in PCR.

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

3. Vortex the reaction mixture for 3 seconds, then centrifuge briefly.
4. Dispense 15 µL of reaction mixture into each reaction well of a MicroAmp™ Optical 96-Well Reaction Plate or each MicroAmp™ tube.
5. Adjust the sample input amount and volume if needed:
  - If total sample input amount is >1.0 ng for 28 cycles or >500 pg for 29 cycles, dilute with low-TE buffer to achieve a 10-µL input volume.
  - If total input volume is <10 µL, adjust the volume with low-TE buffer to achieve a 10-µL input volume.

6. Prepare samples as shown in the following table, then add them to the appropriate well or tube (final reaction volume is 25  $\mu$ L).

Sample	28-cycle protocol	29-cycle protocol
Negative control	10 $\mu$ L of low-TE buffer	10 $\mu$ L of low-TE buffer
Test sample	10 $\mu$ L of DNA (1.0 ng input amount)	10 $\mu$ L of DNA (0.5 ng input amount)
Positive control	10 $\mu$ L of control DNA (0.1 ng/ $\mu$ L)	10 $\mu$ L of control DNA (0.1 ng/ $\mu$ L)

7. Seal the MicroAmp™ Optical 96-Well Reaction Plate with MicroAmp™ Clear Adhesive Film or MicroAmp™ Optical Adhesive Film, or cap the tubes.
8. Amplify the samples.

---

**IMPORTANT!** See “Instrument and software compatibility” on page 15 for a list of optimized thermal cyclers.

---

## Number of PCR cycles to use

All kits are optimized for a specific number of amplification cycles to deliver well-balanced and high quality results. However, increases in the number of low-level DNA samples being submitted for analysis have prompted many laboratories to evaluate a higher number of amplification cycles to improve the sensitivity of the assay. Before increasing the cycle number, perform a comprehensive validation study to establish new performance criteria for the higher cycle number.

Higher cycle numbers can cause the following to occur:

- Exaggerated stochastic effects resulting from low DNA input amounts
- Greater difference between the presence and absence of an allele
- Greater heterozygote peak imbalance
- Possible differences in expected stutter position and percentage
- Possible increase in artifacts and/or background in the profile to accompany the increase in sample allele signal

The Identifier™ Plus kit offers two PCR-cycle-number options:

- **Standard 28-PCR-cycle protocol**—Provides high sensitivity to consistently generate full STR profiles with 125 pg of DNA input. Use with the optimum 1.0 ng DNA input amount in a maximum input volume of 10  $\mu$ L.
- **29-PCR-cycle protocol**—Adds the extra sensitivity when amplifying <125 pg DNA input. Recommended for use when the total DNA input amount is <0.5 ng.

## Perform PCR

**IMPORTANT!** This kit is optimized for use with the validated thermal cyclers that are listed in “Instrument and software compatibility” on page 15.

### 1. Program the thermal cycling conditions.

**IMPORTANT!** Set the thermal cycler you are using as indicated: ProFlex™ PCR System: 9700 Simulation ramping mode (see the *ProFlex™ PCR System Kit Validation User Bulletin* (Pub. No. 100031595); Veriti™ Thermal Cycler: 9600 emulation mode (see *Veriti™ 96-Well Thermal Cycler AmpFℓSTR™ Kit Validation User Bulletin* (Pub. No. 4440754).; GeneAmp™ PCR System 9700 with 96-well silver or gold-plated silver block: Max ramping mode.

Initial incubation step	Cycle (28 or 29 cycles) <sup>[1]</sup>		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE		HOLD	HOLD
95°C, 11 minute	94°C, 20 seconds	59°C, 3 minutes	60°C, 10 minutes	4°C, Up to 24 hours <sup>[2]</sup>

<sup>[1]</sup> See “Number of PCR cycles to use” on page 20.

<sup>[2]</sup> The infinity (∞) setting allows an unlimited hold time.

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

**IMPORTANT!** If you are using adhesive clear film instead of caps to seal the plate wells, place a MicroAmp™ Optical Film Compression Pad (Cat. No. 4312639) on top of the plate to prevent evaporation during thermal cycling. The ProFlex™ PCR System and the Veriti™ Thermal Cycler do not require a compression pad.

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

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

**IMPORTANT!** Protect the amplified DNA from light.



# Perform PCR: CLA Identifiler™ Direct kit

■ Guidelines for cell line preparation, extraction, and purification .....	22
■ Prepare the NUCLEIC-CARD™ matrix with sample .....	23
■ Before you begin .....	24
■ Prepare the amplification reactions .....	25
■ Perform PCR .....	26

## Guidelines for cell line preparation, extraction, and purification

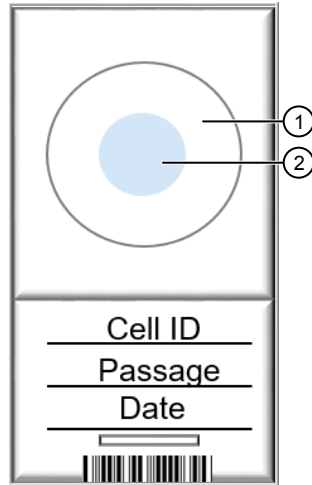
Follow your standard laboratory operating protocol and consider the following guidelines:

- Prepare the cell line samples with an optimal target cell density of  $1 \times 10^6$  cells/mL
  - For cell density  $>1 \times 10^6$  cells/ mL, dilute with a volume of phosphate buffered saline (PBS) that yields a cell density of  $1 \times 10^6$ .
  - For cell density  $<1 \times 10^6$  cells/ mL, re-centrifuge, then resuspend in a volume of PBS that yields a cell density of  $1 \times 10^6$ .
- 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 spotting on NUCLEIC-CARD™ matrix.
- Obtain cells at an early passage.
- Continue growing cells. 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.

## Prepare the NUCLEIC-CARD™ matrix with sample

**Note:** The allelic ladder is not a sample. Do not include it in PCR.

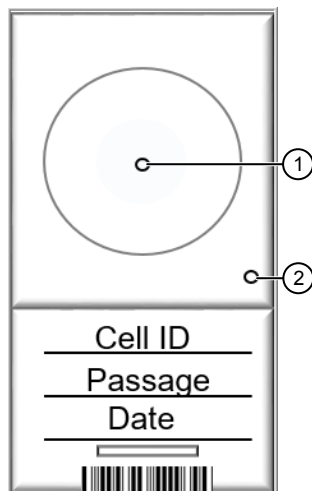
1. Spot 40 µL of the cell suspension in the center of the matrix.



- ① Matrix
- ② 40 µL of the cell suspension spotted in center of matrix

2. Place the spotted card in a laminar flow hood and allow to dry at room temperature for 24 hours.
3. Obtain the punch: Place the tip of a 1.2 mm puncher in the center of the card, hold the barrel of the puncher (do not touch the plunger), gently press and twist 1/4-turn, then eject the disc in to the appropriate well on the reaction plate.

**Note:** Do not add water to the wells on the reaction plate before adding the discs. If you observe static issues with the paper discs, you can prepare and dispense the 25-µL reaction mix into the wells of the reaction plate before adding the punches.



- ① Obtain the punch from the center of the sample spotted on the matrix
  - ② Clean the puncher for the next sample by obtaining a punch from a clean area of the matrix away from the spotted sample
4. Clean the puncher for the next sample by obtaining a punch from a clean area of the matrix away from the spotted sample. Discard the punch.
  5. *(Optional)* To store the spotted NUCLEIC-CARD™, place it in a paper envelope with a desiccant packet. Spotted cards can be stored at room temperature for up to 20 years.

## Before you begin

### Thaw reagents (before first use of the kit)

Thaw the master mix and primer mix.

---

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

---

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

---



## Prepare the amplification reactions

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

If this is the first time you are using the kit, follow the instructions in “Thaw reagents (before first use of the kit)” on page 19 before proceeding.

1. Add samples to the reaction plate:

To these wells ...	Add...
Negative control	1.2 mm blank disc
Test samples	1.2 mm sample disc
Positive control <b>IMPORTANT!</b> Do not add a blank disc to the positive control well.	2 µL of Control DNA 9947A

**Note:** The volumes of positive control are suggested amounts and may be adjusted if peak heights are too high or too low for your optimized cycle number.

2. Vortex the master mix and primer mix for 3 seconds. Before opening the tubes or bottles, remove droplets from the caps by centrifuging the tubes briefly or tapping the bottles on the bench.
3. Pipet the required volumes of components into an appropriately sized polypropylene tube.

Reaction component	Volume per reaction
Master mix	12.5 µL
Primer mix	12.5 µL

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

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

4. Vortex the reaction mix for 3 seconds, then centrifuge briefly.
5. Dispense 25 µL of the reaction mix into each reaction well of a MicroAmp™ Optical 96-Well Reaction Plate.

- Seal the plate with MicroAmp™ Clear Adhesive Film (Cat. No. 4306311) or MicroAmp™ Optical Adhesive Film (Cat. No. 4311971).

---

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

---

**IMPORTANT!** If you are using the GeneAmp™ PCR System 9700 with silver or gold-plated silver block and adhesive clear film instead of caps to seal the plate wells, place a MicroAmp™ Optical Film Compression Pad (Cat. No. 4312639) on top of the plate to prevent evaporation during thermal cycling. Other optimized thermal cyclers do not require a compression pad.

---

- Centrifuge the plate at 3,000 rpm for about 20 seconds in a tabletop centrifuge with plate holders.
- Amplify the samples as described in Chapter 3, “Perform PCR: CLA Identifier™ Direct kit”.

---

**IMPORTANT!** This kit is not optimized for use with the GeneAmp™ PCR System 9700 with the aluminum 96-well block. Use of this thermal cycling platform can adversely affect performance of this kit.

---

## Perform PCR

---

**IMPORTANT!** This kit is optimized for use with the thermal cyclers that are listed in “Instrument and software compatibility” on page 15.

---

- Program the thermal cycling conditions.

**IMPORTANT!** Set the thermal cycler you are using as indicated: ProFlex™ PCR System: 9700 Simulation ramping mode (see the *ProFlex™ PCR System Kit Validation User Bulletin* (Pub. no. 100031595); Veriti™ Thermal Cycler: 9600 emulation mode (see *Veriti™ 96-Well Thermal Cycler AmpFℓSTR™ Kit Validation User Bulletin* (Cat. No. 4440754).; GeneAmp™ PCR System 9700 with 96-well silver or gold-plated silver block: Max ramping mode.

Initial incubation step	26 cycles			Final extension	Final hold
	Denature	Anneal	Extend		
HOLD	CYCLE			HOLD	HOLD
95°C, 11 minutes	94°C, 20 seconds	59°C, 2 minutes	72°C, 1 minute	60°C, 25 minutes	4°C, Up to 24 hours <sup>[1]</sup>

<sup>[1]</sup> The infinity (∞) setting allows an unlimited hold time.

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

---

**IMPORTANT!** If you are using adhesive clear film instead of caps to seal the plate wells, place a MicroAmp™ Optical Film Compression Pad (Cat. No. 4312639) on top of the plate to prevent evaporation during thermal cycling. The ProFlex™ PCR System and the Veriti™ Thermal Cycler do not require a compression pad.

---

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

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

---

**IMPORTANT!** Protect the amplified DNA from light.

---

# 4

## Perform electrophoresis

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- Materials required for electrophoresis ..... 29
- Set up the instrument for electrophoresis (before first use of the kit) ..... 29
- Capillary to plate mapping ..... 30
- Prepare samples for electrophoresis ..... 33

### 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(s)
SeqStudio™	1 per 6 injections	4 samples	23 samples + 1 allelic ladder
3500	1 per 3 injections	8 samples	23 samples + 1 allelic ladder
3500x/	1 per injection	24 samples	23 samples + 1 allelic ladder
3130	1 per 4 injections	4 samples	15 samples + 1 allelic ladder
3130xl	1 per injection	16 samples	15 samples + 1 allelic ladder
3730 <sup>[1]</sup>	2 per injection	48 samples	46 samples + 2 allelic ladders

<sup>[1]</sup> The 48-capillary array is recommended for the 3730xl 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. Perform internal validation studies to verify the required allelic ladder injection frequency, to ensure accurate genotyping of all samples in your laboratory environment.

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

## Materials required for electrophoresis

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

---

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

---

## Set up the instrument for electrophoresis (before first use of the kit)

For procedures to set up the instrument, see the user guide for the instrument.

### Electrophoresis software setup: SeqStudio™

The following table lists the data collection software and the run modules that you can use to analyze PCR products generated by this kit. For details on the procedures, see the documents listed in “Documentation and support” on page 108.

Genetic analyzer	Data Collection Software	Run conditions
SeqStudio™	v1.1.4 or later	<ul style="list-style-type: none"> <li>Run module: FragAnalysis</li> <li>Dye Set G5</li> </ul>

### Electrophoresis software setup: 3500/3500xL

Genetic analyzer	Operating system	3500 Data Collection Software	Run conditions
3500 3500xL	Windows™ 10	v3.3	<ul style="list-style-type: none"> <li>Run module: FragmentAnalysis36_POP7 or FragmentAnalysis50_POP7</li> <li>Dye Set G5</li> </ul>
3500 3500xL	Windows™ 7	v3, v3.1	

## Electrophoresis software setup: 3730/3730xl instruments

Genetic analyzer	Operating system	Data Collection Software	Run conditions
3730 3730xL <sup>[1]</sup>	Windows™ 10	v5	<ul style="list-style-type: none"> <li>Run module: GeneMapper_36_POP7_1 or GeneMapper_50_POP7_1</li> <li>Dye Set G5</li> </ul>
3730 3730xL <sup>[1]</sup>	Windows™ 7	v4	

<sup>[1]</sup> The 48-capillary array is recommended for the 3730xl instrument. The close proximity of capillaries in the 96-capillary array can introduce cross-talk between capillaries and interfere with data interpretation.

## Electrophoresis software setup: 3130/3130xl instruments

Genetic analyzer	Operating system	Data Collection Software	Run modules and conditions
3130 3130x/	Windows™ 7	v4	<ul style="list-style-type: none"> <li>FragmentAnalysis36_POP7_1 or FragmentAnalysis50_POP7_1</li> <li>Injection conditions: 3 kV/5 sec (3 kV/10 sec for xL<sup>[1]</sup>)</li> <li>Dye Set G5</li> </ul>

<sup>[1]</sup> If you observe off-scale peaks, optimize the injection time for your instrument.

## Capillary to plate mapping

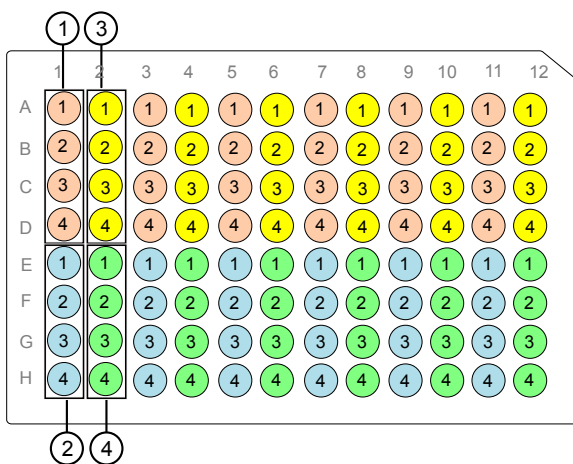
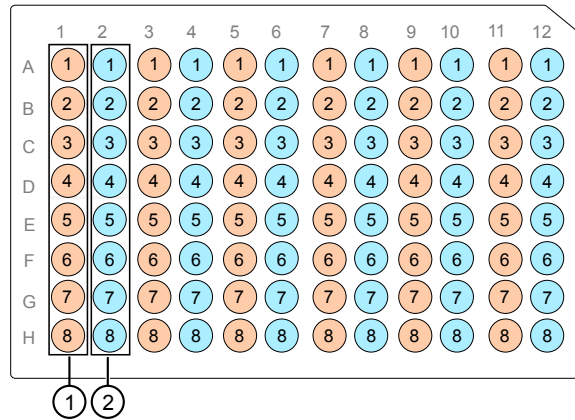


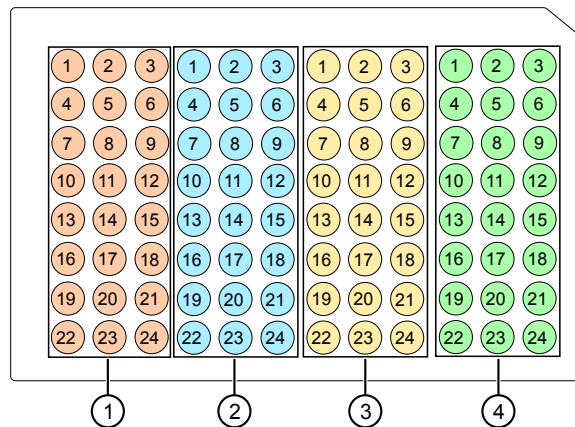
Figure 4 SeqStudio™ capillary-to-plate mapping

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



**Figure 5** 3500 96-well plate capillary-to-plate mapping (8 capillary)

- Injection 1 (wells A1–H1)
- Injection 2 (wells A2–H2)



**Figure 6** 3500xL 96-well plate capillary-to-plate mapping (24 capillary)

- Injection 1 (wells A1–C12)
- Injection 2 (wells D1–F12)
- Injection 3 (wells G1–I12)
- Injection 4 (wells J1–L12)

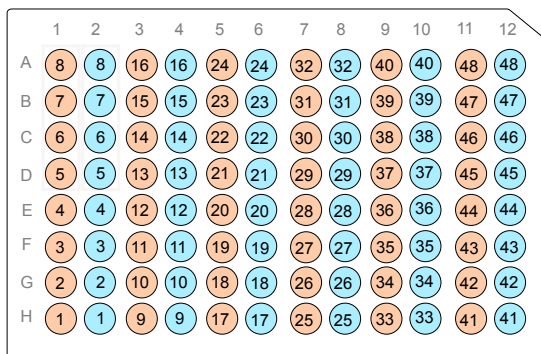


Figure 7 3730/3730xl capillary-to-plate mapping (48-capillary array)

- Injection 1 (wells A1–H1, A3–H3, A5–H5, A7–H7, A9–H9, A11–H11)
- Injection 2 (wells A2–H2, A4–H4, A6–H6, A8–H8, A10–H10, A12–H12)

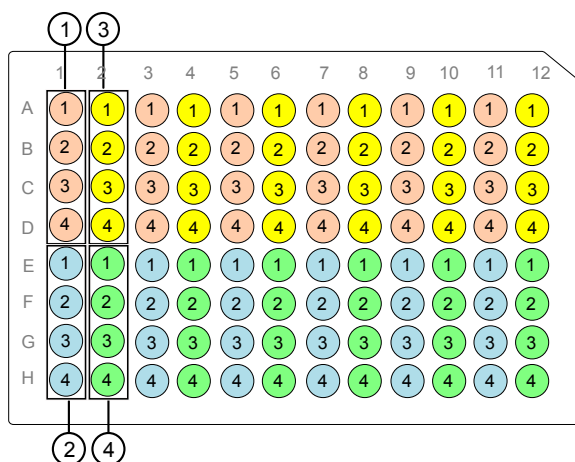


Figure 8 3130 capillary-to-plate mapping

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



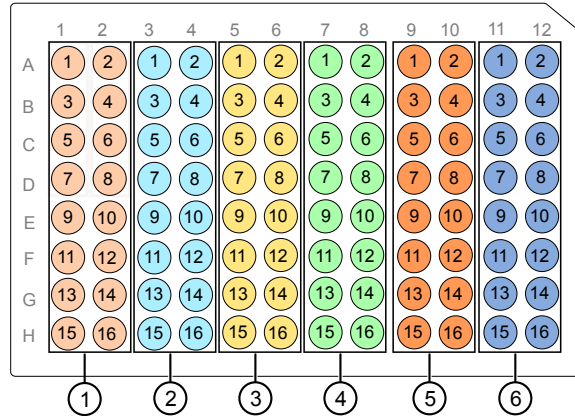


Figure 9 3130xl 96-well plate capillary-to-plate mapping (8 capillary)

- Injection 1 (wells A1–H2)
- Injection 2 (wells A3–H4)
- Injection 3 (wells A5–H6)
- Injection 4 (wells A7–H8)
- Injection 3 (wells A9–H10)
- Injection 4 (wells A11–H12)

## Prepare samples for electrophoresis

Prepare the samples for electrophoresis immediately before loading.

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

Reagent	Volume 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. Into each well of a MicroAmp™ Optical 96-Well Reaction Plate, add:
  - 9 µL of the formamide/size standard mixture
  - 1 µL of PCR product or allelic ladder


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

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.



# Analyze data with Microsatellite Analysis Software

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■ Names and versions of settings files used in this section .....	36
■ Access the Microsatellite Analysis Software and import files .....	36
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■ Review the analysis summary .....	50
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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 109.

## Overview of Microsatellite Analysis Software

Microsatellite Analysis Software is a microsatellite genotyping module available on the Connect cloud-based platform [thermofisher.com/connect](https://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](https://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 41.

1. Go to [thermofisher.com/connect](https://thermofisher.com/connect).
2. Scroll down to the sign in options, then sign in.

Don't have an account?

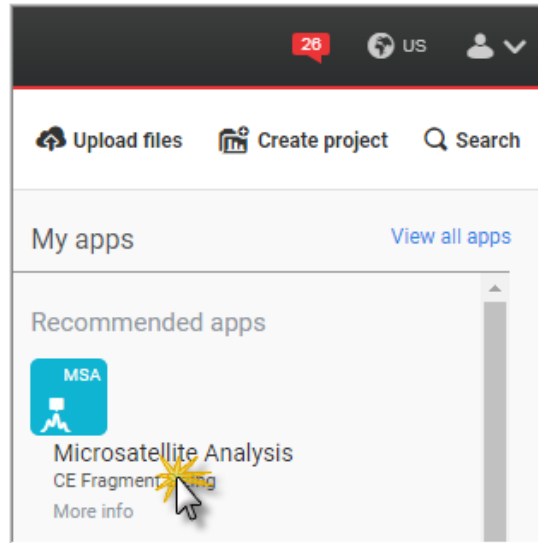
[Sign up now](#)

Already have a Connect user ID?

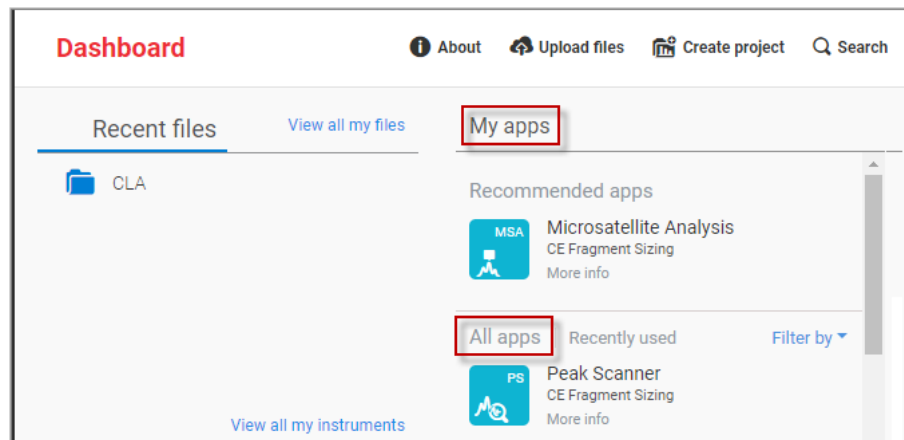
[Sign in](#)

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

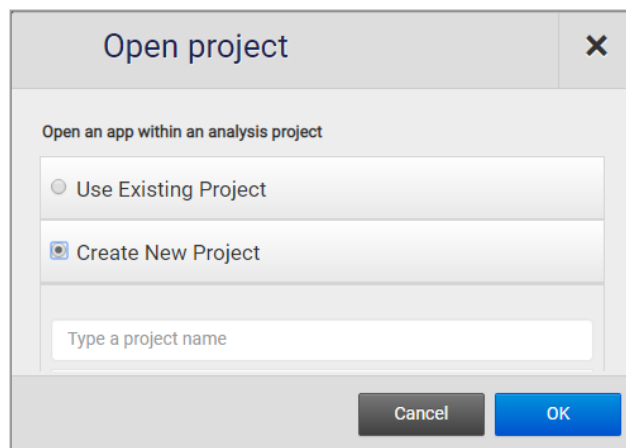
**IMPORTANT!** Click the text, do not click the icon.



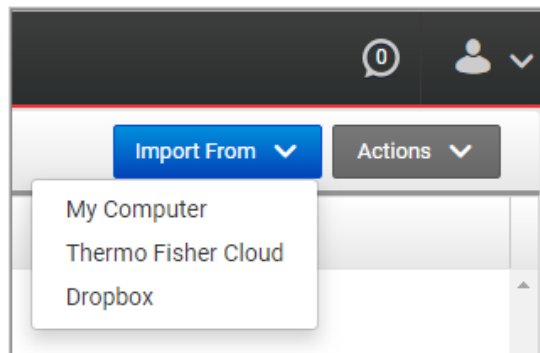
If **Microsatellite Analysis** is not listed under **My Apps**, scroll down in the **All Apps** list.



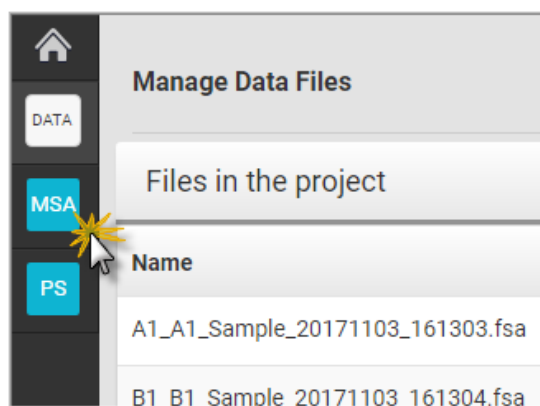
4. In the **Open Project** dialog box, select or create a project, then click **OK**.



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.

The screenshot displays the MSA software interface. At the top, there are tabs for 'Setup' and 'Results'. Below the tabs, a dark header bar contains the text 'Set analysis options then analyze 4 samples' and a 'Template Manager' button. The main content area is divided into several sections:

- Analysis Options:** A row of three dropdown menus: 'Size Standard' (set to GS600LIZ), 'Panel' (set to None), and 'Analysis Settings' (set to Microsatellite Default). Each dropdown has 'View' and 'Save As' buttons.
- Summary:** A blue box on the right stating '4 of 4 samples are prepared for analysis' and a 'Reanalyze Samples' button.
- Overall Summary:** A section with three tables:
  - Analysis Results:**

Analyzed	Unanalyzed	Reanalyze	Total
0	0	4	4
  - Sizing:**

Pass	Suspect	Low Quality	Total
4	0	0	4
  - Genotyping:**

Pass	Suspect	Low Quality	Total
0	0	0	0
- Results by Sample Type:** A section with two tables:
  - Sizing:**

Pass	Suspect	Low Quality	Total
4	0	0	4
  - Genotyping:**

Pass	Suspect	Low Quality	Total
0	0	0	0

- 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 40. If you know that the latest settings are available in the software, skip to page 49.

## 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 41).

---

**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.	42
<b>Optional individual files to import if you did not import a template (alternative method)</b>			
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.	44
Size Standard	XML	Defines the sizes of the fragments present in the size standard.	46
Analysis settings	XML	Defines the settings for peak detection, allele calling, and peak quality flags.	47

### Workflow: Set up Microsatellite Analysis Software

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

“Check settings file versions on your computer” on page 41




“(If needed) Download newer versions of settings files” on page 41

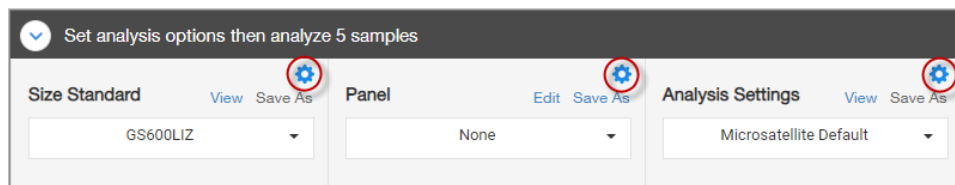


“Import an MSA settings template (preferred method)” on page 42

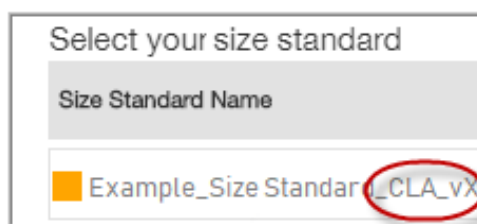


## Check settings file versions on your computer

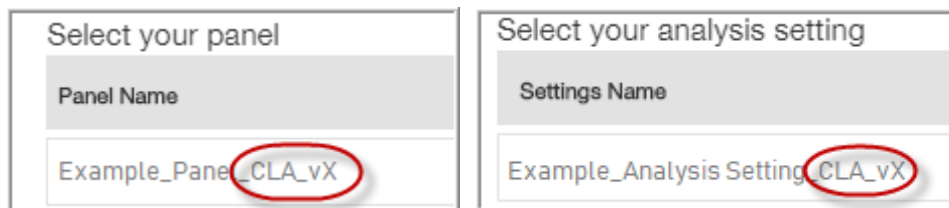
1. Access the Microsatellite Analysis Software as described in “Access the Microsatellite Analysis Software and import files” on page 36.
2. Click  next to **Size Standard**.



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



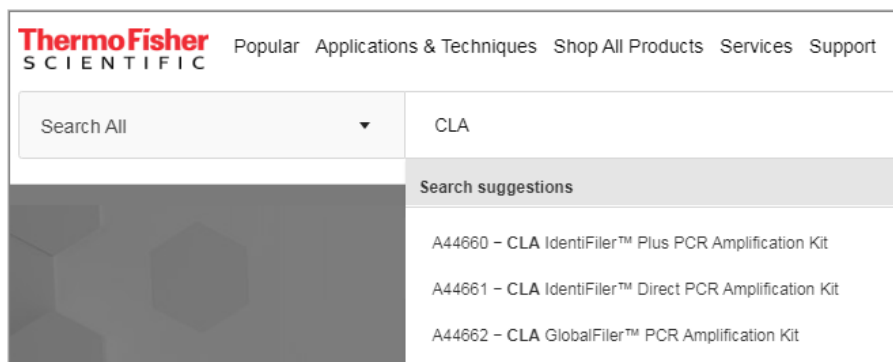
4. Check the version of files for **Panel** and **Analysis Settings**.



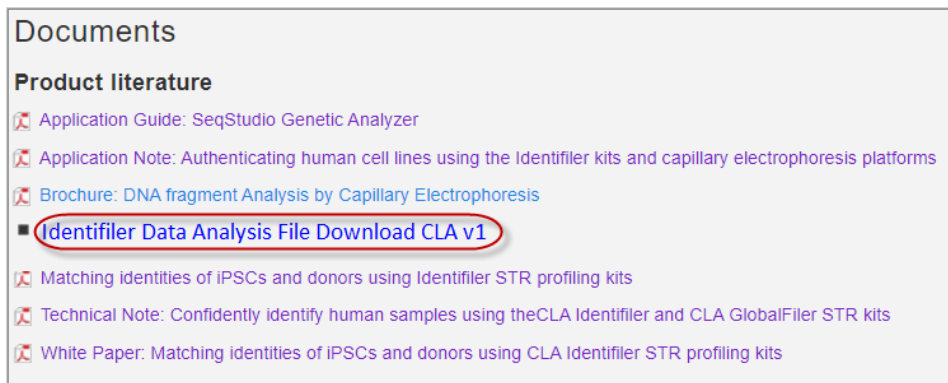
5. Check for newer versions of the files on the website as described in the next procedure.

### *(If needed)* Download newer versions of settings files

1. Go to [www.thermofisher.com](http://www.thermofisher.com).
2. In the search field, type **CLA**, then select the kit of interest.



3. Scroll down to the **Documents** 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, download the new 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.

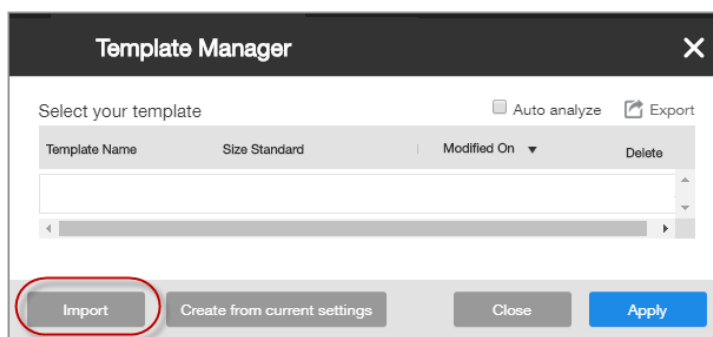
If the size standard, panel, and analysis method libraries do not contain CLA settings, or if you downloaded a new version of the template ZIP file, follow this procedure to import the template ZIP file into the Microsatellite Analysis Software.

**Note:** Alternatively, you can import individual settings. See “Import individual settings (alternative method)” on page 44.

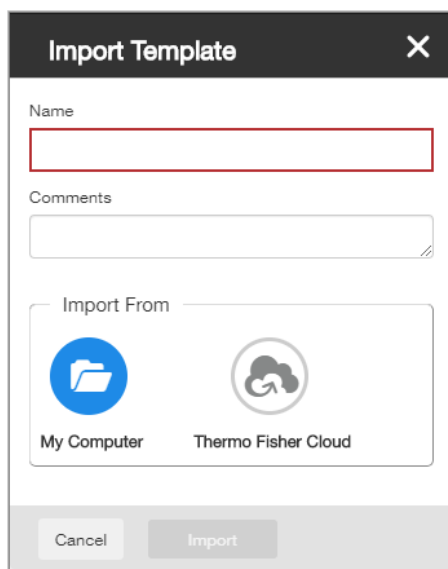
1. Access the Microsatellite Analysis Software as described in “Access the Microsatellite Analysis Software and import files” on page 36.
2. At the top right of the screen, click **Template Manager**.



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

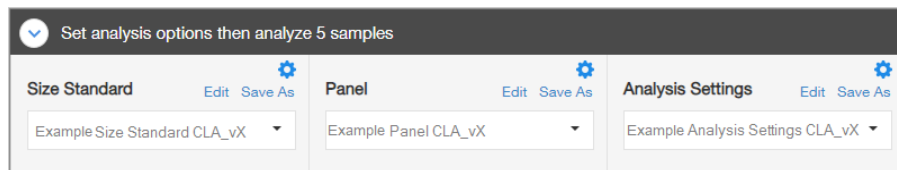


4. In the **Import Template** dialog box, enter a name for the template (include a "CLA\_vX suffix), click **My Computer**, then click **Import**.



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 41), then click **Open**.  
The ZIP file is listed in the **Template Manager**.

- 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.

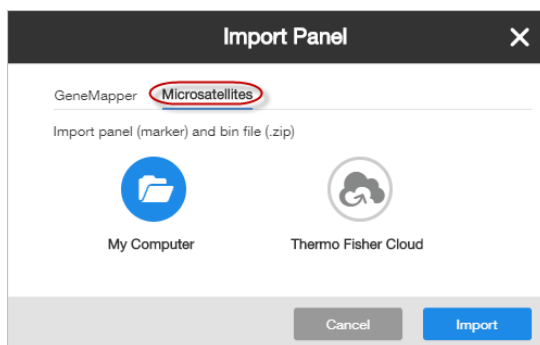
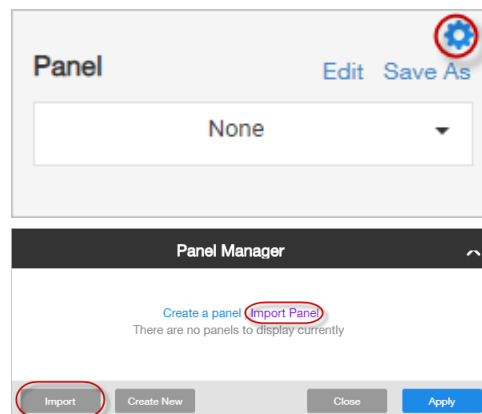
### Import an MSA panel (ZIP file created in the Microsatellite Analysis Software)

**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.

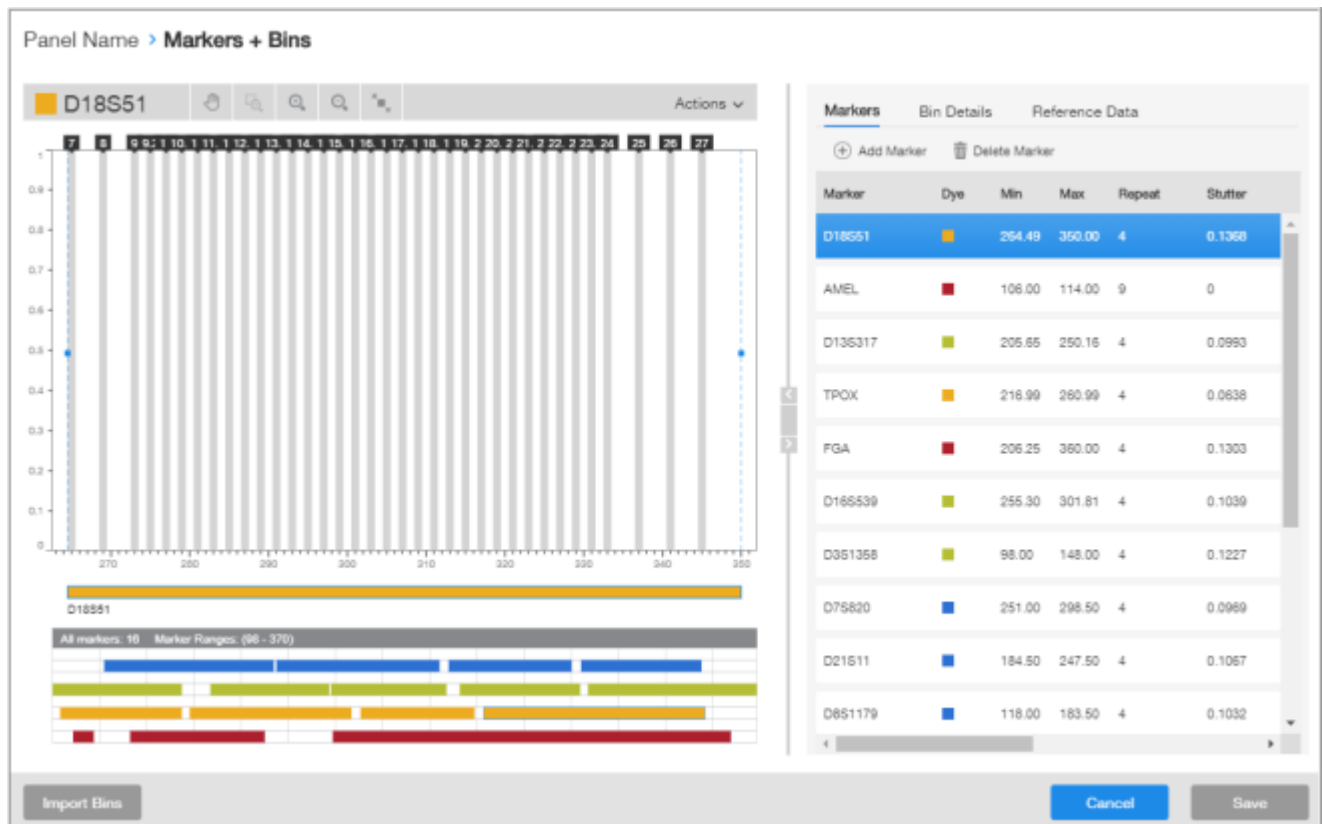
- Access the Microsatellite Analysis Software as described in “Access the Microsatellite Analysis Software and import files” on page 36.
- Click  in the **Panel** pane.
- Click **Import** in the **Panel Manager**.
- Click **Microsatellites** in the **Import Panel** dialog box.



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 41), 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.




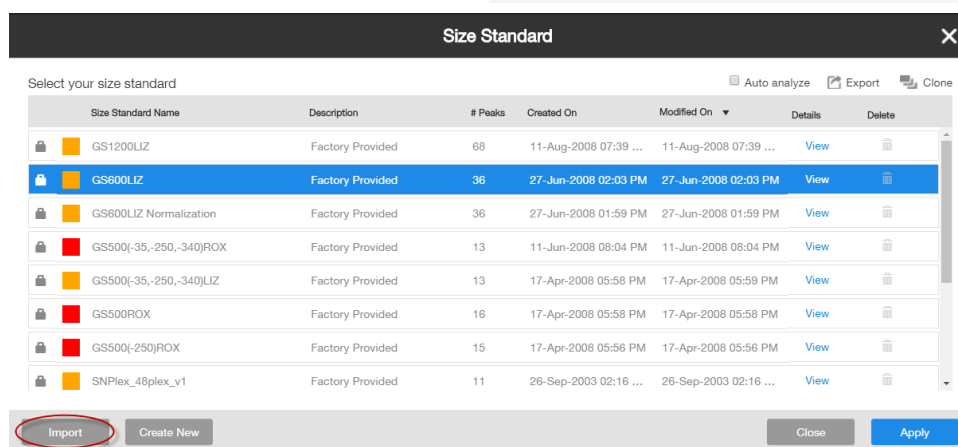
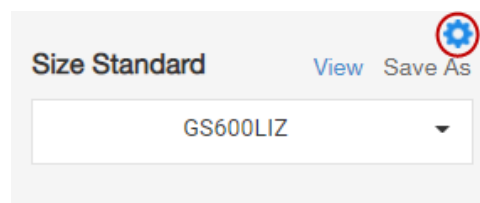
**IMPORTANT!** If the left pane does not show gray bins, click **Import Bins** at the bottom left of the screen, then import the associated bin file.

9. Click **Save**, then click **Back to Library**.
10. Click **Apply** to select the panel for the project.

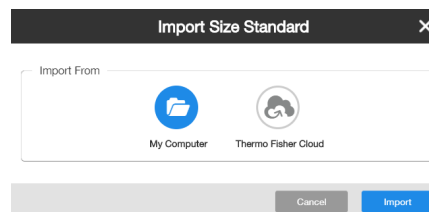
## Import a CLA size standard (XML files provided by Thermo Fisher Scientific)

**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 36.
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**.

**Import Size Standard**

Size Standard Name and Dye Color  
Example\_LS\_Size Standard\_CLA\_vX

Description (optional)  
Local Southern Sizing 60-460

Number of Peaks: 26  
60.00, 80.00, 100.00, 114.00, 120.00, 140.00, 160.00, 180.00, 200.00, 214.00, 220.00, 240.00, 250.00, 260.00, 280.00, 300.00, 314.00, 320.00, 340.00, 360.00, 380.00, 400.00, 414.00, 420.00, 440.00, 460.00

Cancel 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".

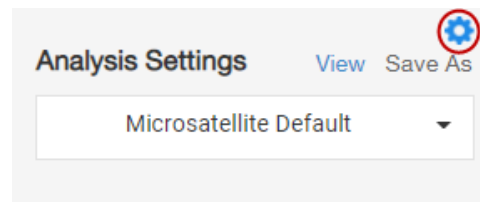
The size standard is added to the **Size Standard** library.

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

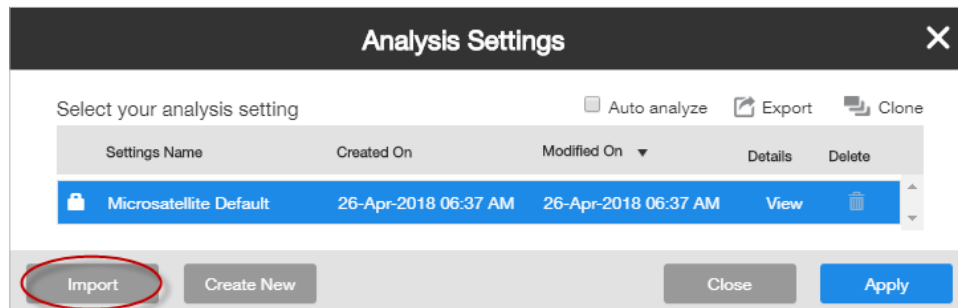
### Import a CLA analysis settings (XML file provided by Thermo Fisher Scientific)

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

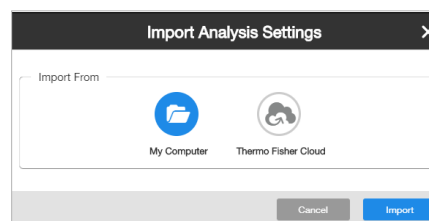
1. Access the Microsatellite Analysis Software as described in “Access the Microsatellite Analysis Software and import files” on page 36.
2. Click  in the **Analysis Settings** pane.



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



- In the **Import Analysis Settings** dialog box, click the location of the files, then click **Import**.
- Navigate to and select the analysis settings XML file for your instrument, then click **Open**.




- 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.


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

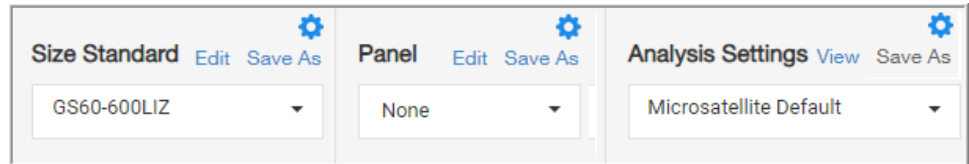
## Plot and table settings

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 .



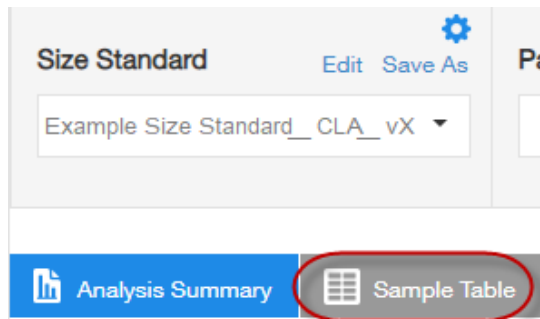
# Select settings and analyze samples in the Microsatellite Analysis Software

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-dependant. Size standard may also be instrument-dependant. Make sure to select an anaysis settings file that corresponds to the instrument on which the imported data was collected.

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



MSA Setup Results

Set analysis options then analyze 5 samples Template Manager Reanalyze Actions

Size Standard: Example Size Standard CLA\_vX Edit Save As Panel: Example Panel CLA\_vX Edit Save As Analysis Settings: Example Analysis Settings CLA\_vX Edit Save As Summary: 0 of 5 samples are prepared for analysis Reanalyze Samples

Analysis Summary **Sample Table** Enter search term X Q

<input type="checkbox"/>	Status	SQ	GQ...	Sample File Name	Sample Name	Sample Type	Instrument Model	Plate Id	Well	Size Standard	Panel	OS	REF	SQI
<input type="checkbox"/>				C1_PosCtrl.fsa	9947A	Positive Control	SeqStudio-3200	96-Well	C01	GS600LIZ				
<input type="checkbox"/>				D1_NegCtrl.fsa	Negative Control	Negative Control		96-Well	D01	GS600LIZ				
<input type="checkbox"/>				B1_Sample_02.fsa	M4A4	Sample		96-Well	B01	GS600LIZ				
<input type="checkbox"/>				A1_Sample_01.fsa	HeLa	Sample		96-Well	A01	GS600LIZ				
<input type="checkbox"/>				E1_Ladder.fsa	Allelic Ladder	Allelic Ladder		96-Well	E01	GS600LIZ				

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.

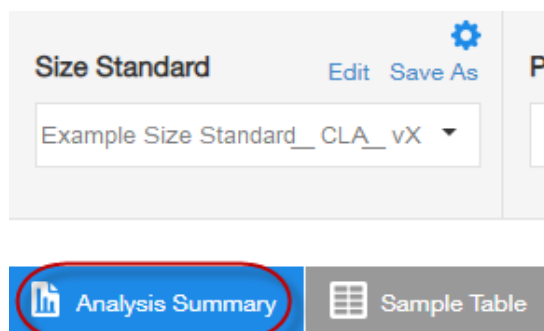
**Note:** For more information about the columns in the sample table, click ?.

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**.

<input checked="" type="checkbox"/>	Status	SQ	GQ ▲	Sample Name	Sample Type	Instrument Model	Well	Size Standard	Panel	OS
<input checked="" type="checkbox"/>	<span style="color: green;">✔</span>	<span style="color: yellow;">●</span>	<span style="color: yellow;">●</span>	Negative Control	<span style="color: gray;">/</span> Negative Control <span style="color: gray;">/</span>		D01	Example Size Std	Example Panel	<span style="color: yellow;">●</span>
<input checked="" type="checkbox"/>	<span style="color: green;">✔</span>	<span style="color: yellow;">●</span>	<span style="color: red;">●</span>	9947A	<span style="color: gray;">/</span> Positive Control <span style="color: gray;">/</span>		C01	Example Size Std	Example Panel	<span style="color: yellow;">●</span>
<input checked="" type="checkbox"/>	<span style="color: green;">✔</span>	<span style="color: yellow;">●</span>	<span style="color: red;">●</span>	M4A4	<span style="color: gray;">/</span> Sample <span style="color: gray;">/</span>		B01	Example Size Std	Example Panel	<span style="color: yellow;">●</span>
<input checked="" type="checkbox"/>	<span style="color: green;">✔</span>	<span style="color: yellow;">●</span>	<span style="color: red;">●</span>	HeLa	<span style="color: gray;">/</span> Sample <span style="color: gray;">/</span>		A01	Example Size Std	Example Panel	<span style="color: yellow;">●</span>
<input checked="" type="checkbox"/>	<span style="color: green;">✔</span>	<span style="color: yellow;">●</span>	<span style="color: red;">●</span>	Allelic Ladder	<span style="color: gray;">/</span> Allelic Ladder <span style="color: gray;">/</span>		E01	Example Size Std	Example Panel	<span style="color: yellow;">●</span>

## Review the analysis summary

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



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

The screenshot displays the software interface with two main sections. The top section, 'Overall Summary', contains three summary tables. The first table, 'Analysis Results', shows 5 Analyzed, 0 Unanalyzed, 0 Reanalyze, and 5 Total. The second table, 'Sizing', shows 5 Pass (green), 0 Suspect (yellow), 0 Low Quality (red), and 5 Total. The third table, 'Genotyping', shows 0 Pass (green), 0 Suspect (yellow), 5 Low Quality (red), and 5 Total. A circled '1' points to this section. The bottom section, 'Results by Sample Type', is divided into 'Allelic Ladder' and 'Sample'. Each has two sub-tables for 'Sizing' and 'Genotyping'. In the 'Allelic Ladder' section, the 'Sizing' table shows 1 Pass (green), 0 Suspect (yellow), 0 Low Quality (red), and 1 Total. The 'Genotyping' table shows 0 Pass (green), 0 Suspect (yellow), 1 Low Quality (red), and 1 Total. In the 'Sample' section, the 'Sizing' table shows 4 Pass (green), 0 Suspect (yellow), 0 Low Quality (red), and 4 Total. The 'Genotyping' table shows 0 Pass (green), 0 Suspect (yellow), 4 Low Quality (red), and 4 Total. A circled '2' points to this section.

- 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

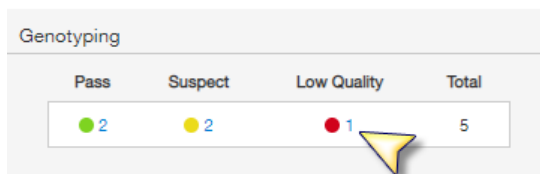
Review the EPT (ElectroPherogram Telemetry) plot to identify instrument performance issues that can affect size quality.




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.



Pass	Suspect	Low Quality	Total
 2	 2	 1	5

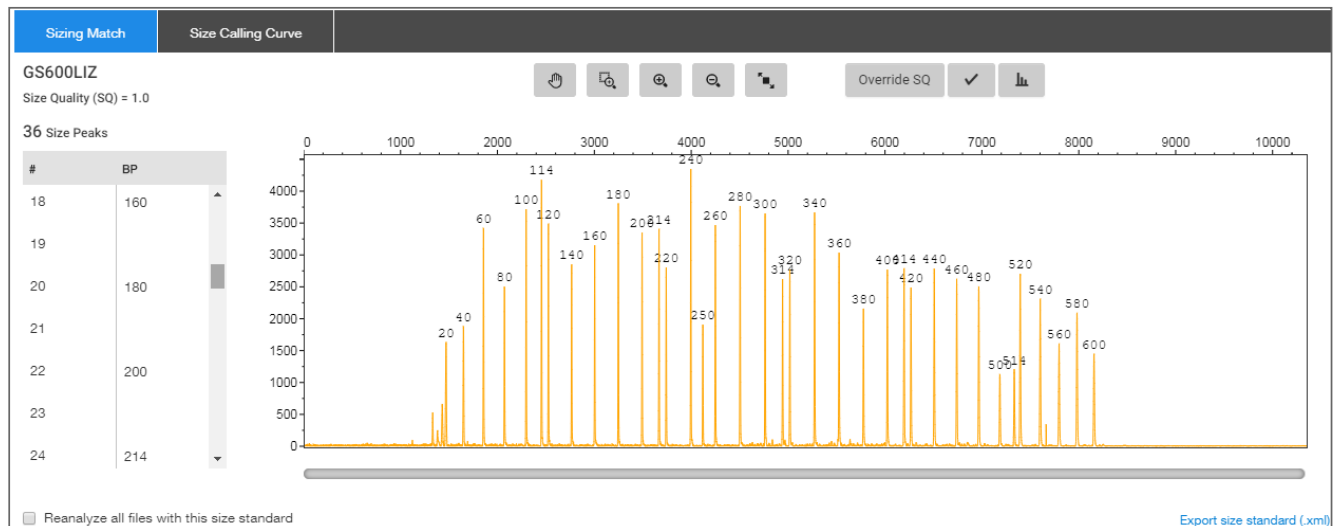
3. Modify the size matches as needed.

## 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	<ol style="list-style-type: none"> <li>1. In the <b>Results</b> screen, click the <b>Size Matching</b> tab.</li> <li>2. Select a sample from the sample list to view the peak assignments in the Sizing Match pane at the bottom of the screen.</li> </ol>
View in Size Match Editor	<ol style="list-style-type: none"> <li>1. In the <b>Results</b> screen, click the <b>Sizing</b> tab.</li> <li>2. Select one or more samples from the sample list.</li> <li>3. Click <b>Actions</b> ▶ <b>View Size Match Editor</b>.</li> </ol>



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	<ol style="list-style-type: none"> <li>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.</li> <li>2. Select the same peak, then click  (auto adjust).</li> </ol> <p>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.</p>
Delete a peak	<ol style="list-style-type: none"> <li>1. Click the size value in the list, or click a peak or peak size label in the plot, then select <b>Delete</b> in the dropdown list.</li> <li>2. Select the same peak, then click .</li> </ol> <p>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.</p>

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 Identifier™ Plus kit and CLA Identifier™ Direct kit	
	SeqStudio™, 3500, 3130	3730
Baseline Window Size	51 (default)	51 (default)
Peak Window Size	15 (default)	11
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.

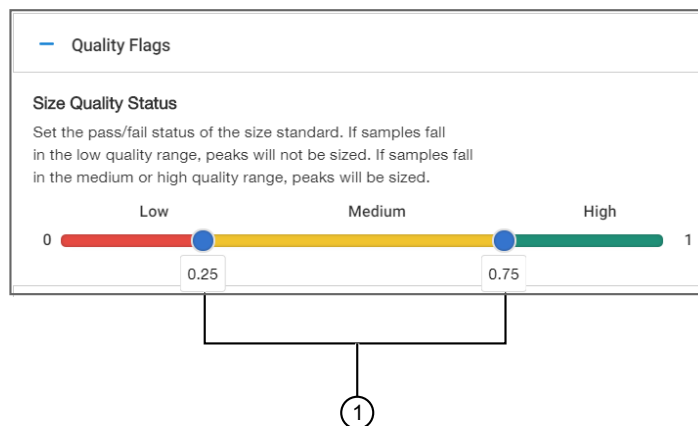


Figure 10 Quality Flag ranges




- 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 )

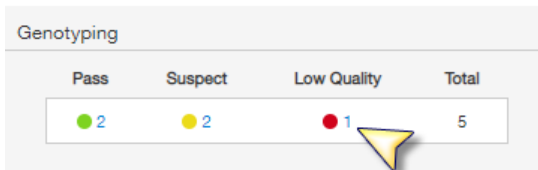
PQVs are flags that reflect the quality of results.   are displayed for GQ (genotyping quality) if any peak in the sample does not meet expected thresholds.  is displayed for individual PQVs in the genotypes table.




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.



Pass	Suspect	Low Quality	Total
 2	 2	 1	5

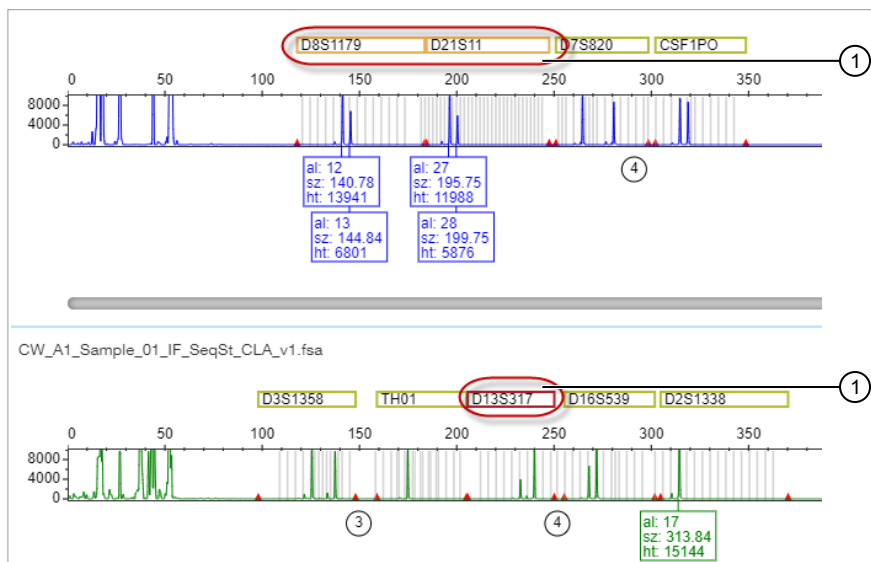




- ① Genotypes plot
- ② Genotypes table

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

- 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.



- Yellow marker header
- Red marker header

- Proceed to “Review PQVs of samples with Suspect and Low Quality GQ or PQV values” on page 58.

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

- Display the genotypes results screen (see “Review samples with Suspect and Low Quality GQ values” on page 56).
- 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).

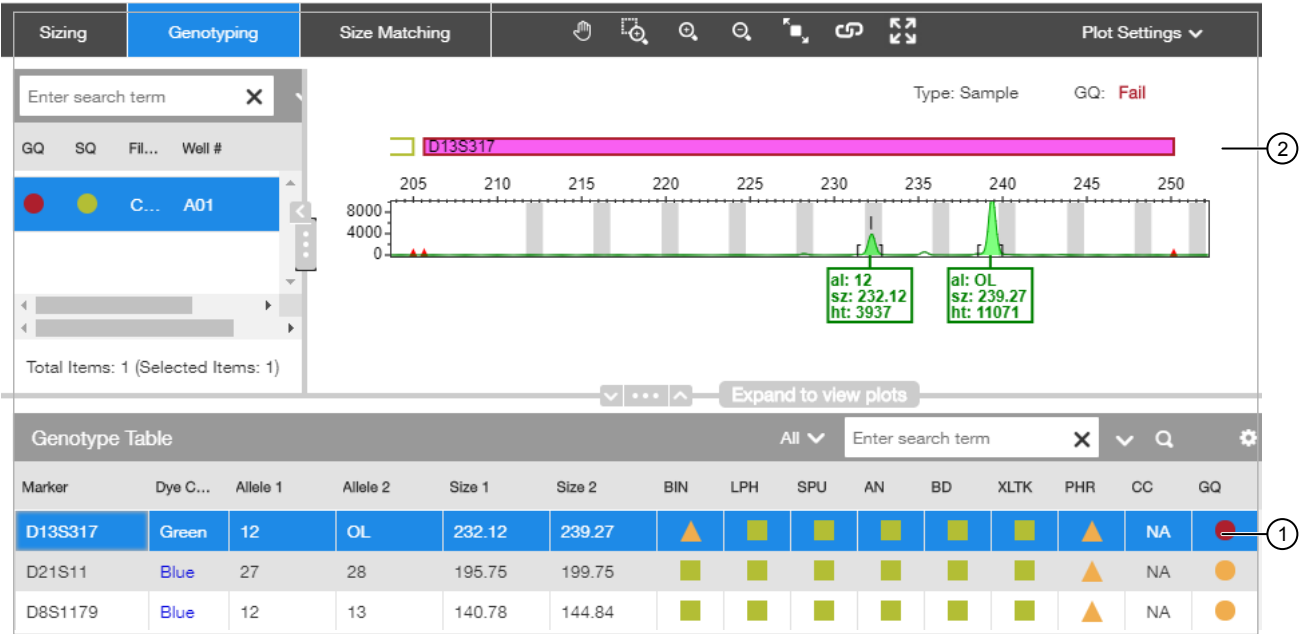


Figure 11 Example 1

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

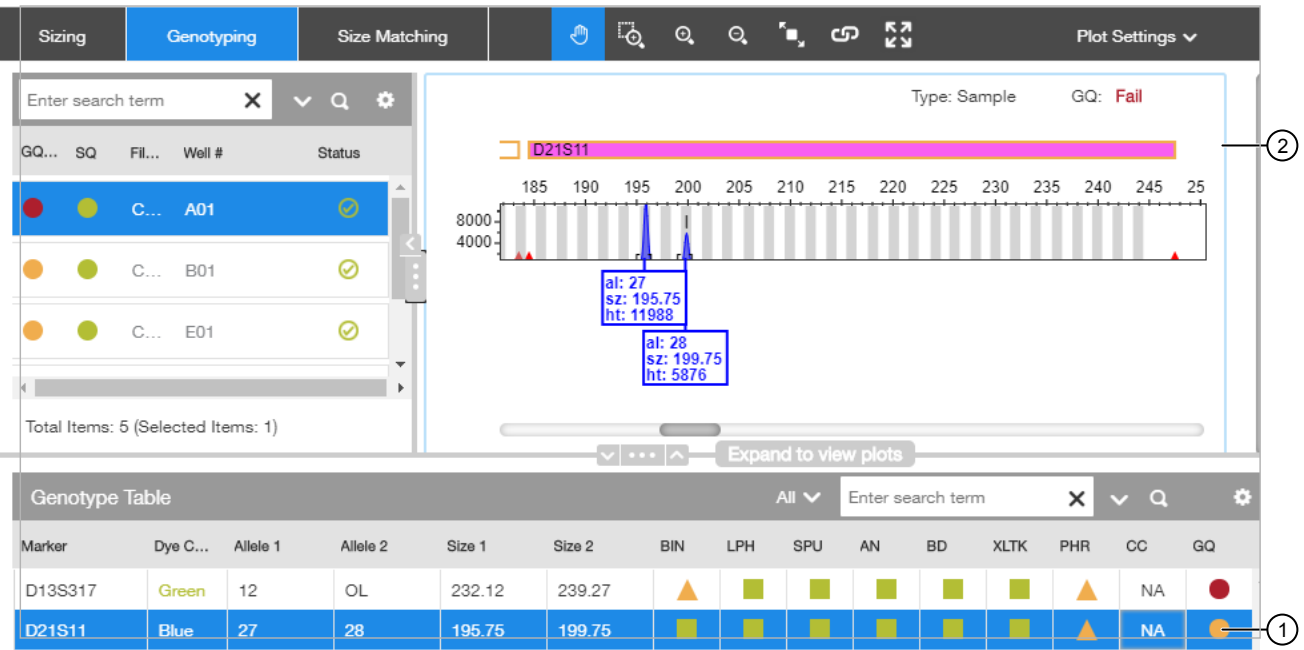


Figure 12 Example 2

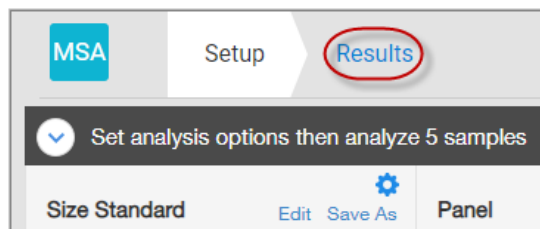
- ① Double-click a row
- ② Associated marker is selected in the plot

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

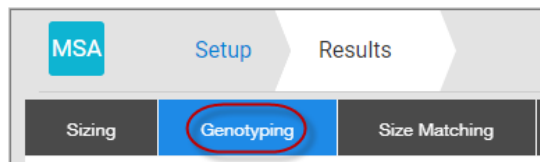
## Export the genotypes table

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.

The screenshot shows the ATCC website's STR Database page. At the top, there is a navigation bar with links for 'Login', 'Create a Profile', 'Quick Order', a shopping cart icon with '(0 items)', 'USA', and a phone number '1.800.638.6597'. The ATCC logo and tagline 'Credible leads to Incredible™' are on the left. A search bar is on the right. Below the navigation bar, there are links for 'Products', 'Services', 'Standards', 'Resources', 'Support', and 'About'. The main content area is titled 'SEARCH THE STR DATABASE' and includes a 'Please log in to search' button circled in red. A sidebar on the left contains a 'WHAT'S IN YOUR FLASK?' section with a 'Learn more' link and a fingerprint image.

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.



# Analyze data with GeneMapper™ Software

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- Names and versions of settings files used in this section ..... 63
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- Display and zoom genotype plots ..... 85
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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 109.

## 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 66).

---

**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.  <b>IMPORTANT!</b> 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.	67
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.	70
Size Standard	XML	Defines the sizes of the fragments present in the size standard.	69
Table Settings	XML	Hide or show columns, filter results, sort order.	
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.	71



## Workflow: Set up GeneMapper™ Software

“Check panel and bin file versions on your computer” on page 65



“(If needed) Download newer versions of panel and bin files” on page 66



“Import panel and bins” on page 67



“Import analysis method” on page 70



“Import the size standard” on page 69

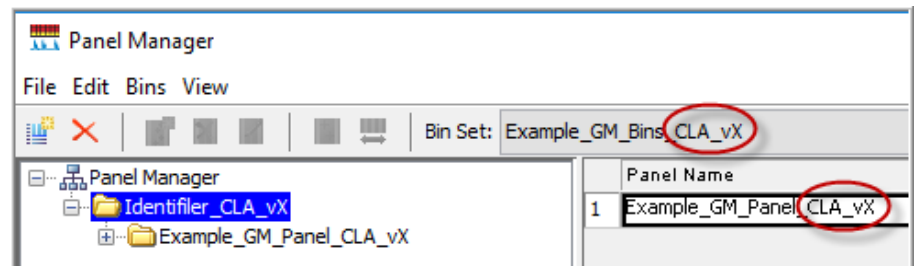
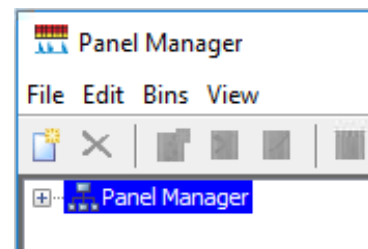


“Import table settings and plot settings” on page 71

## 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.

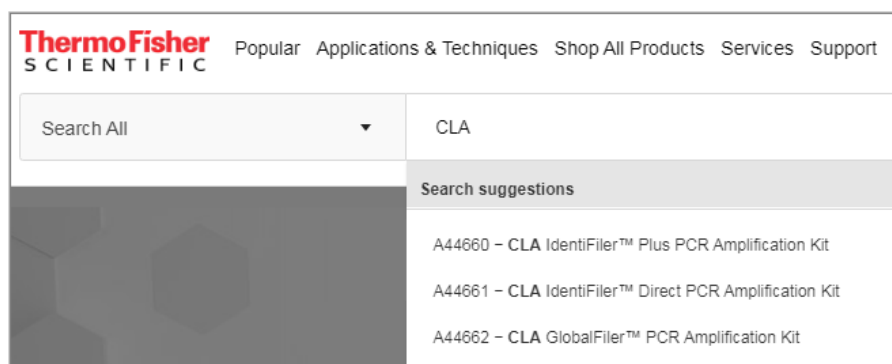


- a. Select **Panel Manager**, then select **File ▶ Import Panels** to open the **Import Panels** dialog box.

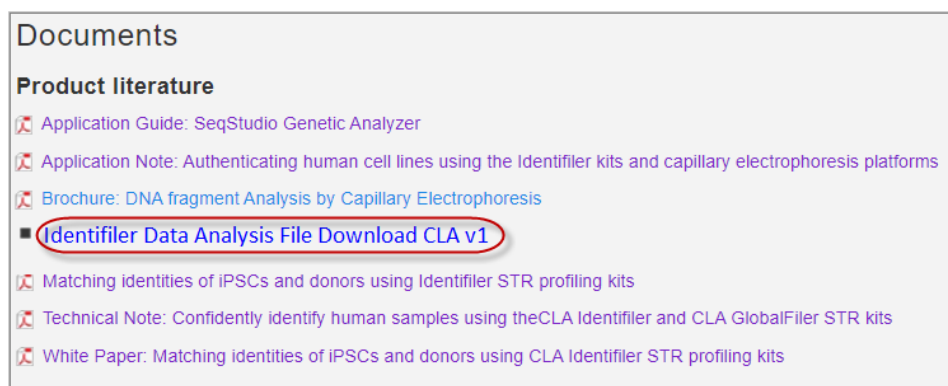
- b. Navigate to, then open the **Panels** folder, then check the version of panel, bin, and stutter files installed.
5. Check for newer versions of the files on the website as described in the next procedure.

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

1. Go to [www.thermofisher.com](http://www.thermofisher.com).
2. In the search field, type **CLA**, then select the kit of interest.



3. Scroll down to the **Documents** 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, download the new 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 IF\_Analysis\_Settings\_CLA\_vX folder, which contains the settings needed to analyze data in the GeneMapper™ Software.

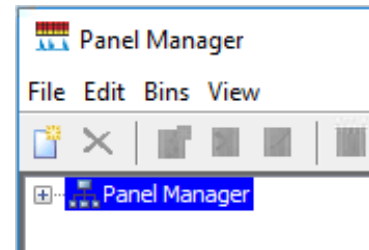
## Import panel and bins

**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.

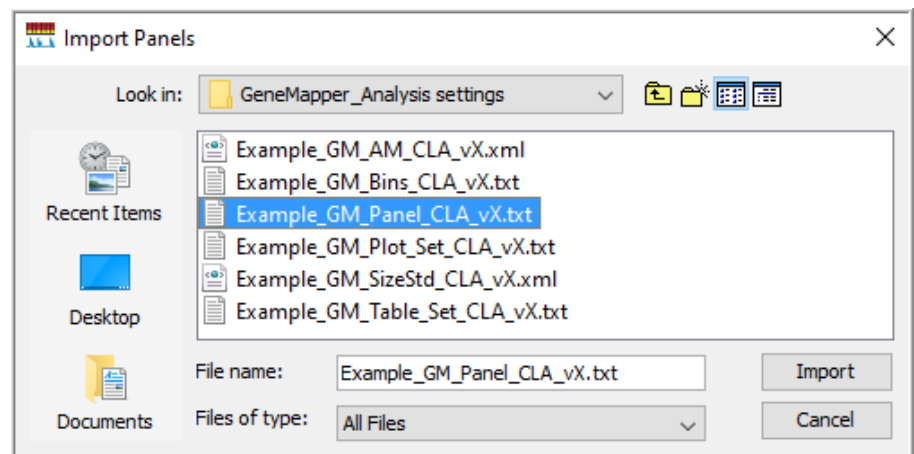
1. Start the GeneMapper™ Software, then log in with the appropriate user name and password.
2. Select **Tools ▶ Panel Manager**.

3. In the left pane, click **Panel Manager**.

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

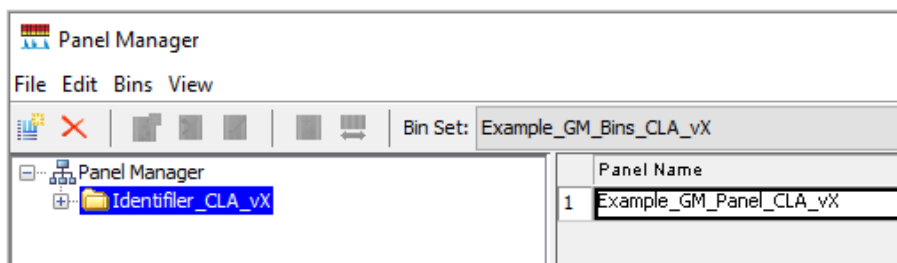


4. Import the panel file:
  - a. Select **File ▶ Import Panels** to open the **Import Panels** dialog box.
  - b. Navigate to, then select, the appropriate GM\_Panel\_CLA\_vX.txt you obtained in “(If needed) Download newer versions of panel and bin files” on page 66.



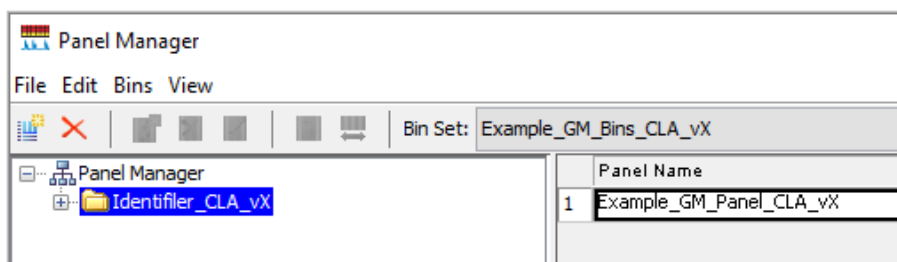
c. Click **Import**.

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



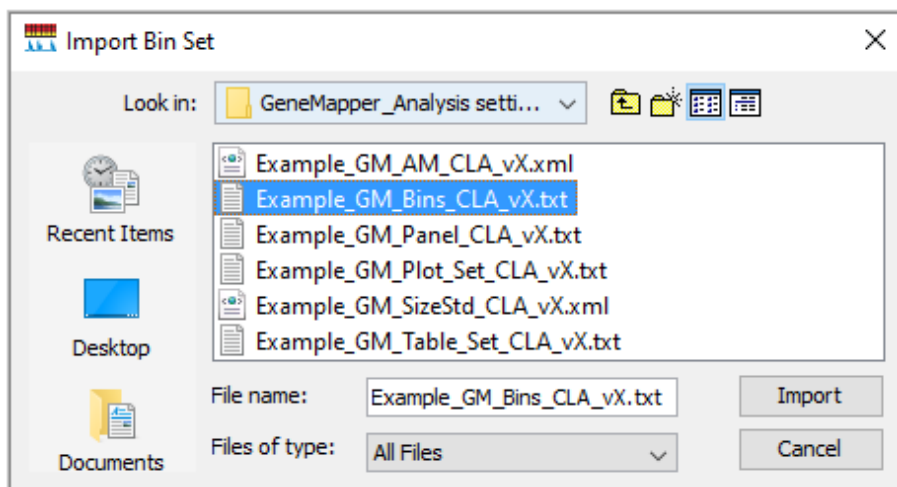
5. Import the bins file:

a. Select the Identifiler folder in the navigation pane.



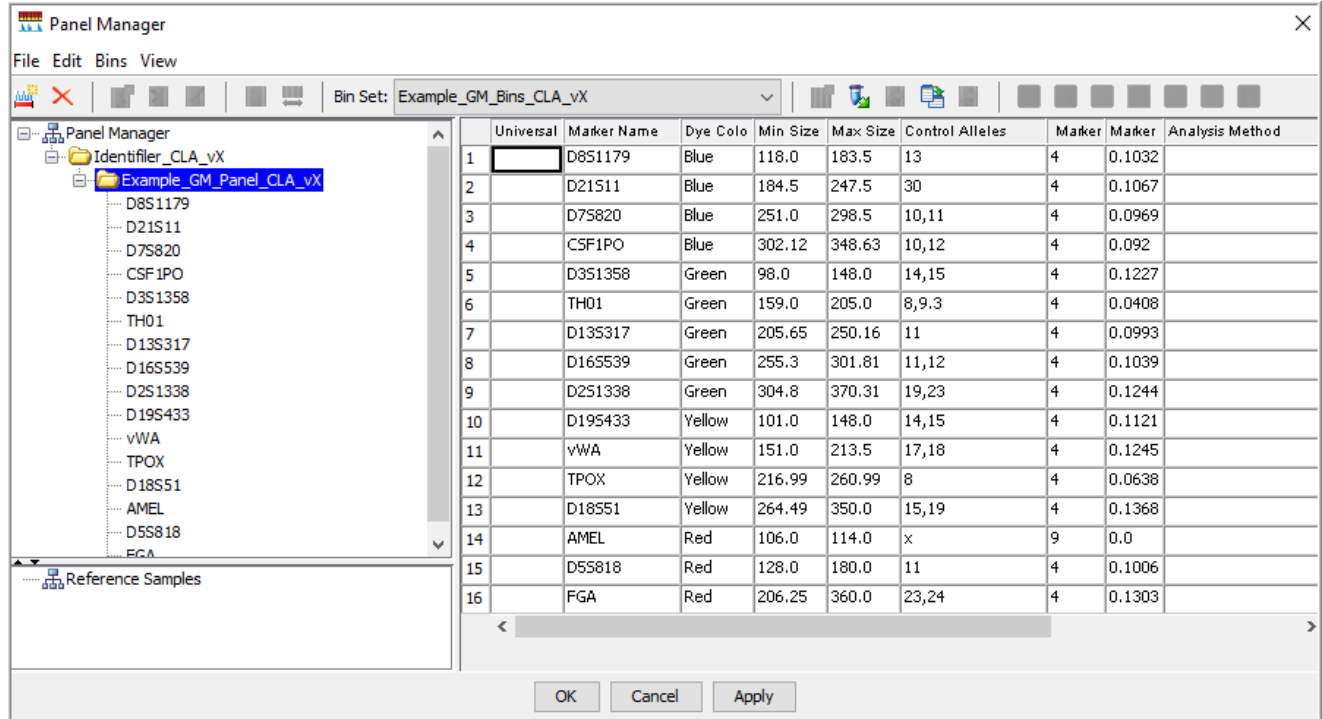
b. Select **File > Import Bin Set** to open the **Import Bin Set** dialog box.

c. Navigate to, then select, the appropriate GM\_Bins\_CLA\_vX.txt file.



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

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



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

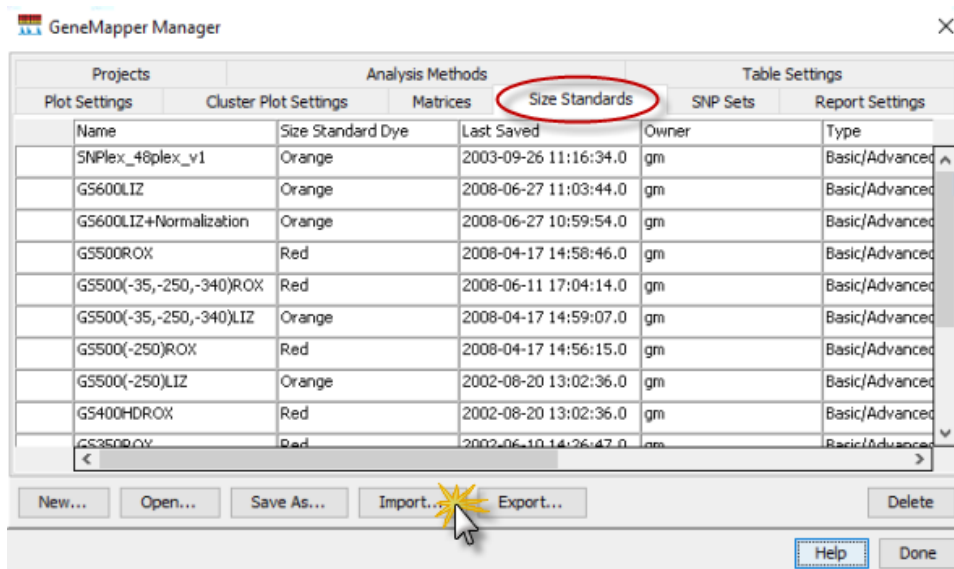
**IMPORTANT!** If you close the **Panel Manager** without clicking **Apply**, the panels, bin sets, and marker stutter are not imported into the software database.

## Import the size standard

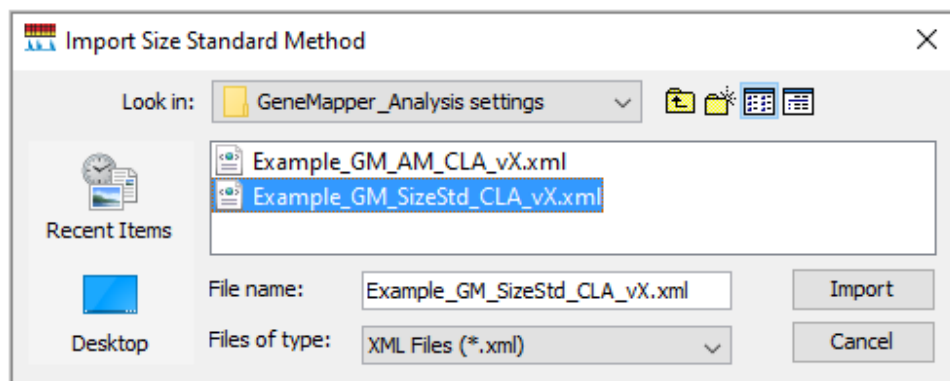
Import the latest size standard from the website into the GeneMapper™ Software database:

- In the GeneMapper™ Software, select **Tools** ▶ **GeneMapper Manager**.
- 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 66.

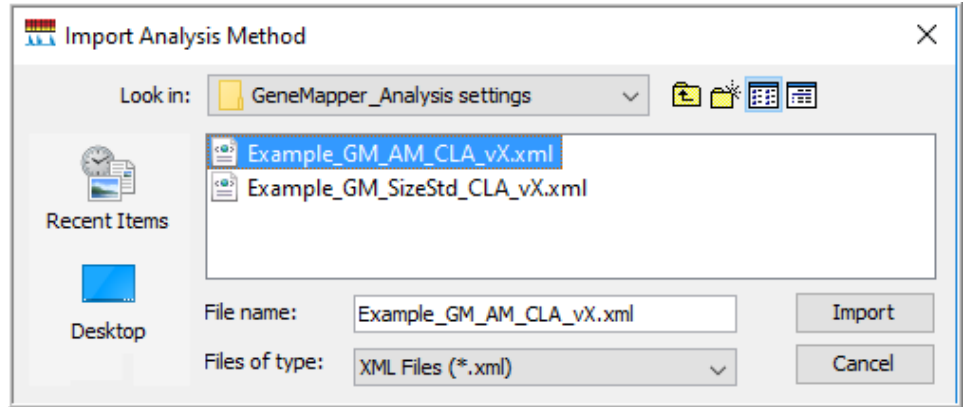


5. Click **Import**.
6. Click **Done**.

## 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 66.



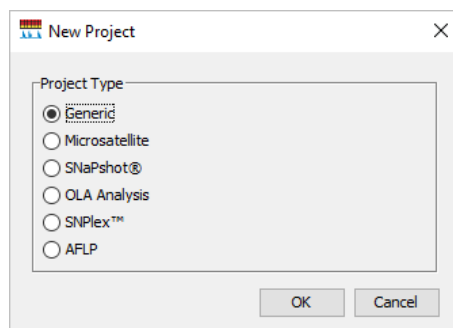
5. Click **Done**.

## Import table settings and plot settings

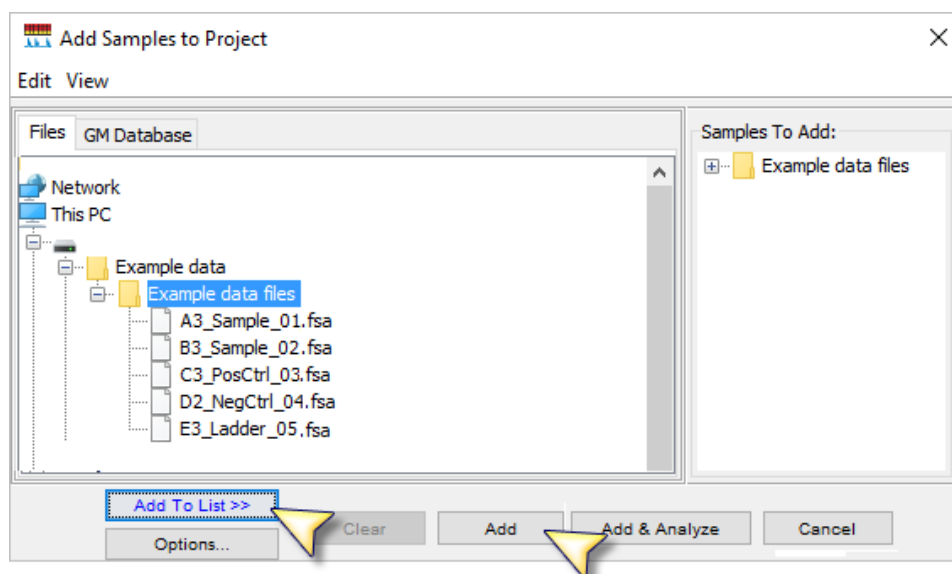
1. Start the GeneMapper™ Software, then log in with the appropriate user name and password.
2. Select **Tools** ▶ **GeneMapper Manager**.
3. In the **Table Settings** tab, click **Import**.
4. Navigate to, then select, the appropriate GM\_Table\_Set\_CLA\_vX.xml file you obtained in “(If needed) Download newer versions of panel and bin files” on page 66.
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.

Samples		Genotypes	
	Status	Sample Name	Sample Type
1		Sample01	Sample
2		Sample02	Sample
3		Sample03	Positive Control
4		Sample04	Allelic Ladder
5		Sample05	Primer Focus
			Negative Control

**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 64.

**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 64.

- 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 Standard	SQ	Well
1		A1	Sample	Example CLA Ar	Example_CI	Example CLA		A01
2		B1	Sample	Example CLA Ar	Example_CI	Example CLA		B01
3		C1	Sample	Example CLA Ar	Example_CI	Example CLA		C01
4		D1	Sample	Example CLA Ar	Example_CI	Example CLA		D01
5		E1	Sample	Example CLA Ar	Example_CI	Example CLA		E01
6		F1	Sample	Example CLA Ar	Example_CI	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 Standard	Run Name	Run Date & Time	SQ
1		A1_A1_Sample_2	A1	Sample	Example CLA Ar	Example_CI	Example CLA	GF SeqStudio	2018-06-11 15:38:51	
2		B1_B1_Sample_2	B1	Sample	Example CLA Ar	Example_CI	Example CLA	GF SeqStudio	2018-06-11 15:38:51	
3		C1_C1_Sample_2	C1	Sample	Example CLA Ar	Example_CI	Example CLA	GF SeqStudio	2018-06-11 15:38:51	
4		D1_D1_Sample_2	D1	Sample	Example CLA Ar	Example_CI	Example CLA	GF SeqStudio	2018-06-11 15:38:51	
5		E1_E1_Sample_2	E1	Sample	Example CLA Ar	Example_CI	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 82).

**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	Sampl	Panel	Marker	Allele 1	Allele 2	Size 1	Size 2	Height 1	Height 2	PHR	AN	QG
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.

Info Raw Data EPT Data

---

**Sample Information**

Sample File : A1\_A1\_Sample\_20180611  
Sample Origin Path : D:\Customer Data\Mart  
Jackson\A1\_A1\_Sample\_20180611\_153851.fsa  
Status Message : Good  
File Source : Disk media  
  
Size Standard Normalization : No

---

**Error Message**

Message : None

---

**Current Settings**

Sample Type : Sample  
Panel : Example\_CLA\_Panel\_G  
Size Standard : Example CLA Size Star  
Matrix : None  
Analysis Method : Example CLA Analysis  
Peak Detection Mode : Advanced  
Analysis range (data point) start: 2000 stop: 10000  
Sizing range (bp) start: 60.0 stop: 500.0  
Baseline window (data point): 33

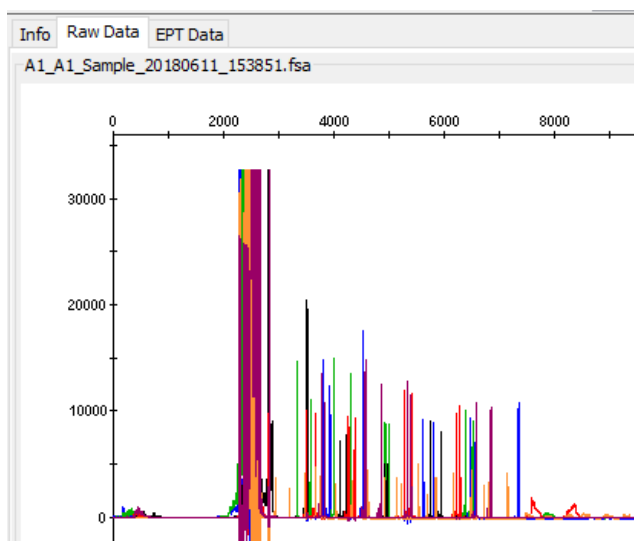
2. To return to the **Project** window, click **Project** in the left pane.

## Examine EPT and raw data

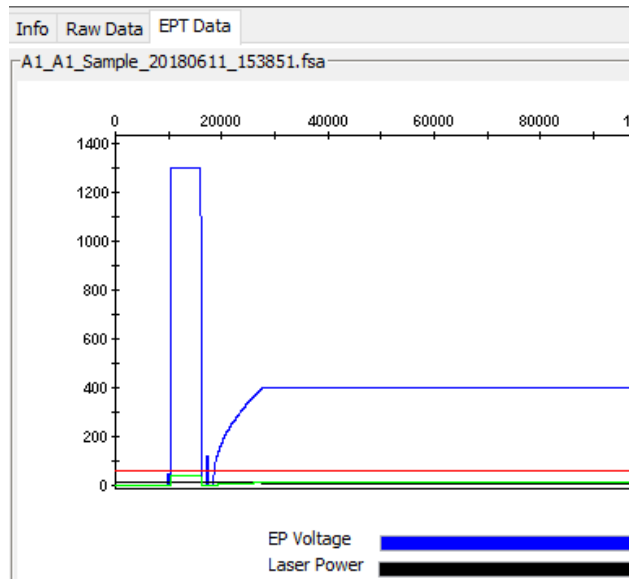
Review the EPT (ElectroPherogram Telemetry) plot to identify instrument performance issues that can affect size quality.

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.



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



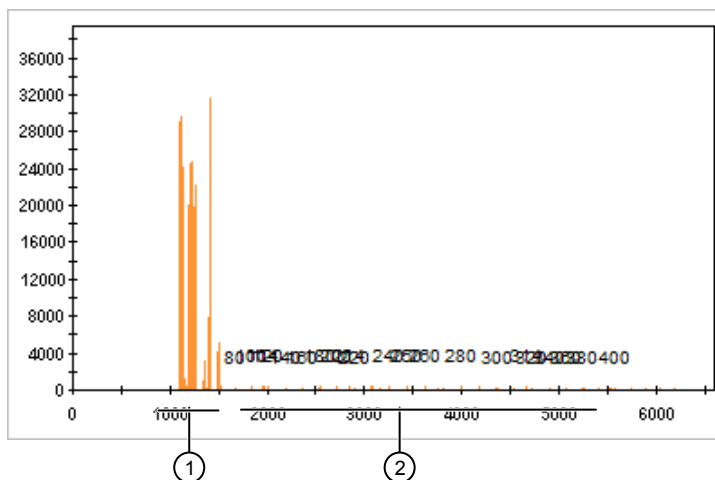
- To return to the **Project** window, click **Project** in the left pane.

## Review size matches

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

Status	Sample Name	Sample Type	Analysis Method	Panel	Size Standard	SQ	Lane	Well
	Sample01	Negative Contrc	Example_AM_CL	Example_Panel_C	Example_SizeStd	🟢	4	D02
	Sample02	Sample	Example_AM_CL	Example_Panel_C	Example_SizeStd	🔴	1	A03
	Sample03	Sample	Example_AM_CL	Example_Panel_C	Example_SizeStd	🟢	2	B03
	Sample04	Positive Control	Example_AM_CL	Example_Panel_C	Example_SizeStd	🟢	3	C03
	Sample05	Allelic Ladder	Example_AM_CL	Example_Panel_C	Example_SizeStd	🟢	5	E03

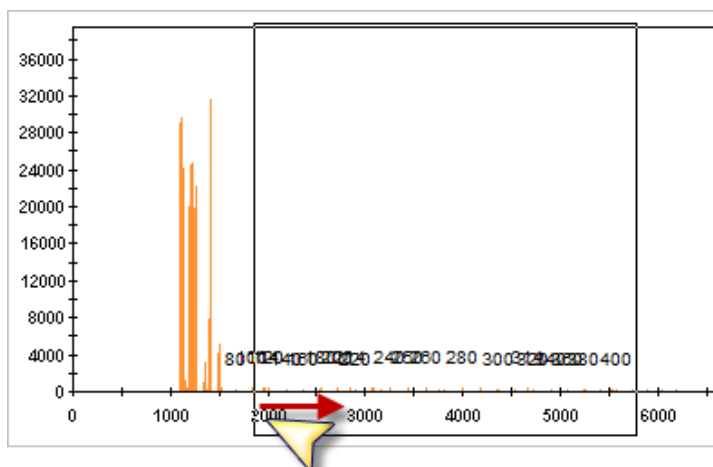
The **Size Match Editor** is displayed.



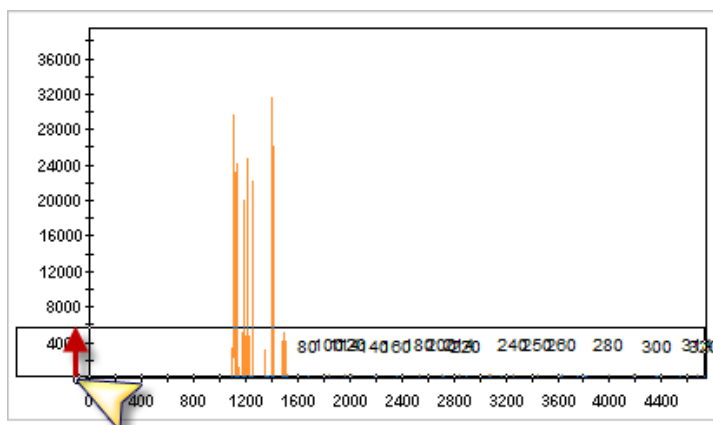
- ① Primer peaks
- ② Size standard peaks

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.

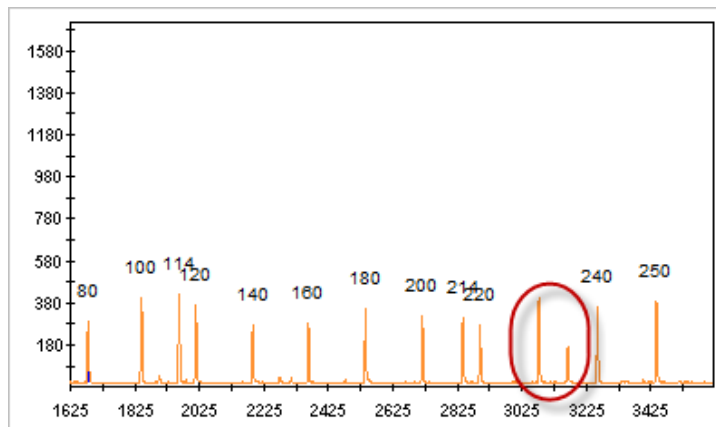


You may have to repeat these steps a few times to zoom on the size standard peaks.

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 2 on page 11). Determine if all size standard peaks are correctly labeled.

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

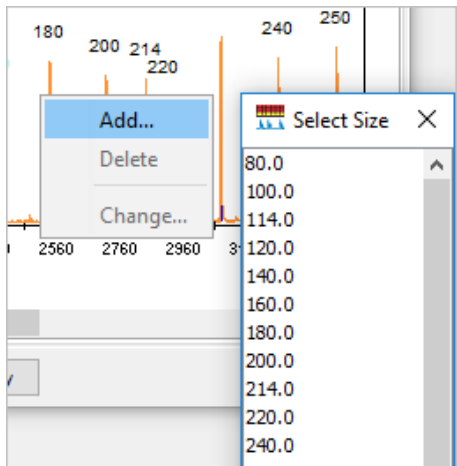


4. Add or delete peaks, or change values, as needed (see “Modify size matches” on page 80).

## 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 77).
2. Use Figure 2 on page 11 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	<p>Right-click an unlabeled peak, select <b>Add</b>, then select a size value.</p> <p><b>Note:</b> If you cannot select a peak, it indicates the peak is below the <b>Peak Amplitude Threshold</b> in the analysis method and is not detected.</p> 
Delete a size value from a peak	Right-click a labeled peak, then select <b>Delete</b> .
Override the SQ	After you correct all size assignments, click <b>Override SQ</b> .

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



## Modify the analysis settings

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 Identifiler™ Plus kit and CLA Identifiler™ Direct kit	
	SeqStudio™, 3500, 3130	3730
Baseline Window Size	51 (default)	51 (default)
Peak Window Size	15 (default)	11
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 82).

**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	Sampl	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_2i	H6	Example_CLA_Pa	AMEL							NA		
3	A1_A1_Sample_2	A1	Example_CLA_Pa	CSF1PO							NA	NA	
4	C4_C4_Sample_2i	C4	Example_CLA_Pa	CSF1PO	9	11	295.06	303.08	2309	5290			
5	C5_C5_Sample_2i	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).

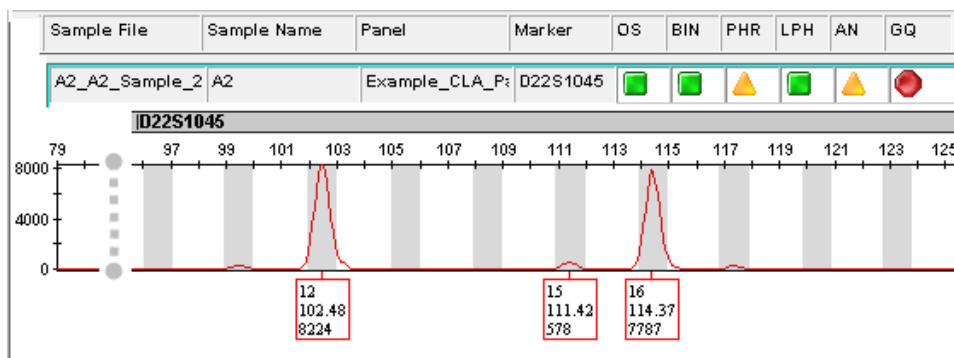


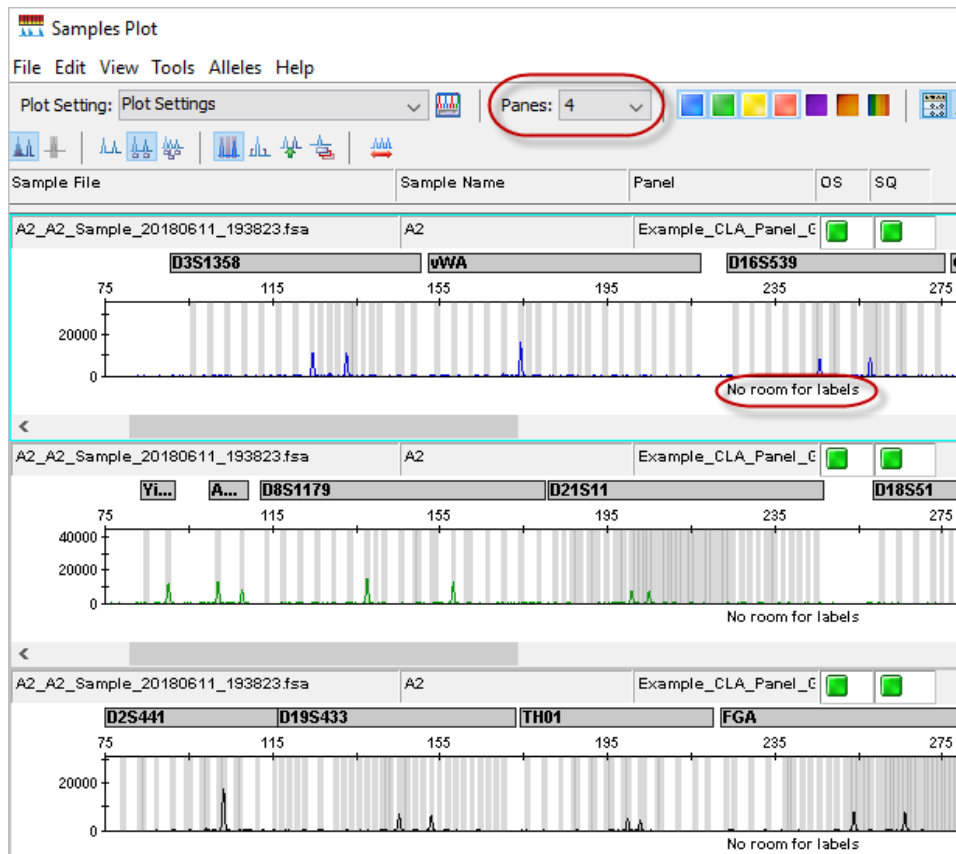


Figure 13 Example 1

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

- 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 **Panels** setting to **2**.

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

GeneMapper® Software Online Help

Hide Back Forward Print

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## Samples/Genotypes Plot

The Samples and Genotypes Plots allow you to display electropherogram data and perform a variety of tasks. The tab/table that is window (the Samples or Genotypes tab) determines the uses to display electropherogram data.

**Note:** To display the Samples or Genotypes Plot, select in the GeneMapper window, then click (Analysis > Display Electropherogram).

**You can use the Samples/Genotypes Plot to:**

- > Display electropherogram plots
- > Modify the plot
- > Zoom the plot

**Labels**

- > Delete labels
- > Display the allele histogram
- > Make room for labels
- > Modify labels

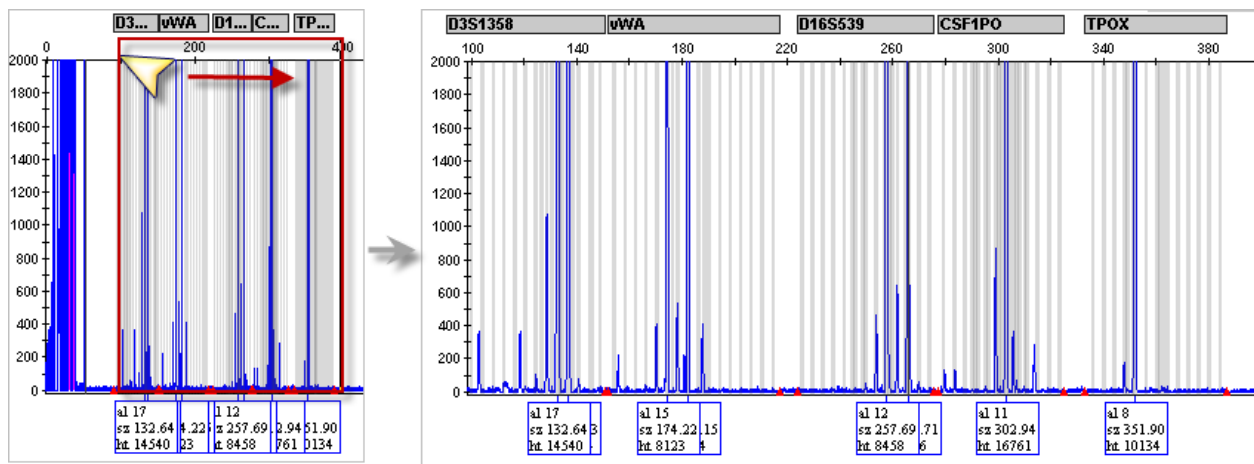
**Bins**

- > Add bins
- > Delete bins
- > Edit bins
- > Modify bins

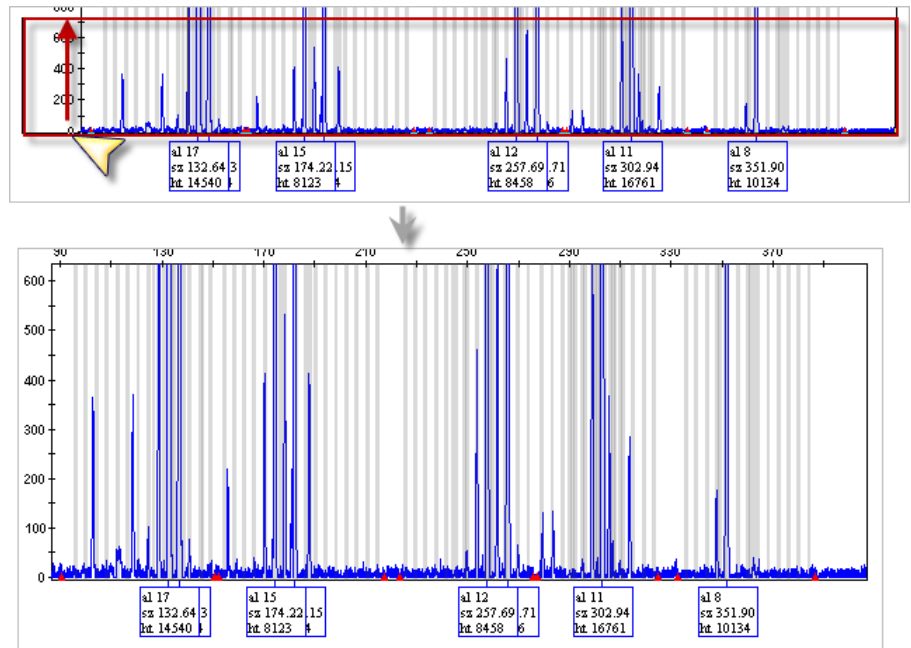
**Genotype Calls**

- > Modify alleles
- > Rename peaks

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.




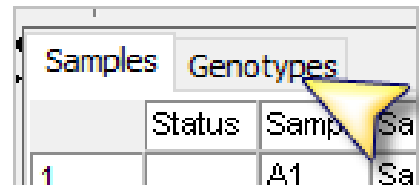
- To zoom on the y- axis, place the cursor on the 0 y-axis, then click-drag up.



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

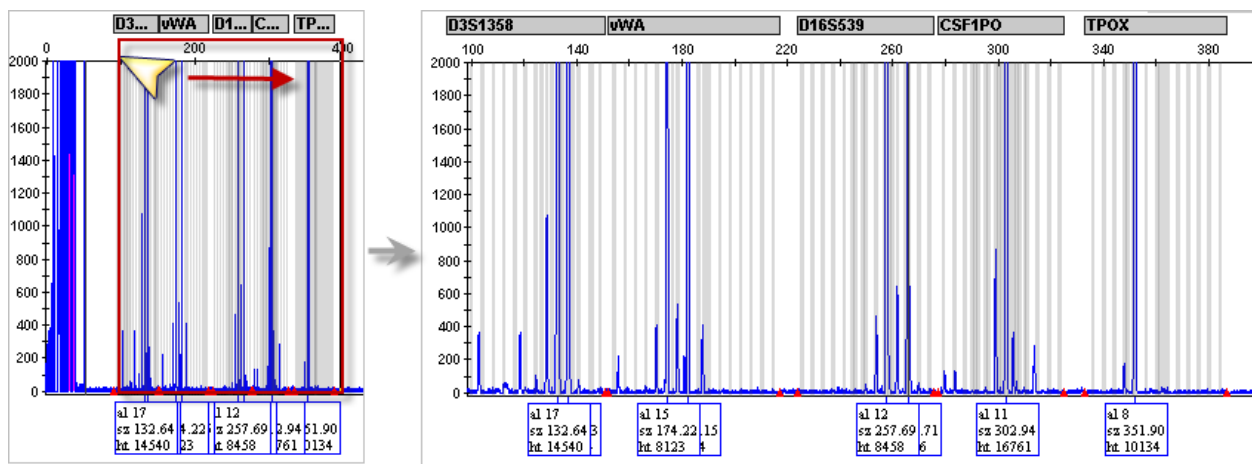
## Display and zoom genotype plots

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**).

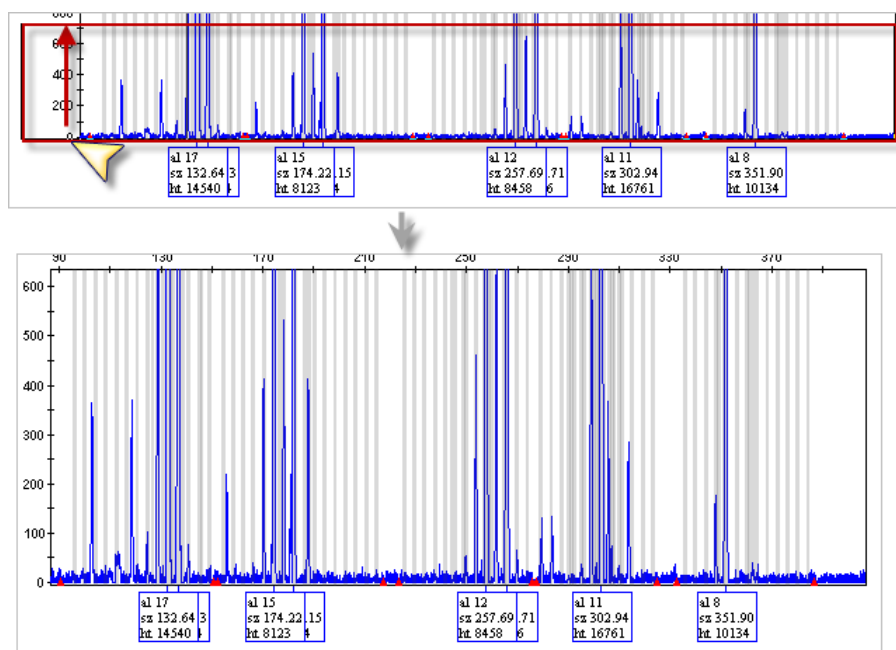


4. To zoom on the plot, select **View ▶ Zoom**, or:

- 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.

Samples		Genotypes											
	Sample File	Sampl	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.

ATCC | Credible leads to Incredible™

Login | Create a Profile | Quick Order | 🛒 (0 items) | USA ▼ | 1.800.638.6597

Search by Keyword or Catalog 🔍

Products Services Standards Resources Support About

Home ▶ STR Database

**WHAT'S IN YOUR FLASK?**

Most journals are making cell line authentication a prerequisite for publication and funding agencies a requirement for grant support.  
[Learn more ▶](#)

**SEARCH THE STR DATABASE**

As part of our continuing efforts to characterize and authenticate the cell lines in the Cell Biology collection, ATCC has developed a comprehensive database of short tandem repeat (STR) DNA profiles for all of our human cell lines. [View our brief tutorial before starting.](#)

1. [STR Profiling Analysis](#)
2. [Matching Algorithm](#)
3. [Interrogating the Database](#)

**Please log in to search**

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.



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## 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 definition

Stutter is a well-characterized PCR artifact that refers to the appearance of a minor peak one repeat unit smaller than the target STR allele product, or less frequently, one repeat larger (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).

Plus-stutter is normally less frequently observed than minus-stutter in STR loci with tetranucleotide repeats.

For the STRs in the CLA Identifiler™ Plus and Identifiler™ Direct PCR Amplification Kits (tetranucleotide repeats), minus stutter peaks can be observed at 4 bp below an allele peak. Plus stutter peaks can be observed at 4 bp above an allele peak.

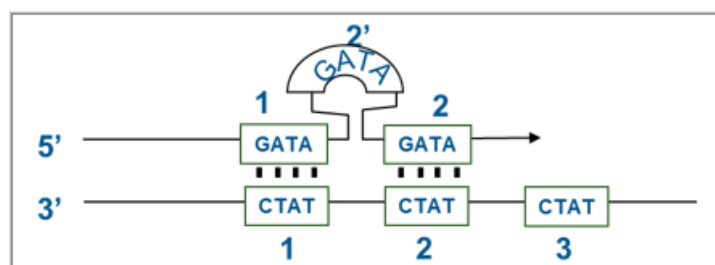


Figure 14 Plus stutter is caused by slippage in the top DNA strand

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).


## 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 <b>Use marker-specific stutter ratio if available</b> is selected, the minus stutter ratio in the <b>Panel Manager</b> is used instead of the minus stutter ratio in the analysis method.  <b>Note:</b> The CLA analysis method ( <b>Allele tab</b> ) specifies <b>Use marker-specific stutter ratio</b> .	Analysis method <b>Allele tab</b>

Parameter	Description	Location
Stutter distance	<p>Size window around an expected stutter peak (because a stutter peak may not migrate at an exact size).</p> <p>Example: A stutter distance of 3.25 to 4.75 specifies a <math>\pm 0.75</math> bp window around the possible <math>-4</math> bp stutter peak.</p> <p>The GeneMapper™ Software provides settings for minus and plus stutter peaks.</p>	Analysis method Allele tab
Stutter filter (not a setting)	<p>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 <b>Panel Manager</b>, the peak is filtered (not labeled in the electropherogram).</p> <p><b>Note:</b> To be analyzed, a peak must exceed the <b>Peak Amplitude Threshold</b> in the analysis method <b>Peak Detector</b> tab.</p>	NA






## 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 51.
  - GeneMapper™ Software—“Examine low-quality sizing results” on page 74.

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 56.
  - GeneMapper™ Software—“Examine low-quality genotyping results” on page 56.

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 56.
  - GeneMapper™ Software—“About PQVs (Process Quality Value symbols)” on page 82.

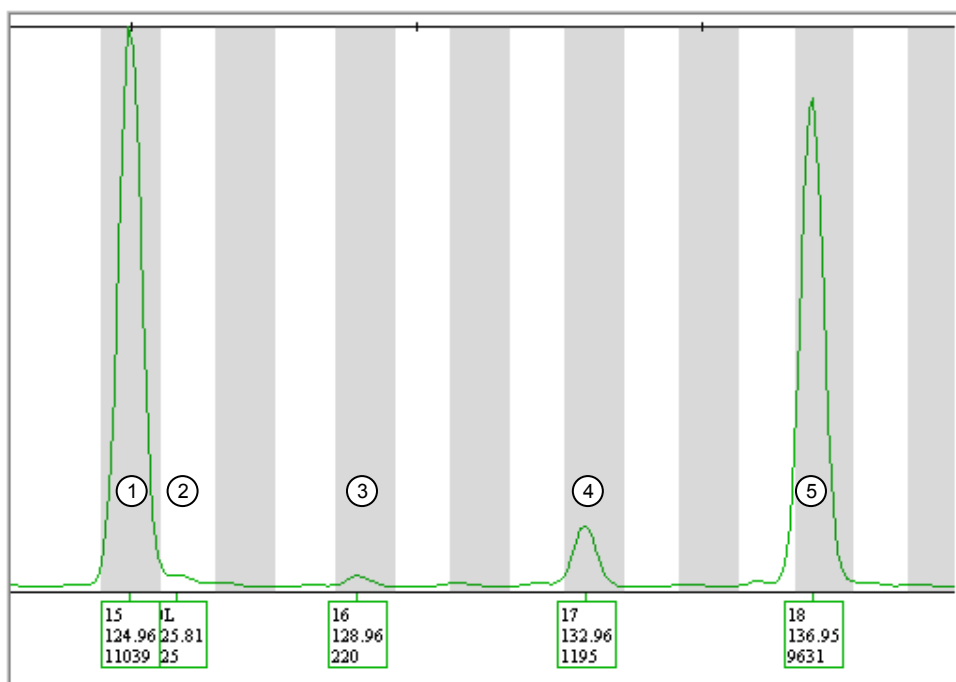
For more information, see the following sections in this chapter.

- 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 shoulder and stutter peak examples (N+1, N-4, and N+4)

**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.



- ① Allele peak 15
- ② OL shoulder peak (+1 bp from allele peak 15)
- ③ Labeled plus stutter peak (+4 bp from allele peak 15). It is not filtered (and is therefore labeled) because no plus stutter distance is set in the GeneMapper™ Software analysis method.
- ④ Labeled minus stutter peak (-4 bp from allele peak 18). It is not filtered (and is therefore labeled) because the stutter ratio exceeds the stutter ratio set in the panel. [Stutter ratio for the labeled 17 peak:  $1195/9631=0.1240$ ; Stutter ratio for the marker in the panel: 0.1227]
- ⑤ Allele peak 18

## 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 sometimes observed in the D7S820 and AMEL markers.

**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.

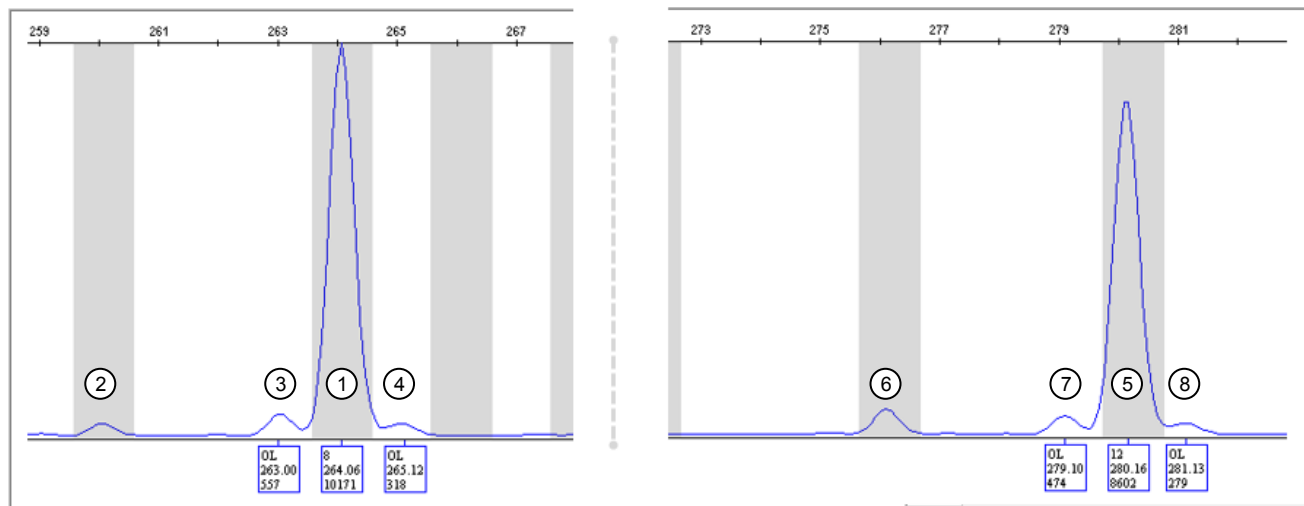


Figure 15 D7S820 marker

- ① Allele peak 8
- ② Filtered minus stutter peak (-4 bp from allele peak 8)
- ③ OL shoulder peak (-1 bp from allele peak 8)
- ④ OL shoulder peak (+1 bp from allele peak 8)
- ⑤ Allele peak 12
- ⑥ Filtered minus stutter peak (-4 bp from allele peak 12)
- ⑦ OL shoulder peak (-1 bp from allele peak 12)
- ⑧ OL shoulder peak (+1 bp from allele peak 12)

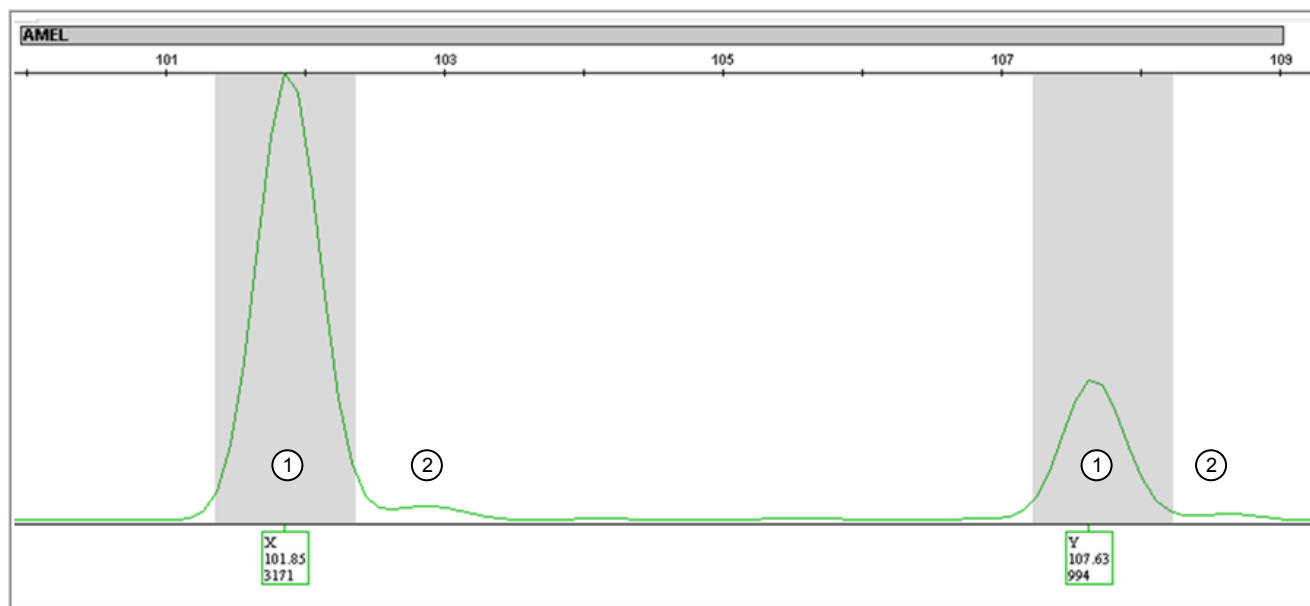


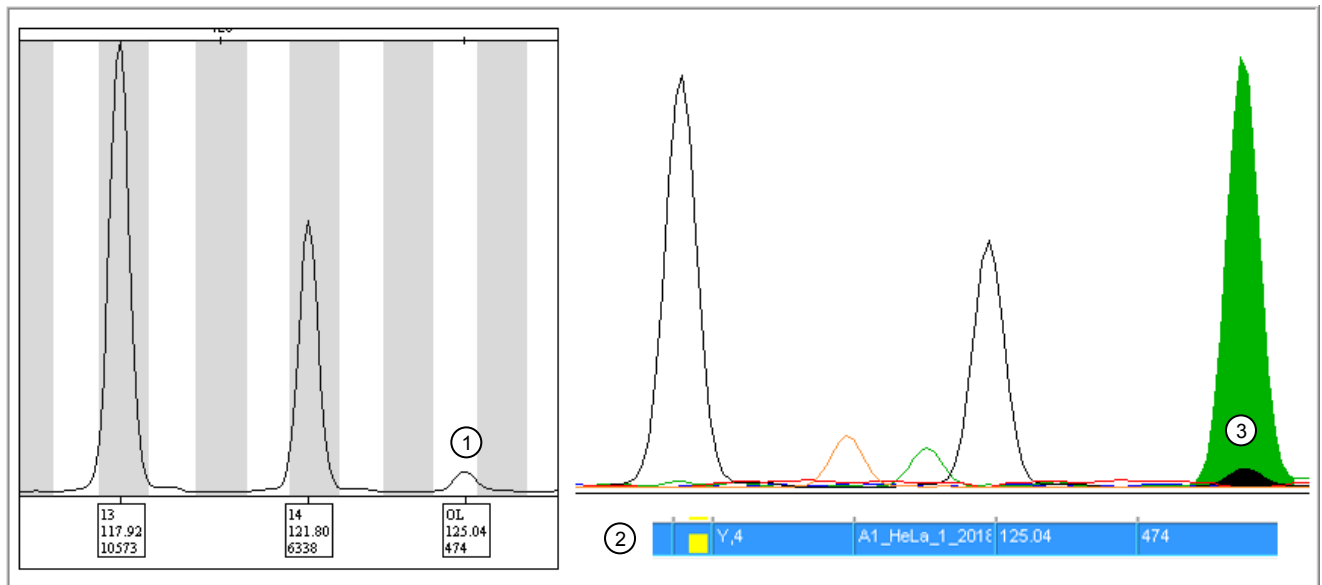
Figure 16 N+1 shoulder peaks in SeqStudio™ Genetic Analyzer data AMEL marker

- ① Allele peak
- ② N+1 shoulder peak

## D19S433: off-ladder alleles caused by dye pullup





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 appears under an allele peak  $\pm 0.5$  bp, it may be a pull-up peak.



In this example, the OL peak in the yellow dye (displayed in black in the software) is at the same bp as the green dye peak, and is therefore possibly a pull-up peak instead of a true DNA peak.







- ① Genotypes plot
- ② Samples plot with all dye channels displayed showing that the OL peak in the yellow dye may be caused by dye pull-up from the green 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: <ul style="list-style-type: none"> <li>• Microsatellite Analysis Software—page 52 and page 53</li> <li>• GeneMapper™ Software—page 77 and “Modify size matches” on page 80</li> </ul>
	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. <ul style="list-style-type: none"> <li>• Microsatellite Analysis Software—page 52 and page 53</li> <li>• GeneMapper™ Software—page 77 and “Modify size matches” on page 80</li> </ul>
	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: <ul style="list-style-type: none"> <li>• Microsatellite Analysis Software—page 49</li> <li>• GeneMapper™ Software—page 72</li> </ul>
	No allelic ladder is specified for the project.	Change at least one allelic ladder in the project to <b>Sample Type Allelic Ladder</b> . See: <ul style="list-style-type: none"> <li>• Microsatellite Analysis Software—page 49</li> <li>• GeneMapper™ Software—page 72</li> </ul>
	You manually created the analysis method, panel, and bins in the GeneMapper™ Software.  <b>IMPORTANT!</b> 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 <b>Sample Type Allelic Ladder</b> . See: <ul style="list-style-type: none"> <li>• Microsatellite Analysis Software—page 49</li> <li>• GeneMapper™ Software—page 72</li> </ul>



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: <ul style="list-style-type: none"> <li>• Microsatellite Analysis Software—page 52</li> <li>• GeneMapper™ Software—page 77</li> </ul>
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 <b>Peak Amplitude Threshold</b> 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 <b>Peak Amplitude Threshold</b> 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 <b>Peak Amplitude Threshold</b> 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 Chapter 2, “Perform PCR: CLA Identifiler™ Plus kit” or Chapter 3, “Perform PCR: CLA Identifiler™ Direct kit”.

Observation	Possible cause	Recommended action
Allelic dropout or imbalance in samples and size standard <i>(continued)</i>	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.



# Materials required but not supplied

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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.

## STR kit materials

Item	Source
CLA Identifiler™ Plus PCR Amplification Kit	A44660
CLA Identifiler™ Direct PCR Amplification Kit	A44661

## Sample preparation required materials

Table 1 Identifiler™ Plus kit

Item	Source
Low-TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0)	Teknova T0223 or see "Prepare low-TE buffer" on page 18.
Hi-Di™ Formamide, 25-mL	4311320



**Table 2 Identifiler™ Direct kit**

Item	Source
NUCLEIC-CARD™ matrix, 1 spot	MLS <sup>[1]</sup>
NUCLEIC-CARD™ COLOR matrix, 1 spot	MLS
Uni-Core™ Punch, 1.2 mm	MLS

<sup>[1]</sup> Major laboratory supplier

## 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

### Veriti™ Thermal Cycler

Item	Source
Veriti™ 96-Well Thermal Cycler	4479071
(Optional) Tabletop centrifuge with 96-Well Plate Adapters	MLS

### GeneAmp™ PCR System 9700

Item	Source
GeneAmp™ PCR System 9700, 96-Well Silver	N8050001
GeneAmp™ PCR System 9700, 96-Well Gold-Plated	4314878
Silver 96-Well Sample Block	N8050251
Gold-Plated 96-Well Block	4314443



## Genetic analyzer required materials

### SeqStudio™ Genetic Analyzer

Item	Source
(Optional) SeqStudio™ Plate Manager and App	Available on <a href="https://apps.thermofisher.com">apps.thermofisher.com</a> or for download at <a href="https://www.thermofisher.com">thermofisher.com</a>
(Optional) Handheld Barcode Scanner	4488442
SeqStudio™ Genetic Analyzer Cartridge v2	A41331
SeqStudio™ Genetic Analyzer Cathode Buffer Container	A33401
Reservoir Septa (for cathode buffer)	A35640
MicroAmp™ Optical 96-Well Reaction Plate	4316813
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4326659
MicroAmp™ Optical 8-Tube Strip, 0.2 mL	4316567
Septa for SeqStudio™ Genetic Analyzer, 96 well	A36541
Septa for SeqStudio™ Genetic Analyzer, 8 strip	A36543
MicroAmp™ 96-Well Tray/Retainer Set (Adapter for 8-Tube Strip)	403081
Hi-Di™ Formamide	4401457
DS-33 Matrix Standard Kit (Dye Set G5)	4345833

### 3500/3500xL Series Genetic Analyzer

Item	Source
3500/3500xL Data Collection Software v3.3 (Windows™ 10)	A40059, A40376
3500/3500xL Data Collection Software v3.1 (Windows™ 7)	4405186, 4405187
Anode buffer container (ABC)	4393927
Cathode buffer container (CBC)	4408256
POP-7™ Polymer (960 samples) for 3500/3500xL Genetic Analyzers	4393714
POP-7™ Polymer (384 samples) for 3500/3500xL Genetic Analyzers	4393708
POP-7™ Polymer (96 samples) for 3500/3500xL Genetic Analyzers	A26073
DS-33 Matrix Standard Kit (Dye Set G5)	4345833



Item	Source
Conditioning reagent	4393718
8-Capillary array, 36 cm for 3500 Genetic Analyzers	4404683
8-Capillary array, 50 cm for 3500 Genetic Analyzers	4404685
24-Capillary array, 36 cm for 3500xL Genetic Analyzers	4404687
24-Capillary array, 50 cm for 3500xL Genetic Analyzers	4404689
96-well retainer & base set (Standard) 3500/3500xL Genetic Analyzers	4410228
8-Tube retainer & base set (Standard) for 3500/3500xL Genetic Analyzers	4410231
8-Strip Septa for 3500/3500xL Genetic Analyzers	4410701
96-Well Septa for 3500/3500xL Genetic Analyzers	4412614
Septa Cathode Buffer Container, 3500 series	4410715

## 3730/3730xL Series Genetic Analyzer

Item	Source
3730xL Data Collection Software v5 (Windows™ 10)	4475133
3730/3730xL Data Collection Software v4 (Windows™ 7)	4405186, 4405187
3730 DNA Analyzer 48-Capillary Array, 36-cm	4331247
3730 DNA Analyzer 48-Capillary Array, 50-cm	4331250
3730xL DNA Analyzer 96-Capillary Array, 36-cm	4331244
3730xL DNA Analyzer 96-Capillary Array, 50-cm	4331246
POP-7™ Polymer 1 x 28 mL	4363929
POP-7™ Polymer 10 x 28 mL	4363935
3730 Running Buffer (10X) 500 mL	4335613
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
Hi-Di™ Formamide	4311320
Polymer Block Cleaning Kit	4335860



## 3130/3130xl Series Genetic Analyzer

Item	Source
3130 Data Collection Software v4	4475105
3130xl Data Collection Software-v4	4475126
96-Well Plate Septa	4315933
Reservoir Septa	4315932
3100/3130 xl Genetic Analyzer Capillary Array, 16-capillary 36-cm	4315931
3100/3130 xl Genetic Analyzer Capillary Array, 16-capillary 50-cm	4315930
3100/3130 Genetic Analyzer Capillary Array, 4-capillary 36-cm	4333464
3100/3130 Genetic Analyzer Capillary Array, 4-capillary 50-cm	4333466
POP-7™ Polymer for 3130/3130xl Genetic Analyzers	4363785, 4352759
Running Buffer, 10X	402824
DS-33 Matrix Standard Kit (Dye Set G5)	4345833
MicroAmp™ Optical 96-Well Reaction Plate	N8010560

## 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	<a href="https://apps.thermofisher.com">apps.thermofisher.com</a>



## 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-Cap Strips	4323032
MicroAmp™ 96-Well Tray/Retainer Set	403081
MicroAmp™ 96-Well Base	N8010531
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Adhesive Film	4311971
MicroAmp™ Optical 96-Well Reaction Plate	N8010560

### Laboratory supplies

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

<sup>[1]</sup> Major laboratory supplier





# Safety



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**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, see the “Documentation and Support” section in this document.
-

## Chemical safety

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**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:  
**<https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf>**
  - World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:  
**[www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf](http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf)**
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# Documentation and support

## Related documentation

Document	Publication number
<b>Thermal cyclers</b>	
<i>ProFlex™ PCR System User Guide</i>	MAN0007697
<i>Veriti™ Thermal Cycler User Guide</i>	4375799
<i>GeneAmp™ PCR System 9700 Base Module User Manual</i>	4303481
<b>SeqStudio™ Genetic Analyzer</b>	
<i>SeqStudio™ Genetic Analyzer Instrument and Software User Guide v1.2</i>	MAN0018646
<b>3500 Series Genetic Analyzer</b>	
<i>3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v3.3 User Guide</i>	100079380
<i>3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software 3.1 User Guide</i>	100031809
<b>3730/3730xl DNA Analyzer</b>	
<i>3730xl DNA Analyzer with 3730xl Data Collection Software 5 User Guide</i>	100077621
<i>Applied Biosystems™ 3730/3730xl DNA Analyzers Getting Started Guide</i>	4478016
<b>Analysis software</b>	
<i>Microsatellite Analysis Software User Guide</i>	MAN0017825
<i>GeneMapper™ Software 6 Installation and Administration User Guide</i>	100079198
<i>GeneMapper™ Software 5 Installation and Administration User Guide</i>	4476603
<p><b>Note:</b> Version 4.1 user documentation applies to Version 5 and Version 6 software. No features have changed in the newer versions of software.</p> <p><b>Note:</b> Newer versions of the documents may be available on <a href="http://www.thermofisher.com">www.thermofisher.com</a>.</p>	

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

## Application notes

Document	url
<i>Application note: Authenticating human cell lines using the Identifiler kits and capillary electrophoresis platforms</i>	Download from <b><a href="http://www.thermofisher.com/CLA">www.thermofisher.com/CLA</a></b> .
<i>Application note: Matching identities of iPSCs and donors using CLA Identifiler STR profiling kits</i>	
<i>Application note: Matching identities of iPSCs and donors using Identifiler STR profiling kits</i>	
<i>Application note: Confidently identify human samples using the CLA Identifiler and CLA GlobalFiler STR kits</i>	

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3730/3730xl instrument

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