applied biosystems

TrueMark™ MSI Assay

USER GUIDE

For Microsatellite Instability Research

for use with:
SeqStudio™ Flex Series Genetic Analyzer
SeqStudio™ Genetic Analyzer
3500/3500xL Genetic Analyzer
TrueMark™ MSI Analysis Software

Catalog Numbers A45295
Publication Number MAN0018868
Revision C

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

ThermoFisher SCIENTIFIC

Life Technologies Ltd | 7 Kingsland Grange | Woolston, Warrington WA1 4SR | United Kingdom
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0018868 C (English)

Revision	Date	Description
C	3 June 2025	• SeqStudio™ Flex Series Genetic Analyzer and the corresponding workflow were added. • "TRADEMARKS" on page 2, "Required materials not supplied" on page 9, and "Related documentation" on page 76 were updated.
B00	11 March 2024	Document secondary title was updated.
A.0	13 December 2019	New document for the TrueMark™ MSI Assay.

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

Contents

1 Product information

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

Product description

Kit overview

The Applied Biosystems™ TrueMark™ MSI Assay detects the presence of microsatellite instability in DNA samples through multiplex PCR and fragment analysis. Fragment analysis is performed on the Applied Biosystems™ SeqStudio™ 8 Flex Genetic Analyzer, Applied Biosystems™ SeqStudio™ 24 Flex Genetic Analyzer, Applied Biosystems™ SeqStudio™ Genetic Analyzer, or Applied Biosystems™ 3500/3500xL Genetic Analyzer. Data is analyzed using the Applied Biosystems™ TrueMark™ MSI Analysis Software for easy reporting of results.

About the primers

The TrueMark™ MSI Assay primers are manufactured using the same synthesis and purification improvements as the primers in our forensic DNA profiling kits. These improvements enhance the assay signal-to-noise ratio and simplify the interpretation of results.

Dyes used in the kit

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

Markers amplified by the kit

Table 1 TrueMark™ MSI Assay markers

Marker designation	Chromosomal location	Type	Dye label
BAT-25	4q12	MSI	6-FAM™
NR-24	2q11.1		
NR-21	14q11.2		
BAT-40	1p12		VIC™
CAT-25	7q34		
NR-22	11q24.2		NED™
NR-27	11q22.2		
ABI-19	1q42.3		
ABI-20B	1q21.3		
ABI-17	17p12		SID™
ABI-16	17p13.2		
BAT-26	2p21		
ABI-20A	12q24.13		
TH01	11p15.5	Human Identification (HID)	VIC™
PentaD	21q22.3		TAZ™

Standards and controls that are required

For the TrueMark™ MSI Assay, the panel of standards needed for PCR amplification and PCR product sizing are:

- **TrueMark™ MSI Assay Amplification Control**—A negative MSI and PCR amplification control for evaluating the efficiency of the amplification step. TrueMark™ MSI Assay Amplification Control is included in the kit. See “TrueMark™ MSI Assay Amplification Control profile” on page 8.
- **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. Order the GeneScan™ 600 LIZ™ Size Standard v2.0 (Cat. No. 4408399) separately.

TrueMark™ MSI Assay Amplification Control profile

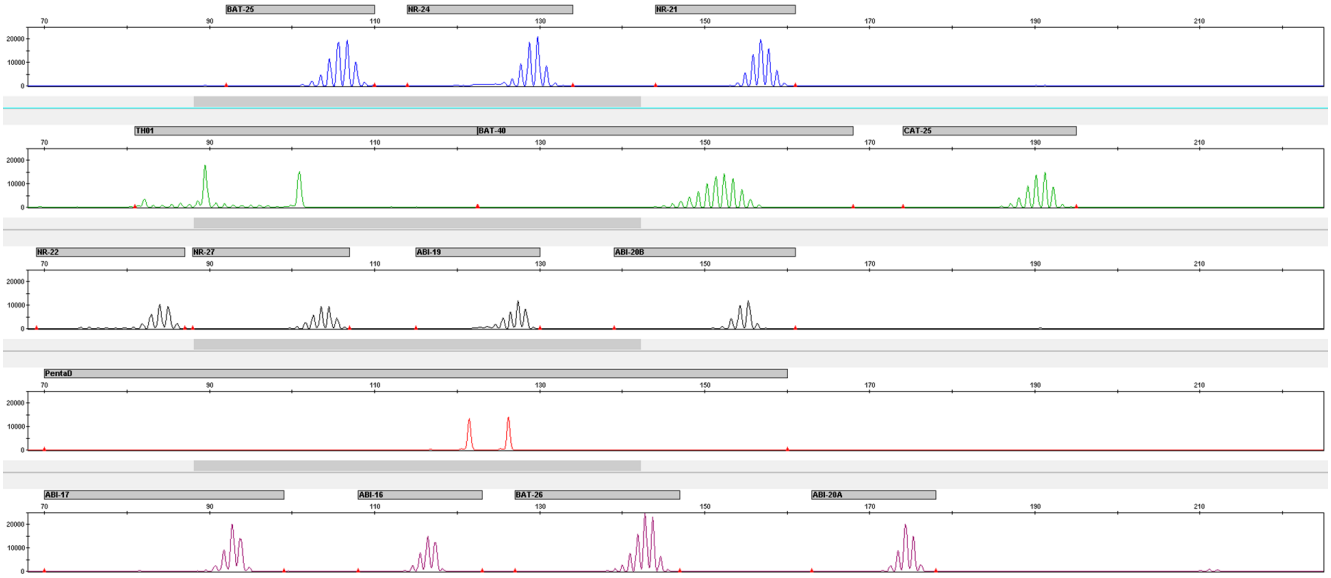


Figure 1 TrueMark™ MSI Assay Amplification Control (2 ng) amplified at 29 PCR cycles with the TrueMark™ MSI Assay and analyzed on an Applied Biosystems™ SeqStudio™ Genetic Analyzer (Y-axis scale 0 to 25,000)

Contents and storage

Table 2 TrueMark™ MSI Assay (Cat. No. A45295)

Contents	Amount	Storage
TrueMark™ MSI Assay Master Mix	480 µL	–25°C to –15°C After first use, store at 2–8°C. Store for ≤6 months or the expiration date of the kit (whichever comes first).
TrueMark™ MSI Assay Primer Mix	130 µL	
TrueMark™ MSI Assay Amplification Control	120 µL	
TrueMark™ MSI Assay No-Template Control	520 µL	

Required materials not supplied

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

Table 3 Materials for generating PCR products

Item	Source
Instruments, one of the following thermal cyclers[1]	
ProFlex™ 96-well PCR System	4484075
ProFlex™ 2 × 96-well PCR System	4484076
ProFlex™ 3 × 32-Well PCR System	4484073
Equipment	
Adjustable micropipettors	**MLS**
Benchtop microcentrifuge	**MLS**
Vortex mixer	**MLS**
Plates and other consumables	
RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE, or equivalent	AM1975
TE Buffer	12090015
MicroAmp™ 96-Well Tray	N8010541
MicroAmp™ 96-Well Tray/Retainer Set	403081
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4306737
MicroAmp™ Clear Adhesive Film, or equivalent	4306311
Aerosol-resistant pipette tips	**MLS**
Other plastic consumables	**thermofisher.com/plastics**

[1] You can use an equivalent thermal cycler. If using an equivalent thermal cycler, optimize the protocols for your thermal cycler.

Table 4 Materials for capillary electrophoresis

Item	Source
Instruments, one of the following genetic analyzers	
SeqStudio™ Flex Series Genetic Analyzer with: • SeqStudio™ Flex Series Instrument Software v1.1.1 or later • *(Recommended)* SeqStudio™ Plate Manager	Contact your local sales office

Table 4 Materials for capillary electrophoresis *(continued)*

Item	Source
SeqStudio™ Genetic Analyzer with: • SeqStudio™ Genetic Analyzer Instrument Software v1.1.4 or later • *(Recommended)* SeqStudio™ Plate Manager	Contact your local sales office
3500/3500xL Genetic Analyzer with: • 3500 Series Data Collection Software v3 or later (Windows™ 7 or 10 operating system) • 50 cm POP-7™ polymer array	
Equipment	
Adjustable micropipettors	**MLS**
Benchtop microcentrifuge	**MLS**
Vortex mixer	**MLS**
General reagents	
GeneScan™ 600 LIZ™ Size Standard v2.0	4408399
Hi-Di™ Formamide	4311320
DS-36 Matrix Standard Kit (Dye Set J6)	4425042
Reagents for the SeqStudio™ Flex Series Genetic Analyzer	
SeqStudio™ Flex Genetic Analyzer 8-Capillary Array	A49106
SeqStudio™ Flex Genetic Analyzer 24-Capillary Array	A49107
Anode Buffer Container (ABC), for 3500/SeqStudio™ Flex	4393927
Cathode Buffer Container (CBC) for the 3500/SeqStudio™ Flex	4408256
Conditioning Reagent, for 3500/SeqStudio™ Flex	4393718
POP-7™ Polymer, for 3500/SeqStudio™ Flex	A26073
96-Well Standard Retainer & Base Set, for SeqStudio™ Flex	A49316
Septa for 96-Well Plates, for 3500/SeqStudio™ Flex	4412614
Reagents for the SeqStudio™ Genetic Analyzer	
Septa for SeqStudio™ Genetic Analyzer, 96 well	A35641
SeqStudio™ Cartridge or SeqStudio™ Cartridge v2	A33671 or A41331
SeqStudio™ Cathode Buffer Container	A33401
Cathode Buffer Container Reservoir Septa for SeqStudio™ Genetic Analyzer	A35640

Table 4 Materials for capillary electrophoresis *(continued)*

Item	Source
Reagents for the 3500/3500xL Genetic Analyzer	
3500 Genetic Analyzer 8-Capillary Array, 50 cm	4404685
3500xL Genetic Analyzer 24-Capillary Array, 50 cm	4404689
Anode Buffer Container (ABC), for 3500/SeqStudio™ Flex	4393927
Cathode Buffer Container (CBC) for the 3500/SeqStudio™ Flex	4408256
Conditioning Reagent, for 3500/SeqStudio™ Flex	4393718
POP-7™ Polymer, for 3500/SeqStudio™ Flex	A26073
Retainer & Base Set (Standard) for 3500/3500xL Genetic Analyzer, 96 well	4410228
Septa for 96-Well Plates, for 3500/SeqStudio™ Flex	4412614
Plates and other consumables	
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4306737
MicroAmp™ Clear Adhesive Film, or equivalent	4306311

Table 5 Materials for data analysis

Item	Source
TrueMark™ MSI Analysis Software	Download the software from **downloads.thermofisher.com/TrueMark MSI Analysis Software.zip**
(Optional) GeneMapper™ Software (v5.0 or later)	Contact your local sales office.

Workflow

TrueMark™ MSI Assay

Prepare one of the following genetic analyzers for capillary electrophoresis:

- SeqStudio™ Flex Series Genetic Analyzer (page 13)
- SeqStudio™ Genetic Analyzer (page 20)
- 3500/3500xL Genetic Analyzer (page 25)

Prepare and run the samples (page 35)

Analyze the data with the TrueMark™ MSI Analysis Software (page 41)

2 Prepare for capillary electrophoresis

This chapter outlines guidelines and procedures for preparing the capillary electrophoresis instrument for use with the TrueMark™ MSI Assay. For detailed instructions, see the user guide for your instrument (“Related documentation” on page 76).

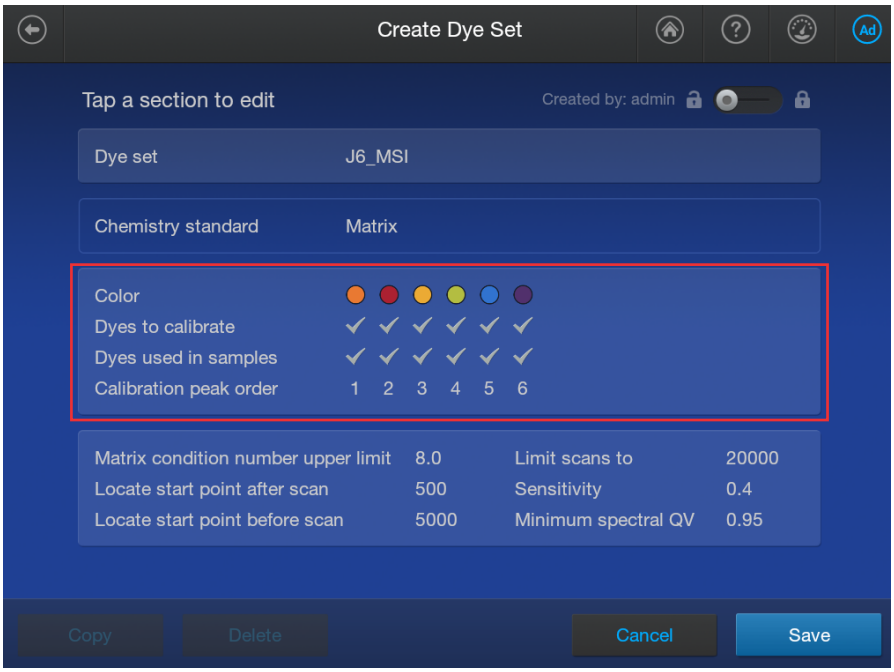
SeqStudio™ Flex Series Genetic Analyzer

For optimal performance, we recommend that you create a custom dye set with **Auto-Spectral** setting turned off and perform manual calibration. For detailed instructions about how to prepare the SeqStudio™ Flex Series Genetic Analyzer for capillary electrophoresis, see the *SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide* (Pub. No. 100104689).

Prepare for spectral calibration

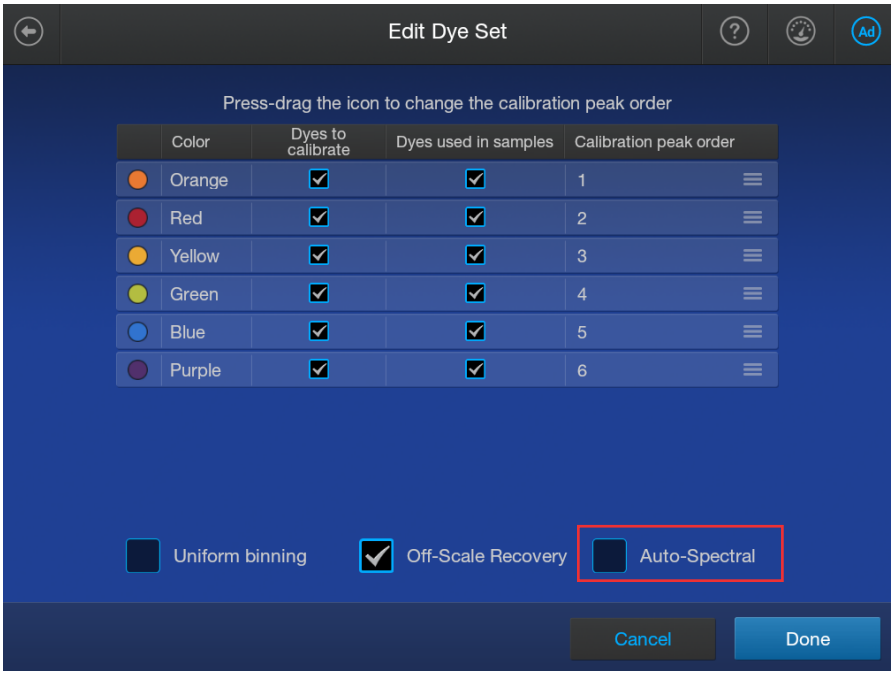
1. Create a custom dye set.
    a. Make a copy of the system **J6 (DS-36)** dye set, then modify the system-generated name as needed (e.g. ***J6_MSI***).

b. In the **Create Dye Set** screen, tap the dye selection pane.



The **Edit Dye Set** screen opens.

c. In the **Edit Dye Set** screen, de-select **Auto Spectral**.



d. Tap **Done**, then tap **Save**.

2. Prepare the instrument for spectral calibration as described in the *SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide* (Pub. No. 100104689).

3. Prepare the dye set calibration standards and plate as described in the *DS-36 Matrix Standard Kit (Dye Set J6) Product Information Sheet* (Pub. No. 4426042).

Perform spectral calibration

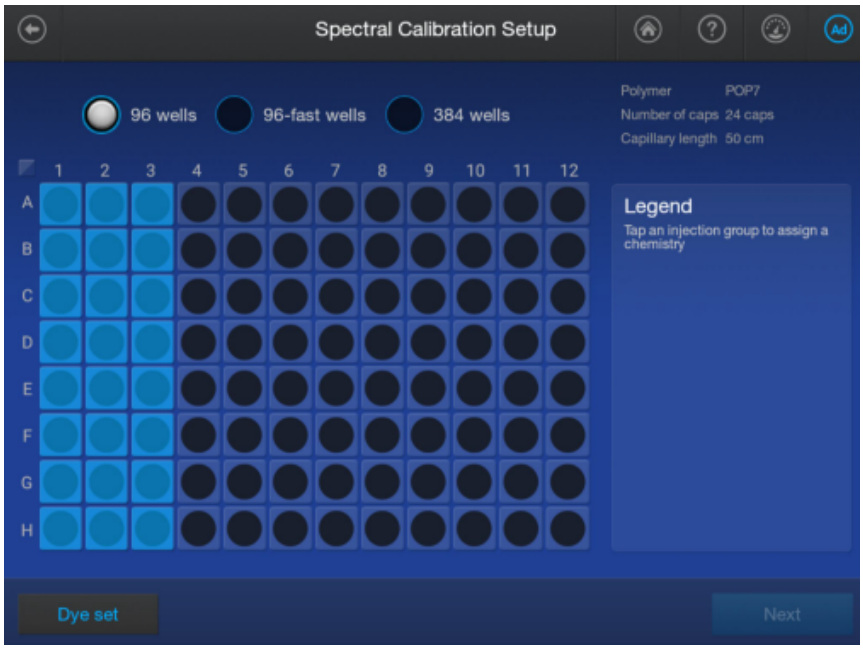
The spectral calibration takes approximately 33 minutes to complete (excluding the time it takes for the oven to preheat).

1. Load the calibration plate into the instrument.

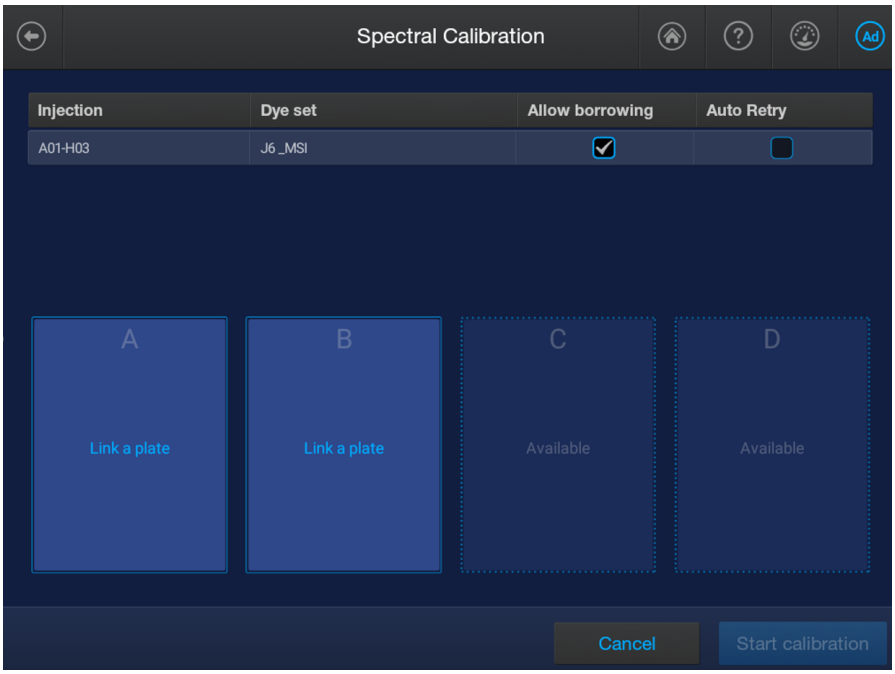
2. In the instrument home screen, tap ⋯ **Actions** ▸ **Maintenance** ▸ **Calibration** ▸ **Spectral Calibration**.

3. Tap the screen to select the plate type you are using for calibration.

4. Tap the injection group for the dye set in the plate, then tap **Dye set**.



5. In the **Dye Set for Chemistry Standard** screen, select the custom dye set that you created in step 1 on page 13 (e.g. **J6_MSI**), then tap **Next**.
   The home screen is displayed with capillary information sharing settings.



6. Tap the plate position that corresponds to the location of the spectral calibration plate in the instrument.

7. Tap **Start calibration**.
   The home screen is displayed. The status of the spectral calibration is shown in the appropriate plate position. The spectral calibration run is also listed in the **Run queue**.

When the spectral calibration finishes, the status changes to **Completed** in the home screen and the **Run queue**.

Create the file name convention

A file name convention specifies the naming convention for sample data files. The assay-specific file name convention can be specified in the injection properties when creating a plate file (see “Create a plate file” on page 19). For information about sample naming requirements for the TrueMark™ MSI Assay, see “Sample naming requirements for TrueMark™ MSI Analysis Software” on page 35.

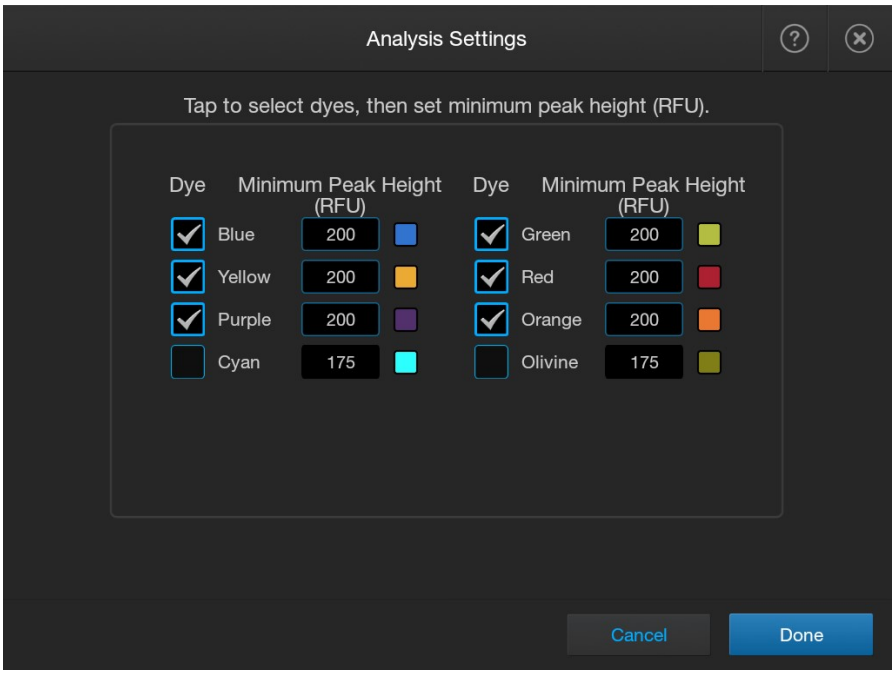
1. In the instrument home screen, tap ⋯ **Actions** ▸ **Library** ▸ **File name conventions**.
2. In the **Manage File Name Conventions** screen, tap the **Fragment_Default** name convention, then tap **Open**.
3. In the **View File Name Convention** screen, in the lower left corner of the screen, tap **Copy**.
4. In the **Copied File Name Convention Name** screen, enter the name for the TrueMark™ MSI Assay file name convention (e.g. ***Fragment_MSI***), then tap **Enter**.
5. Tap the **File name convention preview** pane, then tap **Attributes**.
6. In the **Edit File Name Convention** screen, select the **Sample name** attribute and any additional attributes as needed, then tap **Done**.
7. In the **Move attributes to rearrange the file format** screen, move the **Sample name** attribute to the top of the **Current attributes** list by press-dragging ≡ **(Move)**. If needed, rearrange any other attributes, making sure that the **Sample name** attribute remains at the top of the list.
8. Tap **Done**, then tap **Save**.

Create analysis settings

Analysis settings define the settings for peak detection and sizing. You can use the TrueMark™ MSI Assay analysis settings when you create a plate file (“Create a plate file” on page 19) or create a TrueMark™ MSI Assay specific injection protocol (see “Create the injection protocol” on page 18).

1. In the instrument home screen, tap ⋯ **Actions** ▸ **Library** ▸ **Analysis settings**.
   The **Manage Analysis Settings** screen opens.
2. In the **Analysis setting name** column, tap **Fragment_Default**, then click **Open**.
3. In the lower left corner of the screen, tap **Copy** to create a copy of the default analysis settings.
4. In the **Copied Analysis Settings Name** window, enter a name for the TrueMark™ MSI Assay analysis settings (e.g. ***Fragment_MSI***), then tap **Enter**.

5. Tap the **Peak amplitudes** pane to open the dye selection screen, de-select **Cyan** and **Olivine**, then in the **Minimum Peak Height (RFU)** field, enter **200** for each selected dye.



6. Click **Done**, then click **Save** to create the TrueMark™ MSI Assay analysis settings.

Create the injection protocol

An injection protocol contains the following parameters that control the instrument during data collection and the settings for analysis of the data.

- Dye set
- Run module
- Analysis settings
- Size standard

When creating a plate file for the TrueMark™ MSI Assay, you can select the injection protocol to auto-populate the parameters. For more information, see “Create a plate file” on page 19.

1. In the instrument home screen, tap ⋯ **Actions** ▸ **Library** ▸ **Injection protocols**.
2. In the **Manage Injection Protocols** screen, tap to select **Fragment_Protocol_50_POP7xl**, then tap **Open**.
3. In the lower left corner of the **View Injection Protocol** screen, tap **Copy**.
4. In the **Copied Injection Protocol Name** screen, enter the TrueMark™ MSI Assay injection protocol name (e.g. ***Fragment_Protocol_50_POP7xl_MSI***), then tap **Enter**.

5. In the **Create Injection Protocol** screen, select the following parameters.

Parameter	Selection
Application type	**Fragment**
Dye set	Select the custom dye set that you created in step 1 on page 13 (e.g. **J6_MSI**).
Run module	**FragmentAnalysis50_POP7xl**
Analysis settings	Select the analysis settings that you created in "Create analysis settings" on page 17 (e.g. **Fragment_MSI**).
Size standards	**GS600_LIZ(80-400)**

6. Tap **Done**, then tap **Save**.

Create a plate file

1. Create, copy, or import a plate file on the SeqStudio™ Flex Series Genetic Analyzer instrument or in the Plate Manager software, as described in the *SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide* (Pub. No. 100104689).

2. Enter or select the following plate and injection properties.

Property	Selection
Plate barcode	Enter the plate barcode, if available.
Sample name	Enter the names for samples according to the sample naming convention described in "Sample naming requirements for TrueMark™ MSI Analysis Software" on page 35.
Application type	**Fragment analysis**
Injection protocol	Select the injection protocol you created in "Create the injection protocol" on page 18 (e.g. **Fragment_Protocol_50_POP7xl_MSI**). **Note:** If you select the TrueMark™ MSI Assay specific injection protocol, **Dye set**, **Run module**, **Analysis settings**, and **Size standard** parameters are auto-populated in accordance with the injection protocol.
Dye set	Select the custom dye set that you created in step 1 on page 13 (e.g. **J6_MSI**).
Run module	**FragmentAnalysis50_POP7xl**
Analysis settings	Select the analysis settings that you created in "Create analysis settings" on page 17 (e.g. **Fragment_MSI**).
Size standard	**GS600_LIZ_(80-400)**
File name convention	Select the file name convention that you created in "Create the file name convention" on page 17 (e.g. **Fragment_MSI**).

3. Save the plate file, then proceed to Chapter 3, "Prepare and run the samples".

SeqStudio™ Genetic Analyzer

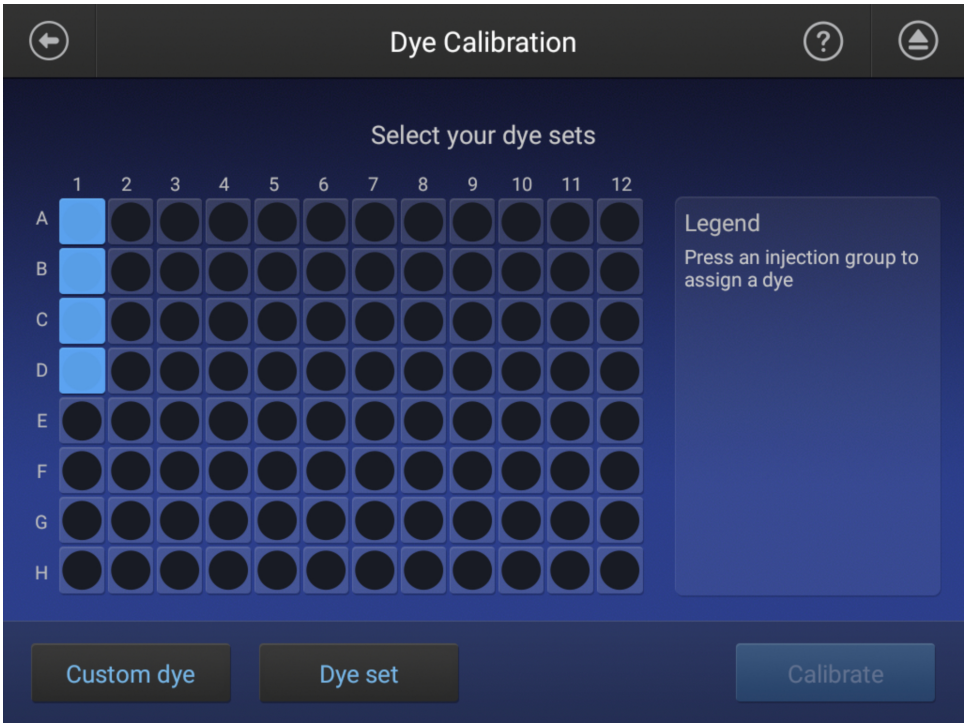
For detailed instructions about how to prepare the SeqStudio™ Genetic Analyzer for capillary electrophoresis, see the *SeqStudio™ Genetic Analyzer Instrument and Software User Guide* (Pub. No. MAN0018646).

Perform a system dye calibration

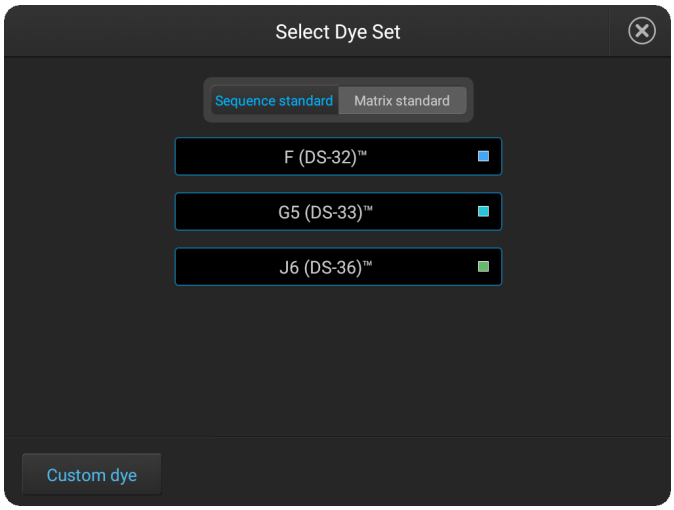
A system dye calibration requires ~30 minutes to complete.

Prepare the dye set calibration standards and plate as described in the *DS-36 Matrix Standard Kit (Dye Set J6) Product Information Sheet* (Pub. No. 4426052).

1. In the home screen, tap **Settings** ▸ **Maintenance and Service** ▸ **Calibration** ▸ **Dye Calibration**.
2. Touch the injection group for the dye set in the plate, then tap **Dye set**.



3. Tap **Matrix Standard**, then select a system dye calibration standard provided with the instrument.



4. Tap **Calibrate**.

   The calibration run starts.

IMPORTANT! If the dye calibration fails:

- The results of the calibration are not saved, and the calibration plate is not moved to **Run History**.
- The instrument does not allow you to rerun the plate setup for a failed calibration. Close the calibration screen, then start a new calibration.

Spectral Quality Value

A spectral Quality Value reflects the confidence that the individual dye emission signals can be separated from the overall measured fluorescence signal. It is a measure of the consistency between the final matrix and the data from which it was computed. A Quality Value of 1.0 indicates high consistency, providing an ideal matrix with no detected pull-up/pull-down peaks.

In rare cases, a high Quality Value can be computed for a poor matrix. This can happen if the matrix standard contains artifacts, leading to the creation of one or more extra peaks. The extra peaks cause the true dye peak to be missed by the algorithm, and can lead to a higher Quality Value than would be computed with the correct peak. Therefore, it is important to visually inspect the spectral calibration profile for each capillary.

Condition number

A Condition Number indicates the amount of overlap between the dye peaks in the fluorescence emission spectra of the dyes in the dye set.

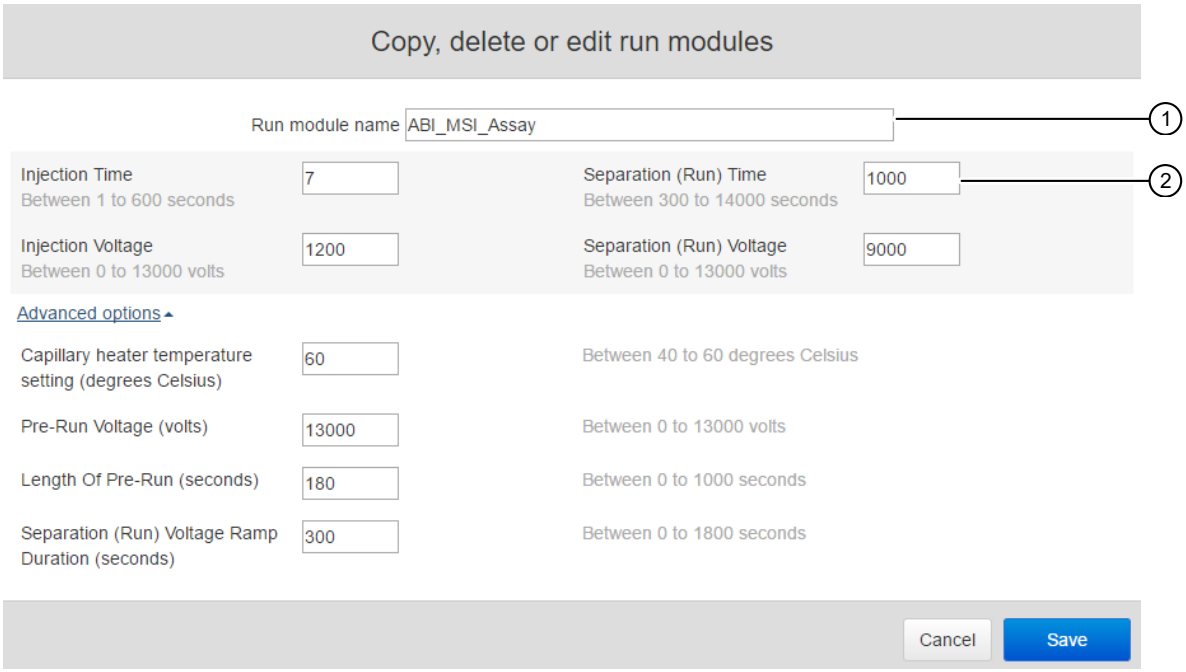
If there is no overlap in a dye set, the Condition Number is 1.0 (ideal conditions), the lowest possible value. The condition number increases with increasing peak overlap.

The ranges that the software uses to determine if a capillary passes or fails are:

Dye Set	Quality Value Minimum	Condition Number Maximum
J6	0.95	8.0

Create the TrueMark™ MSI Assay run module

1. In the SeqStudio™ Plate Manager, open a new or existing plate, then navigate to the **Plate** tab.
2. Click **Actions** ▸ **Manage run modules** from the top right corner of the screen.
3. Select the default **FragAnalysis** run module, then click **Copy**.
4. Enter the **Run module name**—**ABI_MSI_Assay**
5. Change the **Separation (Run) Time** to **1,000** seconds.



① Run module name
② Separation (Run)Time

6. Click **Save**.

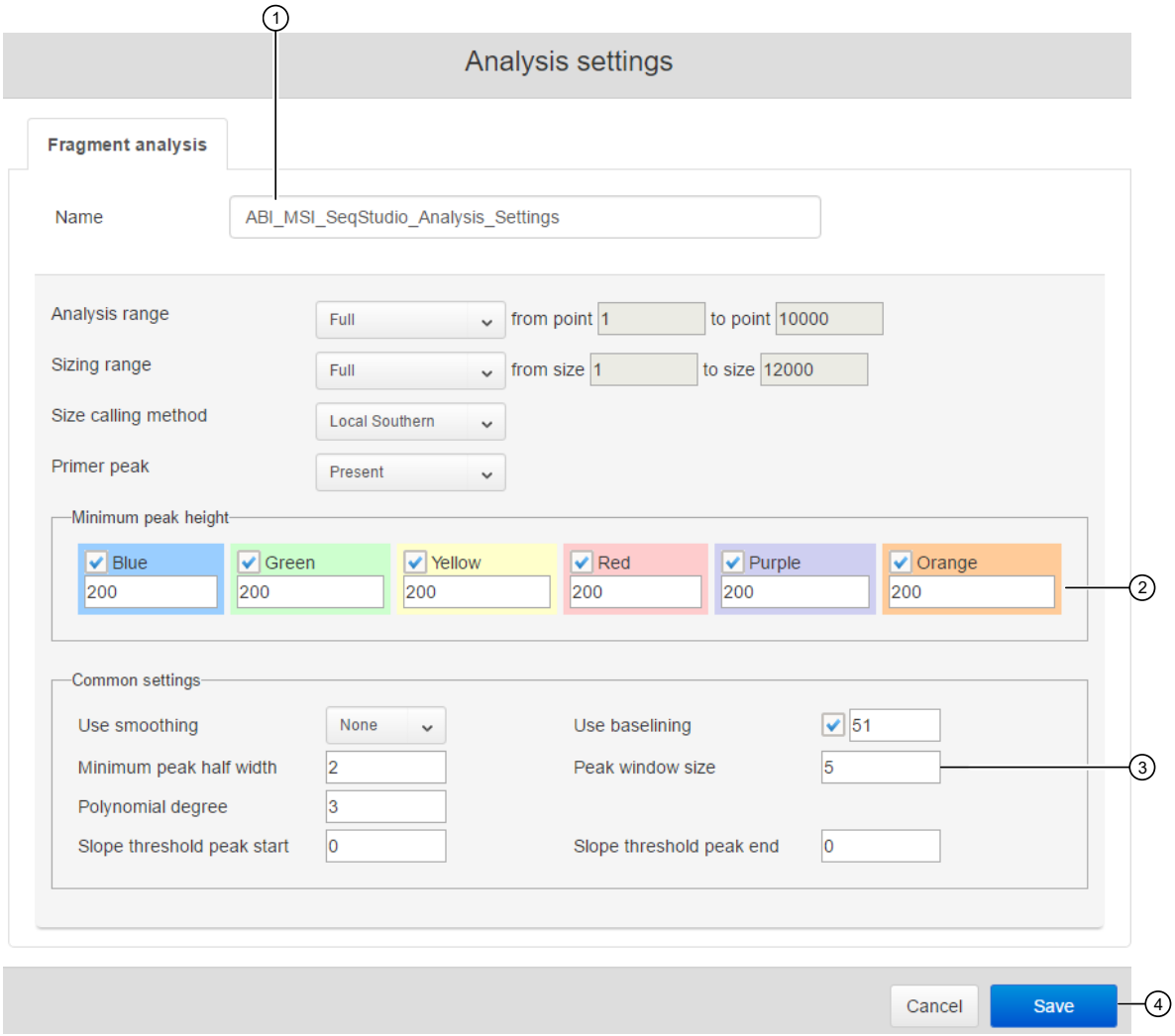
Create the TrueMark™ MSI Assay Size Standards

1. In the SeqStudio™ Plate Manager, open a new or existing plate, then navigate to the **Plate** tab.
2. Click **Actions** ▸ **Manage size standards** from the top right corner of the screen.
3. Select the 80-400 sizes, then save with the name **GS600_LIZ_(80-400)**.

Create a new plate setup

1. Open the SeqStudio™ Plate Manager and select **New**.
2. Enter or accept the default **Plate name**, then click **Next**.
3. Enter the plate properties.
   a. Select **Fragment analysis** as the **Application type**.
   b. Click **Setting Details**, then click **Copy** to make a copy of the default settings to edit.
   c. In the Analysis Settings window, enter a unique **Name** for the MSI analysis settings.
   d. In the Analysis Settings window, change all minimum peak heights to **200**.

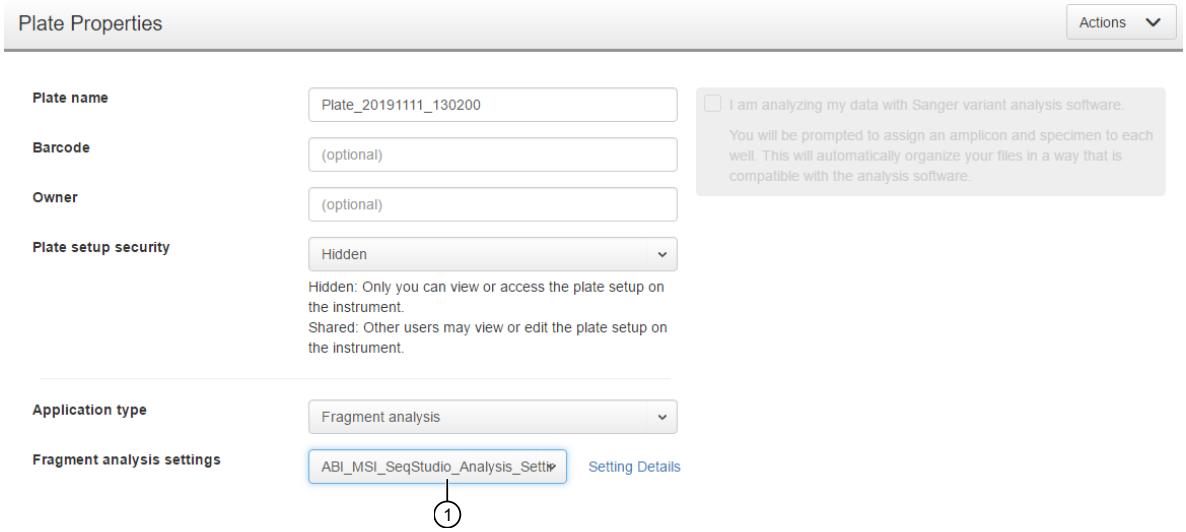
e. In the Analysis Settings window, change the Peak window size to **5**.



① **Name** for the Analysis settings.
② **Minimum peak height**
③ **Peak window size**
④ **Save** button

4. Click **Save** to save the **Analysis Settings**, then **Close**.

5. Ensure that the new **Fragment analysis settings** are selected, then click **Next** to proceed to the Plate tab.



① MSI fragment analysis settings

6. In the Plate tab, select:
   - **Size standard—GS600_LIZ_(80-400)**
   - **Dye set—J6 (DS-36)™**
   - **Run module 1—ABI_MSI_Assay**

   Note: For information on creating a run module, see “Create the TrueMark™ MSI Assay run module” on page 22.

7. Click **Next**.

8. In the **Save the plate setup** window:
   a. Enter or accept the default **Plate name**.
   b. Ensure that the **ABI_MSI_SeqStudio_Analysis_Settings** is selected as the **Fragment analysis settings**.
   c. Click **Save**.

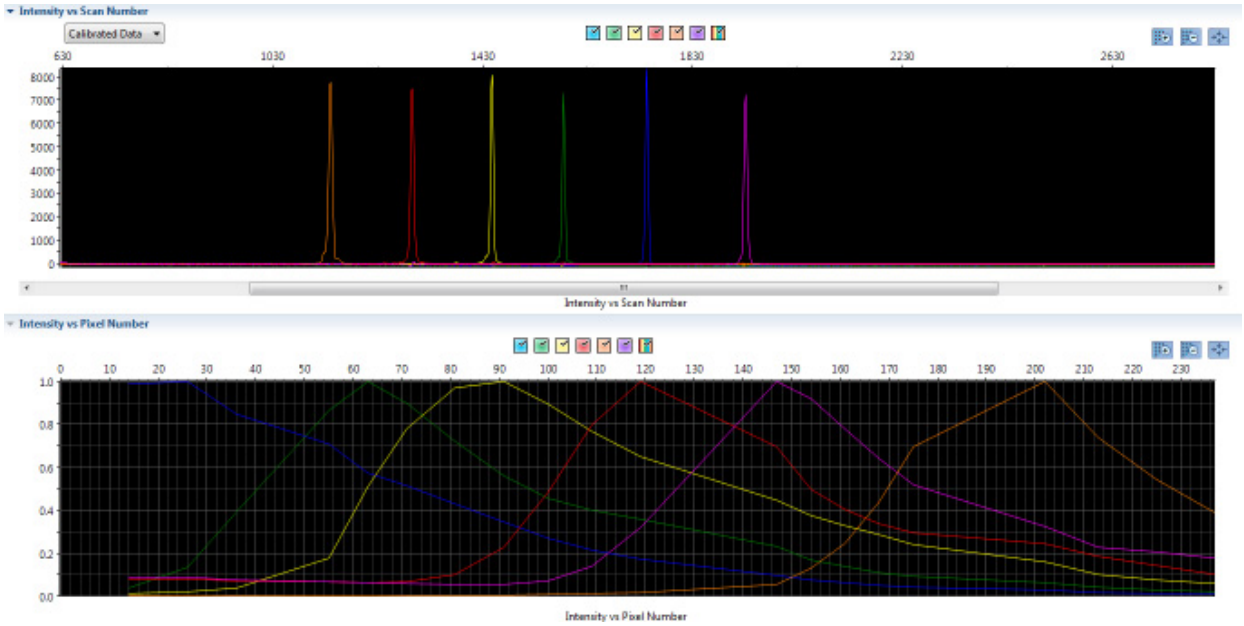
3500/3500xL Genetic Analyzer

For detailed instructions about how to prepare the 3500/3500xL Genetic Analyzer for capillary electrophoresis, see the *3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v3.3 User Guide* (Pub. No. 100079380).

Perform spectral calibration

Perform a spectral calibration using the DS-36 Matrix Standard Kit (Dye Set J6) (Cat. No. 4425042).

The following figure is an example of a passing 6-dye spectral calibration.



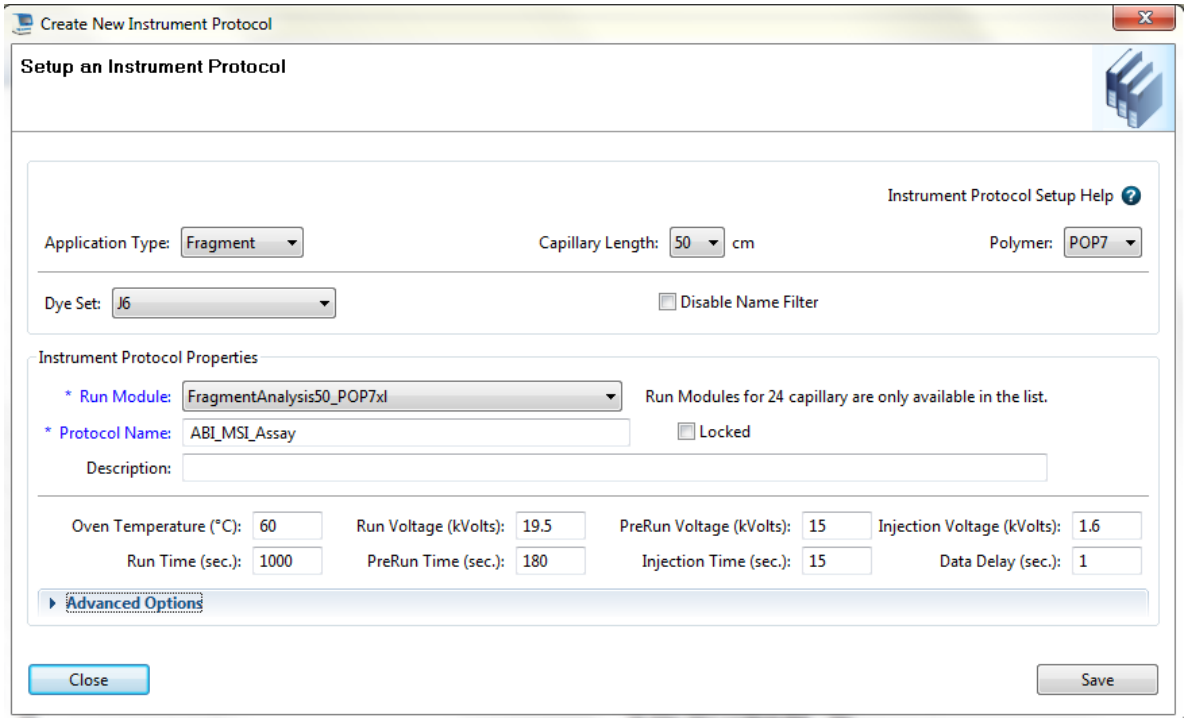
Electrophoresis software setup

(Recommended) Ensure that your genetic analyzer is running on the Windows™ operating system with 3500 Series Data Collection Software v3 or later.

The following instructions cover setting up an assay (ABI_MSI_Assay), instrument protocol (ABI_MSI), and size calling protocol (GS600[80-400]). For more detailed information about the instrument procedures, see the instrument user guide (Appendix D, “Documentation and support”).

Create an instrument protocol

1. Navigate to the **Instrument Protocols** library.
2. Click **New**.
3. Specify the settings listed below.



Setting	Value
Capillary length	50 cm
Polymer	POP7
Dye Set	J6
Run Module	FragmentAnalysis50_POP7xl
Protocol Name	ABI_MSI
Oven Temperature (°C)	60
Run Time (sec)	1,000
Run Voltage (kVolts)	19.5
PreRun Time (sec)	180
PreRun Voltage (kVolts)	15
Injection Time (sec)	15

(continued)

Setting	Value
Injection Voltage (kVolts)	1.6
Data Delay (sec)	1

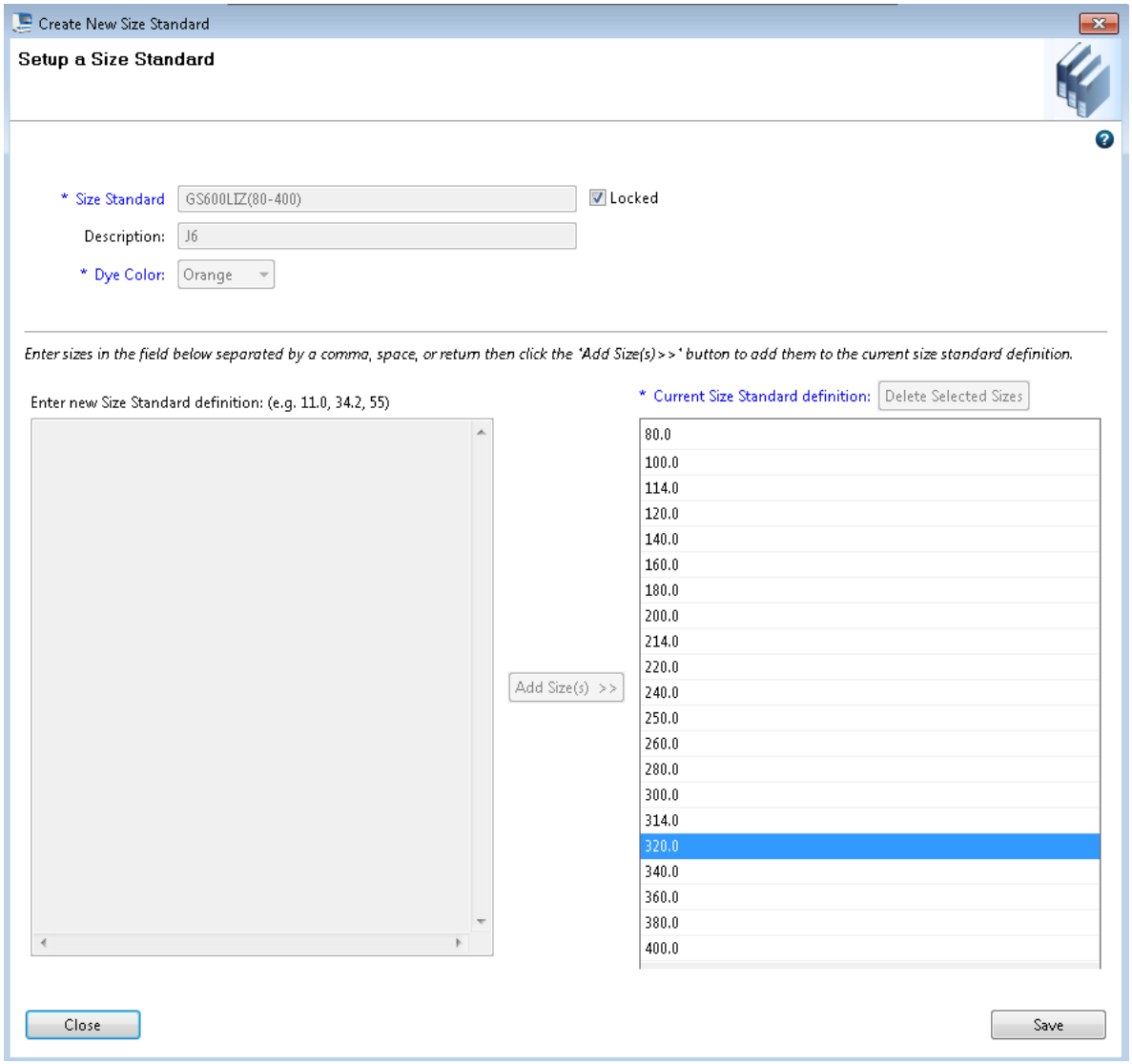
4. Click **Save**.

Create the size standard

A size standard defines the sizes of known fragments. It is used to generate a standard curve. The standard curve is used to determine the sizing of unknown samples. When you create a size calling (fragment analysis) protocol, you add a size standard to the protocol.

1. Navigate to the **Size Standards** library.
2. Click **New**.

3. Specify the settings listed below.

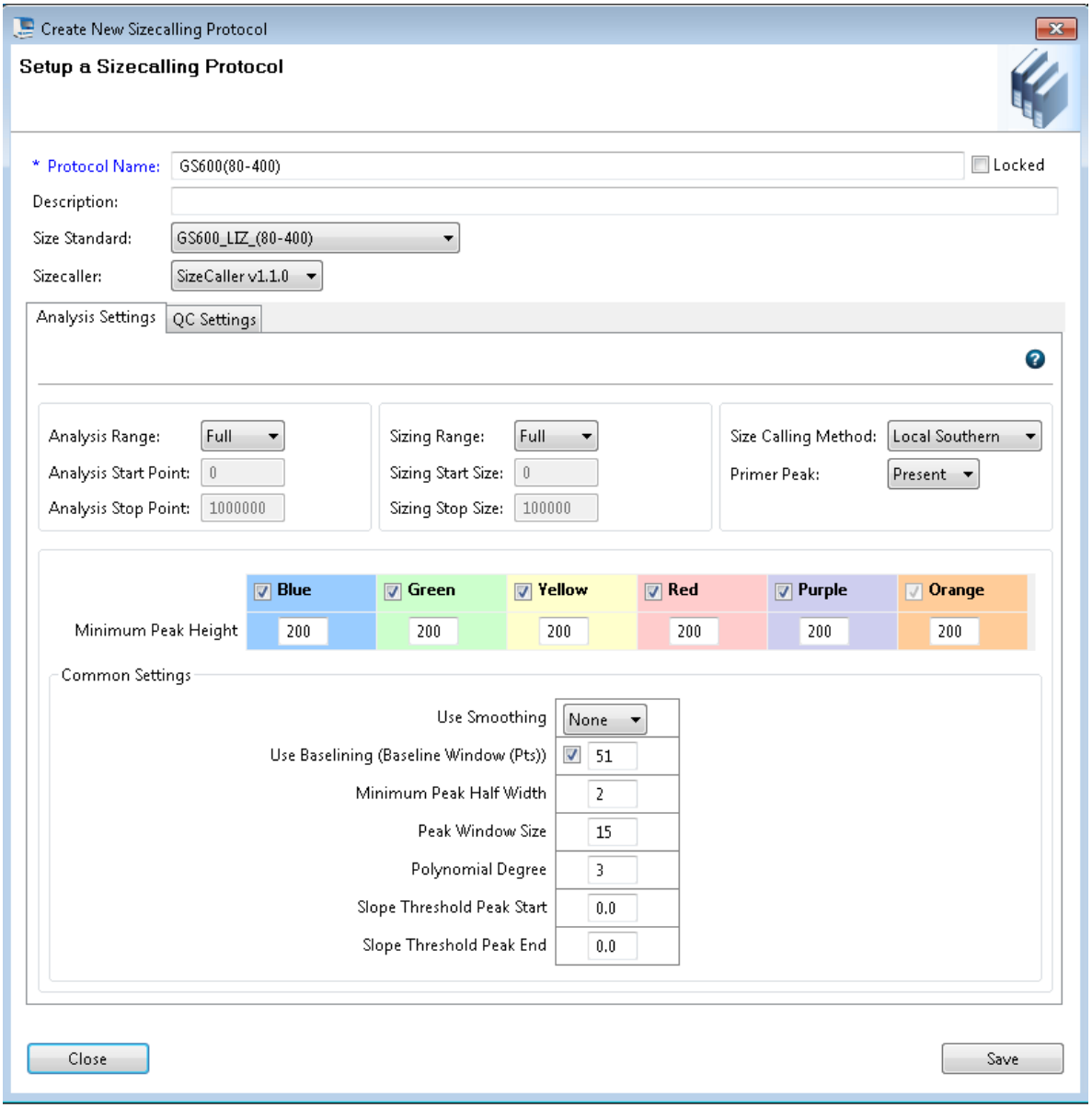


Setting	Value
Size Standard name	GS600LIZ (80-400); select **Locked**
Description	J6
Dye Color	Orange
Size Standards (list in left text box)	80.0, 100.0, 114.0, 120.0, 140.0, 160.0, 180.0, 200.0, 214.0, 220.0, 240.0, 250.0, 260.0, 280.0, 300.0, 314.0, 320.0, 340.0, 360.0, 380.0, and 400.0

4. Click **Save**.

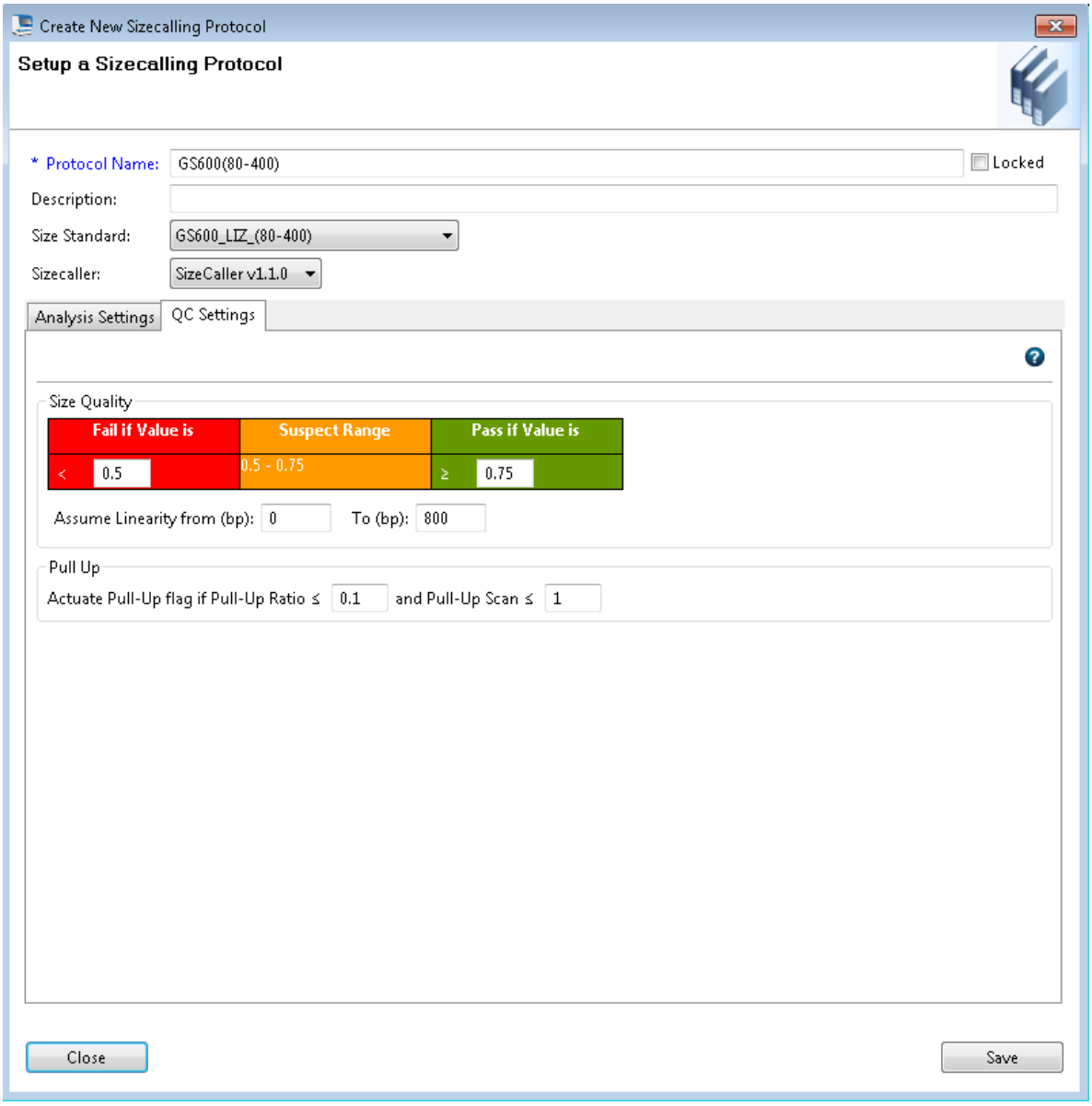
Create the size calling protocol

1. Navigate to the **Sizecalling Protocols** library.
2. Click **New**.
3. Specify the settings listed below.



Setting	Value
Sizecaller	SizeCaller v1.1.0
Analysis Range and Sizing Range	Full
Size Calling Method	Local Southern
Primer Peak	Present
Minimum Peak Height	200
Use Smoothing	None
Use Baselining (Baseline Window) (Pts)	51
Minimum Peak Half Width	2
Peak Window Size	15
Polynomial Degree	3
Slope Threshold Peak Start/End	0.0 (both)

4. Click the **QC Settings** tab, and specify the settings listed below.



Setting	Value
Fail if Value is	<0.5
Suspect Range	0.5–0.75
Pass if Value is	≥0.75
Assume Linearity from (bp) **To (bp)**	0 800
Actuate Pull-Up flag if Pull-Up Ratio and **Pull-Up Scan**	≤0.1 ≤1

5. Click **Save**.

IMPORTANT! Normalization is not applied to samples with Size Quality flags. Specify analysis settings that accurately detect and size the size standard, and QC settings with appropriate pass fail ranges. The 3500 Series Data Collection Software does not support re-analyzing data with new settings.

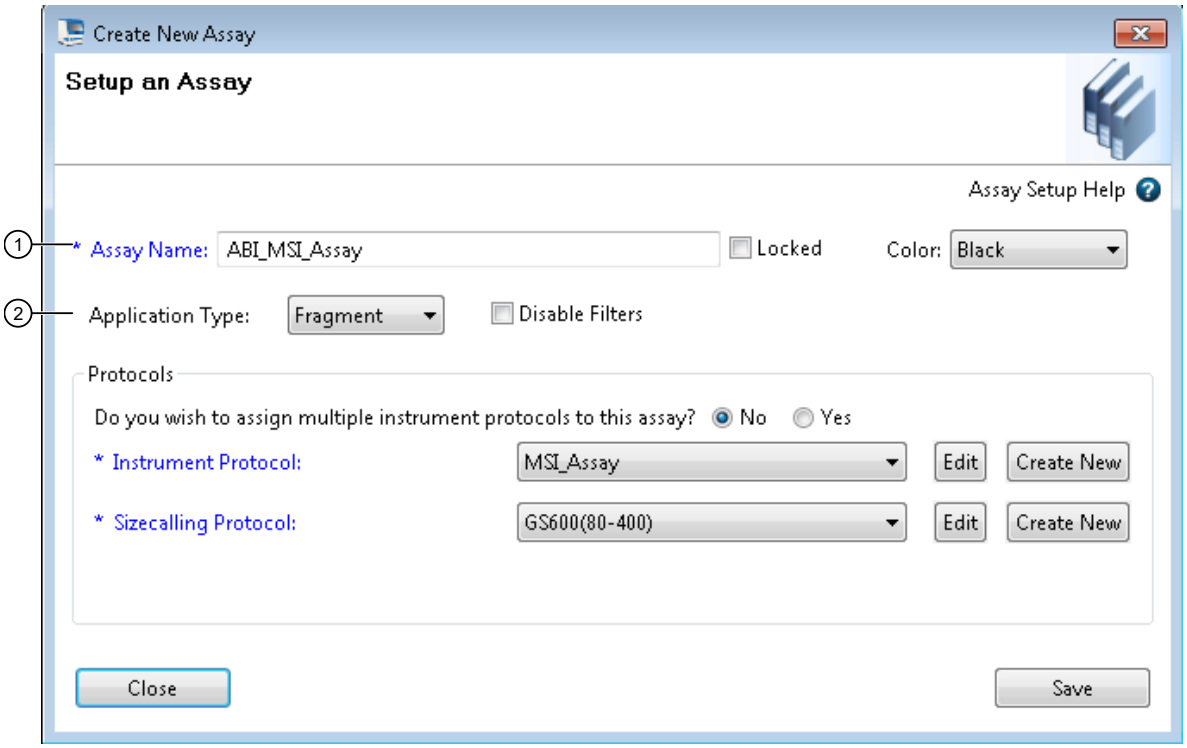
Create the assay

1. Navigate to the **Assays** library.

   The list of items in the library may be filtered based on the library filtering user preference. Click **Disable Filters** to show all items in the list.

2. Click **New**.

3. Specify the settings.



① **Assay Name**—ABI_MSI_Assay
② **Application Type**—Fragment

Setting	Value
Assay Name	ABI_MSI_Assay
Color	Black
Application Type	Fragment
Do you wish to assign multiple protocols to this assay?	No
Instrument Protocol (select from the dropdown list)	ABI_MSI
Sizecalling Protocol (select from the dropdown list)	GS600(80-400)

4. Click **Save**.

3 Prepare and run the samples

Sample naming requirements for TrueMark™ MSI Analysis Software

To be successfully imported into the TrueMark™ MSI Analysis Software, the sample file (FSA) names must follow the correct naming conventions.

In the following examples, "SpecimenID" becomes the main name for identifying the specimen within the software and exports. SpecimenID text cannot contain an underscore (_), because only the text before the first underscore is imported as the specimen ID.

Paired samples convention:

- SpecimenID_T _*.fsa (tumor tissue sample)
- SpecimenID_N _*.fsa (normal adjacent tissue sample from same individual)

Sample file (FSA) names must meet the following conventions.

Sample type	Sample naming conventions	Guidelines
Specimen	For paired samples, the naming convention is: • `<SpecimenID>`_T _<*>.fsa—Tumor tissue sample • `<SpecimenID>`_N _<*>.fsa—Normal tissue sample from the same individual, adjacent to the Tumor tissue sample where: • `<SpecimenID>` is user-defined, but is identical in the Normal (**N**) and Tumor (**T**) tissue samples • `<*>` is user-defined	• The `<SpecimenID>` prefix identifies the specimen within the TrueMark™ MSI Analysis Software and exported file names. Ensure that the `<SpecimenID>` text does not include an underscore (_). • Within a batch, each `<SpecimenID>` must be unique, unless two files will be analyzed as a Tumor-Normal sample pair. If `<SpecimenID>` duplicates are detected, the software imports only the last file, in alphanumeric sort order. For example, if the files are named "SpecimenIDBlue_aaa.fsa" and "SpecimenIDBlue_zzz.fsa", the software imports only "SpecimenIDBlue_zzz.fsa".

(continued)

Sample type	Sample naming conventions	Guidelines
Specimen	For unpaired samples, the naming convention is: <SpecimenID>_<*>.fsa where: <SpecimenID> and <*> are user-defined	• **IMPORTANT!** The TrueMark™ MSI Analysis Software will not import a <SpecimenID>_N_<*>.fsa file if there is no matching <SpecimenID>_T_<*>.fsa file to import. However, a <SpecimenID>_T_<*>.fsa file will be imported even if there is no <SpecimenID>_N_<*>.fsa file to import. • **IMPORTANT!** If you are running replicate reactions on the same plate, assign the replicates a unique <SpecimenID> before the **_T** or **_N** to ensure that the replicates are processed correctly within the software. For example: – "Spec1.rep1_T" and "Spec1.rep1_N" – "Spec1.rep2_T" and "Spec1.rep2_N"
Negative control	NEG<*>.fsa where: <*> is user-defined **Note:** The TrueMark™ MSI Assay Amplification Control sample file name must begin with "NEG" to be properly analyzed. For example, "NEGAmp.1_A12_daytimestamp.fsa".	File names that begin with "NEG" are analyzed as negative control samples. The TrueMark™ MSI Analysis Software displays a ⚠ **(Warning)** Review Flag if it assigns an **Unstable** call to any reportable marker for a negative control sample.
No template control	NTC<*>.fsa where: <*> is user-defined	File names that begin with "NTC" are no template control samples. The TrueMark™ MSI Analysis Software displays a ⚠ **(Warning)** Review Flag if it assigns a call other than **No Call** to any reportable marker for a no template control sample.

Table 6 Examples of files that will or will not import

Files selected for import	Import result
20190917.plate1.tst123_T_A01_datetime.fsa	Tumor/Normal pair of files imported as <SpecimenID> "20190917.plate1.tst123"
20190917.plate1.tst123_N_A02_datetime.fsa	
20190924.plate1.sample1.tumor_T_A02.fsa	Unpaired Tumor file imported as <SpecimenID> "20190924.plate1.sample1.tumor"
20190924.plate1.sample1.normal_N_A01.fsa	Unpaired Normal file is not imported
20190924.plate1.sample1.normal_A01.fsa	Unpaired file imported as <SpecimenID> "20190924.plate1.sample1.normal"

Table 6 Examples of files that will or will not import *(continued)*

Files selected for import	Import result
specimen1_injection1.fsa	Both files have the same `<SpecimenID>`, "specimen1". The software imports only the last file, in alphanumeric sort order. In this example, the software imports only "specimen1_injection2.fsa".
specimen1_injection2.fsa	
specimen2_something.fsa, from the 3500/3500xL Genetic Analyzer	Both files imported as `<SpecimenIDs>` "specimen2" and "specimen3", because each `<SpecimenID>` is unique. A single batch can contain specimens from different plates and instrument types.
specimen3_something.fsa, from the SeqStudio™ Genetic Analyzer	
specimen4_T_something.fsa, from the 3500/3500xL Genetic Analyzer	Neither file imported, because Tumor/Normal pair of files from different instrument types is not supported.
specimen4_N_something.fsa, from the SeqStudio™ Genetic Analyzer	
HiDi_something.fsa	Files with HiDi prefix in filename are rejected

Note: Files are resized upon import. The failure of one file to meet the sizing quality threshold may prevent any file in the batch from being imported. Open the **Import Manager** for more information on the files reporting errors.

DNA sample preparation guidelines

The TrueMark™ MSI Assay accepts DNA that has been extracted by various methods. This workflow has been tested on fixed formalin paraffin-embedded (FFPE) DNA that was extracted using the RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Cat. No. AM1975). DNA inputs of 2–5 ng are recommended to optimize results, and to minimize offscale signals.

All protocols should be optimized with your standard laboratory procedures. For more information, see the *RecoverAll™ Total Nucleic Acid Isolation Kit Protocol* (Pub. No. 1975M).

Set up the PCR reactions

IMPORTANT! Perform all steps on ice.

Thaw the TrueMark™ MSI Assay Primer Mix, TrueMark™ MSI Assay Amplification Control, and TrueMark™ MSI Assay No-Template Control on ice. Thoroughly mix the components by vortexing for 3–5 seconds, then centrifuge for 3–5 seconds before use.

Table 7 Recommended contents per 10 µL reaction

Component	Amount
TrueMark™ MSI Assay Master Mix	4 µL
TrueMark™ MSI Assay Primer Mix	1 µL
DNA	2–5 ng[1]
TrueMark™ MSI Assay No-Template Control	To a final reaction volume of 10 µL

[1] **IMPORTANT!** Tumor and normal sample pairs should have similar amounts of starting DNA in the PCR reactions to minimize PCR artifacts.

1. On ice, prepare sufficient PCR reaction mix for the required number of reactions plus 1 additional reaction for overage.

Table 8 Example reaction mix (1ng/µL DNA)

Component	Volume per reaction	Volume (12 reactions)
TrueMark™ MSI Assay Master Mix	4 µL	48 µL
TrueMark™ MSI Assay Primer Mix	1 µL	12 µL
TrueMark™ MSI Assay No-Template Control	3 µL	36 µL
Total volume	**8 µL**	**96 µL**

2. To the labeled reaction plate, add the following components.
   a. Add 8 µL of PCR reaction mix to each sample, amplification control, or no template control (NTC) well.
   b. Add 2 µL of sample DNA (1 ng/µL) to the sample wells.
   c. Add 2 µL of TrueMark™ MSI Assay No-Template Control to NTC wells.
   d. Add 1 µL of TrueMark™ MSI Assay Amplification Control and 1 µL of TrueMark™ MSI Assay No-Template Control to the amplification control wells.

Note: Input DNA quantity and quality affect fragment analysis results.

3. Cover the plate with adhesive film, then centrifuge for 3–5 seconds to bring the mixture to the bottom of the tube and eliminate air bubbles.

Immediately proceed to “Run the PCR”.

Run the PCR

1. Program the thermal cycling conditions.

   IMPORTANT! If you are using the ProFlex™ 96-well PCR System, select the GeneAmp™ PCR System 9700 simulation mode (**Edit ▸ Manage Steps ▸ Advanced Options ▸ Simulation Mode**).

Step	Temperature	Time	Cycles
Hot start	95°C	11 minutes	1
Denature	94°C	20 seconds	29
Anneal/ Extend	59°C	2 minutes	
Final extension	60°C	25 minutes	1
Hold	4°C	∞	

2. Set the reaction volume to 10 µL, then load the plate into the thermal cycler.
3. Close the heated cover, then start the run.
4. When the run is complete, remove the plate from the thermal cycler.

   IMPORTANT! Protect the amplified DNA from light.

Amplified DNA can be stored at 2°C to 8°C for up to 2 weeks, or long term at –25°C to –15°C.

Prepare samples for capillary electrophoresis

Prepare the samples for electrophoresis immediately before loading.

1. Prepare the mix of Hi-Di™ Formamide and GeneScan™ 600 LIZ™ Size Standard v2.0 for the required number of reactions plus 1 additional reaction for overage.

Component	Volume per reaction	Volume (12 reactions)
GeneScan™ 600 LIZ™ Size Standard v2.0	1 µL	12 µL
Hi-Di™ Formamide	17 µL	204 µL
Total volume	**18 µL**	**216 µL**

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

2. Thoroughly mix the components by vortexing 3–5 seconds, then centrifuge 3–5 seconds before use.

3. Prepare the fragment analysis reactions. To a MicroAmp™ Optical 96-Well Reaction Plate, add the following components.

Component	Volume per reaction	Volume (12 reactions)
Hi-Di™ Formamide and GeneScan™ 600 LIZ™ Size Standard v2.0 mix	18 µL	216 µL
PCR product (see "Run the PCR" on page 39)	2 µL	24 µL
Total volume	**20 µL**	**240 µL**

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

	1	2	3	4	5	6	7	8	9	10	11	12
A	NEG	Specimen7_T	Hi-Di									
B	NTC	Specimen7_N	Hi-Di									
C	Specimen1_T	Specimen8_T	Hi-Di									
D	Specimen2_T	Specimen8_N	Hi-Di									
E	Specimen3_T	Specimen9_T	Hi-Di									
F	Specimen4	Specimen9_N	Hi-Di									
G	Specimen5	Specimen10_T	Hi-Di									
H	Specimen6	Specimen10_N	Hi-Di									
	Injection 1			Injection 2			Injection 3			Injection 4		

Figure 2 Example 3500xL Genetic Analyzer plate layout

4. Seal the reaction plate with MicroAmp™ Clear Adhesive Film.
5. Thoroughly mix the components by vortexing 3–5 seconds, then centrifuge 10–20 seconds before use.
6. Denature the DNA fragments:
   a. Incubate the mixture at 95°C for 3 minutes.
   b. Incubate the mixture at 4°C, or on ice, for 2 minutes.
7. Centrifuge the plate for 10–20 seconds to ensure that all sample mixtures are at the bottom of the wells.
8. Remove the MicroAmp™ Clear Adhesive Film, then seal the plate with a septa.
9. Assemble the plate with the retainer and base, then load onto the capillary electrophoresis instrument.

   Reactions can be run on the SeqStudio™ Flex Series Genetic Analyzer, SeqStudio™ Genetic Analyzer, or 3500/3500xL Genetic Analyzer.

For details on setting up the run, see the user guide for your instrument ("Related documentation" on page 76).

4 Analyze the data with the TrueMark™ MSI Analysis Software

For information on data analysis or troubleshooting with GeneMapper™ Software, see the *TrueMark™ MSI Assay User Guide* (Pub. No. MAN0018868).

Software access restrictions

- The TrueMark™ MSI Analysis Software allows up to five users to be signed in simultaneously. When that limit is reached, no other users are allowed to sign in.
- Your access to functions in the software is based on the permissions associated with your user account. For more information, see the *TrueMark™ MSI Analysis Software User Guide* (Pub. No. MAN0018874).
- If your system is configured for password expiration, you will be periodically prompted to change your password.
- If your system is configured to monitor failed log in attempts, you will be locked out of the software if you incorrectly enter your user name or password more than the specified number of times.

Sign in to the TrueMark™ MSI Analysis Software (all users)

1. *(First sign-in only)* Obtain your user name and password from your TrueMark™ MSI Analysis Software Administrator.

2. On the computer desktop, double-click **(MSI Client)** to start the client.

   IMPORTANT! The MSI Server must already be running on the computer so that the client can connect to the server. The server is configured to automatically start whenever the computer is started.

   If you get a connection error when you start the client, you may need to manually start the server. Try each of the following actions to resolve the connection error; perform the actions in the order listed.

   1. On the computer desktop, double-click **(MSI Server)**.
   2. On the computer desktop, right-click **(MSI Server)**, then select **Run as administrator**.
   3. See the *TrueMark™ MSI Analysis Software User Guide* (Pub. No. MAN0018874).

3. Enter your **User Name** and **Password**, then click **Log in**.

4. *(The first time that you sign in to a new MSI Client)* Accept the End User License Agreement to continue.

The first time that you sign in, the **Home** screen is empty. To begin using the software, you must import sample files (FSA). See “Import sample files” on page 42.

Import sample files

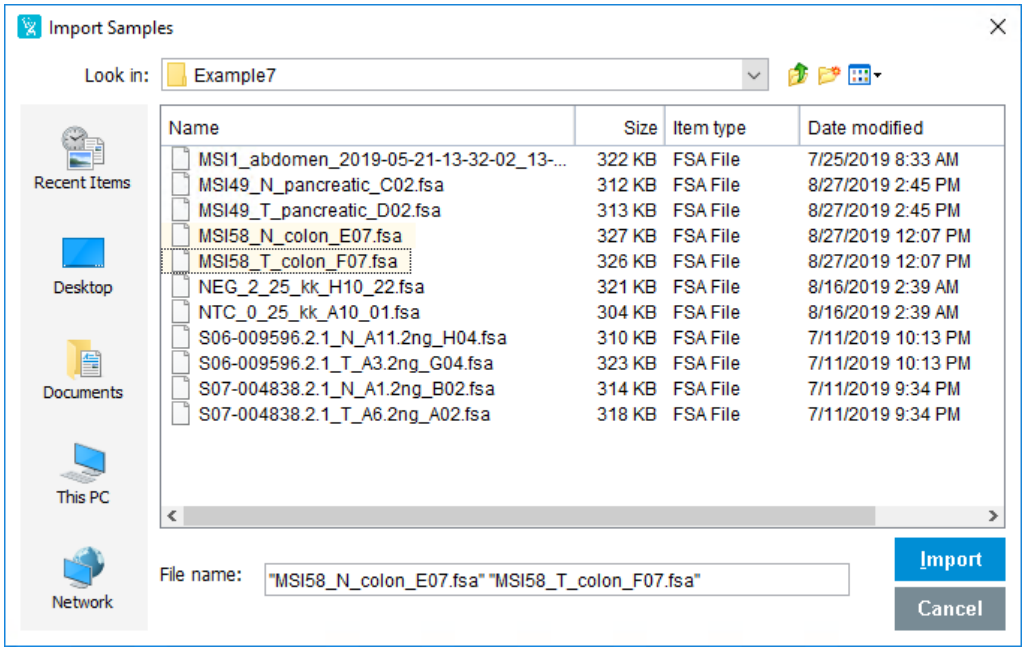
1. In the **Home** screen toolbar, click **Import Samples**.

2. Navigate to and select the sample files (FSA) to import.

   You can select a folder to import all sample files in the folder, or you can select individual sample files. If you select individual sample files, ensure that you select the normal (**N**) and tumor (**T**) sample files for paired samples.

   IMPORTANT! We recommend that you group sample files from different injections or plates in separate folders. If you import tumor and normal sample files that have the same `<SampleID>` but are from different injections or plates into the same batch, the software may pair the samples, which increases the risk of a miscall (false positive).

WARNING! Do not import more than 96 sample files in a single import operation. Importing more than 96 sample files may cause some or all specimens in the batch to be miscalled.



The default location is:

`<installation drive>\Applied Biosystems\MSI Client\User Files\Import`

Note: To change the default save location, see the *TrueMark™ MSI Analysis Software User Guide* (Pub. No. MAN0018874).

3. Click **Import**.

4. In the **Batch Information** dialog box, enter a **Batch Name**, *(optional)* enter the **Instrument ID** and **Operator**, then click **Save**.

Note: It may take several minutes to complete the import process.

The TrueMark™ MSI Analysis Software automatically analyzes the samples at import, using the current analysis settings. The results are displayed in the **Home** screen.

To change the analysis settings, see the *TrueMark™ MSI Analysis Software User Guide* (Pub. No. MAN0018874).

IMPORTANT! Changes to the analysis settings apply only to batches that are imported after you save the changes. To reanalyze samples with new analysis settings, import the samples again into a new batch. Changes do not affect existing batches.

Check for import errors

1. In the **Batches** pane of the **Home** screen, check that the number of imported specimens reflects the number of sample files (FSA) that you selected for import. Each specimen that has paired samples will have two sample files.

2. In the **Home** screen toolbar, check for a warning symbol ⚠ on the **Import Manager** button.

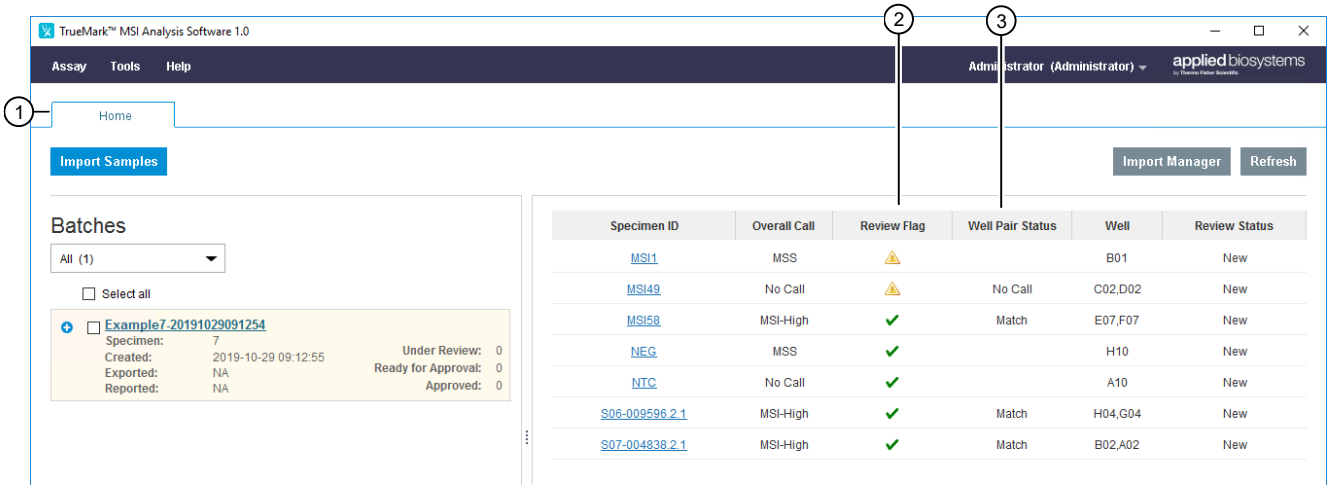
3. If the number of specimens and sample files do not agree, or if ⚠ appears on the **Import Manager** button, see the *TrueMark™ MSI Analysis Software User Guide* (Pub. No. MAN0018874).

IMPORTANT! We recommend that you also check the electropherogram plot title to confirm that the expected sample files were selected for each specimen (see *TrueMark™ MSI Analysis Software User Guide* (Pub. No. MAN0018874)). If you import tumor and normal sample files that have the same `<SampleID>` but are from different injections or plates into the same batch, the software may pair the samples, which increases the risk of a miscall (false positive).

Perform an initial QC of the imported data

1. In the **Home** tab, look for any specimens that display **Review Flag**.

2. In the **Home** tab, check the **Well Pair Status**. This column displays **Match** if the HID markers in the specimen both have similar fragment sizes between the tumor and normal files, indicating the samples likely belong to the same specimen.



① **Home** tab
② Review Flag
③ Well Pair Status

For more detailed information see the *TrueMark™ MSI Analysis Software User Guide* (Pub. No. MAN0018874).

View and interpret the results

Open the **Batch Summary** tab. The **Batch Summary** is populated when data import is complete.

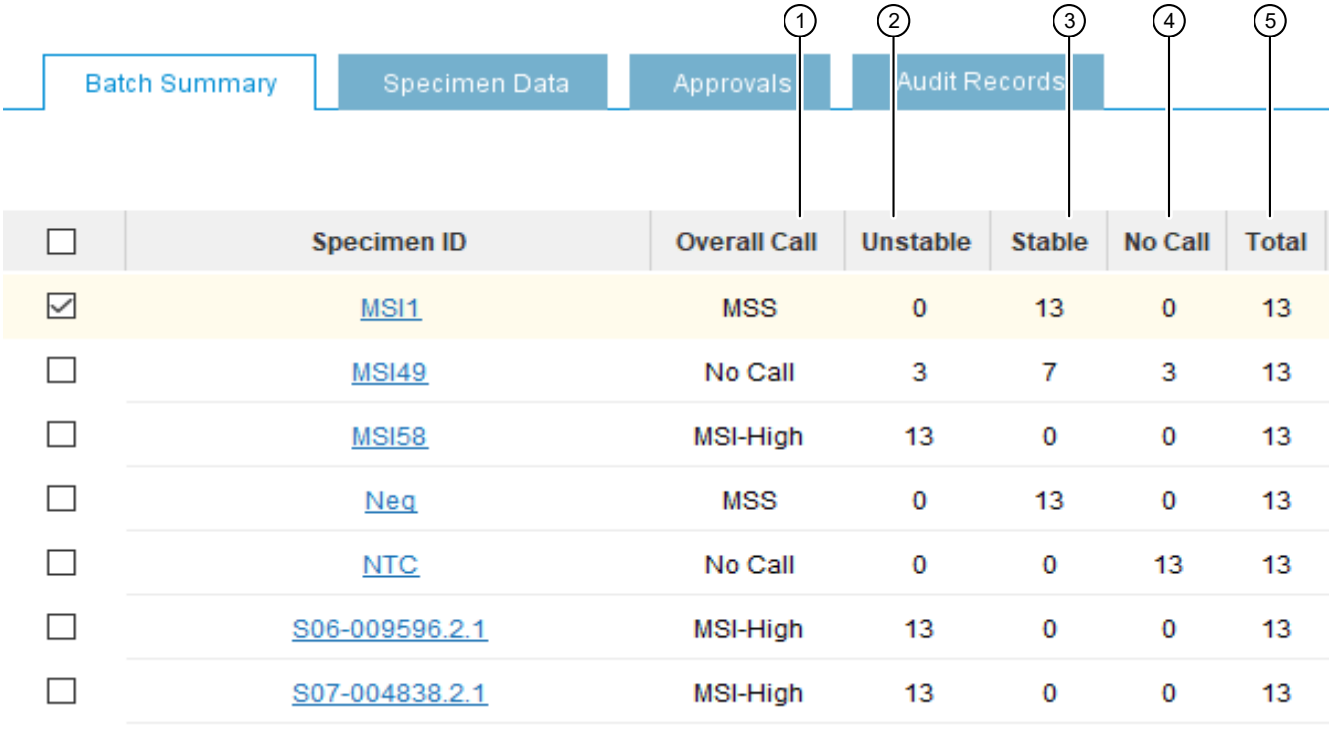
The **Overall Call** for the specimen is based on the percentage of markers reporting instability.

1. View the **Overall Call** for each specimen.
   - **MSS**—Miscrosatellite stable
   - **MSI–Low**—Low levels of microsatellite instability
   - **MSI–High**—High levels of microsatellite instability
   - **No Call**—At least one marker had no call.

   Note: If every marker **No Call** is manually changed to a call of either **Unstable** or **Stable**, then the overall call will be adjusted from **No Call** to **MSS**, **MSI-Low**, or **MSI-High**.

2. View the number of **Unstable**, **Stable**, or **No Call** markers.

Interpret the results



	Specimen ID	Overall Call	Unstable	Stable	No Call	Total
☑	MSI1	MSS	0	13	0	13
☐	MSI49	No Call	3	7	3	13
☐	MSI58	MSI-High	13	0	0	13
☐	Neg	MSS	0	13	0	13
☐	NTC	No Call	0	0	13	13
☐	S06-009596.2.1	MSI-High	13	0	0	13
☐	S07-004838.2.1	MSI-High	13	0	0	13

① **Overall Call** for the specimen
② Number of **Unstable** markers
③ Number of **Stable** markers
④ Number of **No Call** markers
⑤ **Total** number of markers used for the **Overall Call**

See the *TrueMark™ MSI Analysis Software User Guide* (Pub. No. MAN0018874) for more detailed information on viewing individual markers and changing calls.

View the electropherogram traces

1. Click the **Specimen ID** in the **Home** or **Batch Summary** tabs to open the relevant specimen in the **Specimen Data** tab.

2. Review the electropherogram traces in the **Specimen Data** tab.
   a. Click the marker row in the **Marker Details** table to display the relevant dye and size range for that marker.

   Note: The **Marker Details** table can be used to identify which markers have a warning in the **Review Flag** column, indicating that a review of the call is recommended. The **Marker Details** table can also be used to override the **Auto Call** with a **Manual Call**, and/or exclude the marker from the **Overall Call** assessment and from reports and exports.

Representative data

Examples of microsatellite instability in colon tumor tissue samples

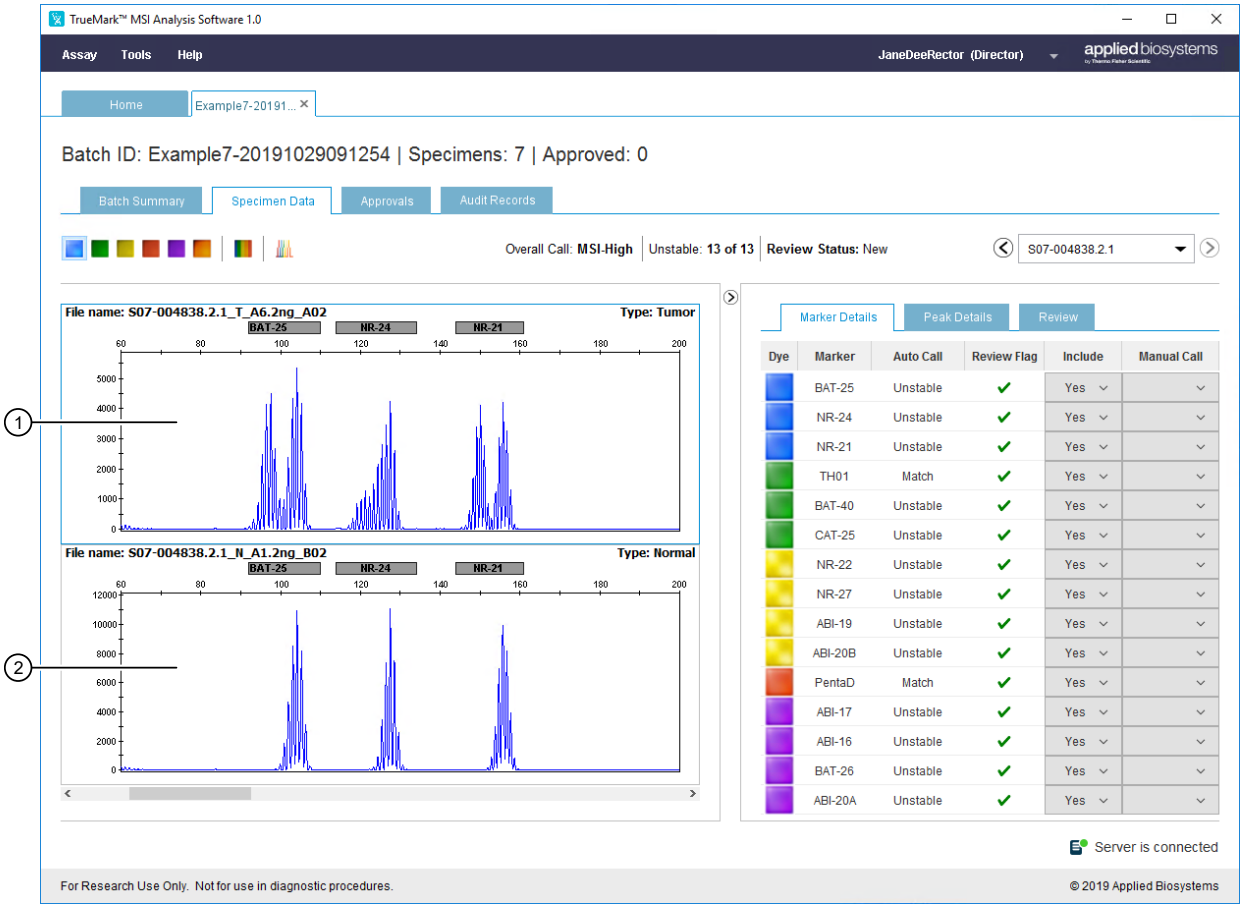


Figure 3 Example electropherogram of a FFPE colon tumor tissue sample.

① Tumor trace—Displays extra peaks that are not present in the normal trace.

② Normal trace—Displays standard peaks for the marker.

The colon tumor tissue sample was amplified with the TrueMark™ MSI Assay, run on a SeqStudio™ Genetic Analyzer, and analyzed with TrueMark™ MSI Analysis Software (Y-axis scale 0–12,000 RFU). The extra peaks in the tumor trace have a smaller fragment size than the main peak for each marker, indicating microsatellite instability in the tumor sample.

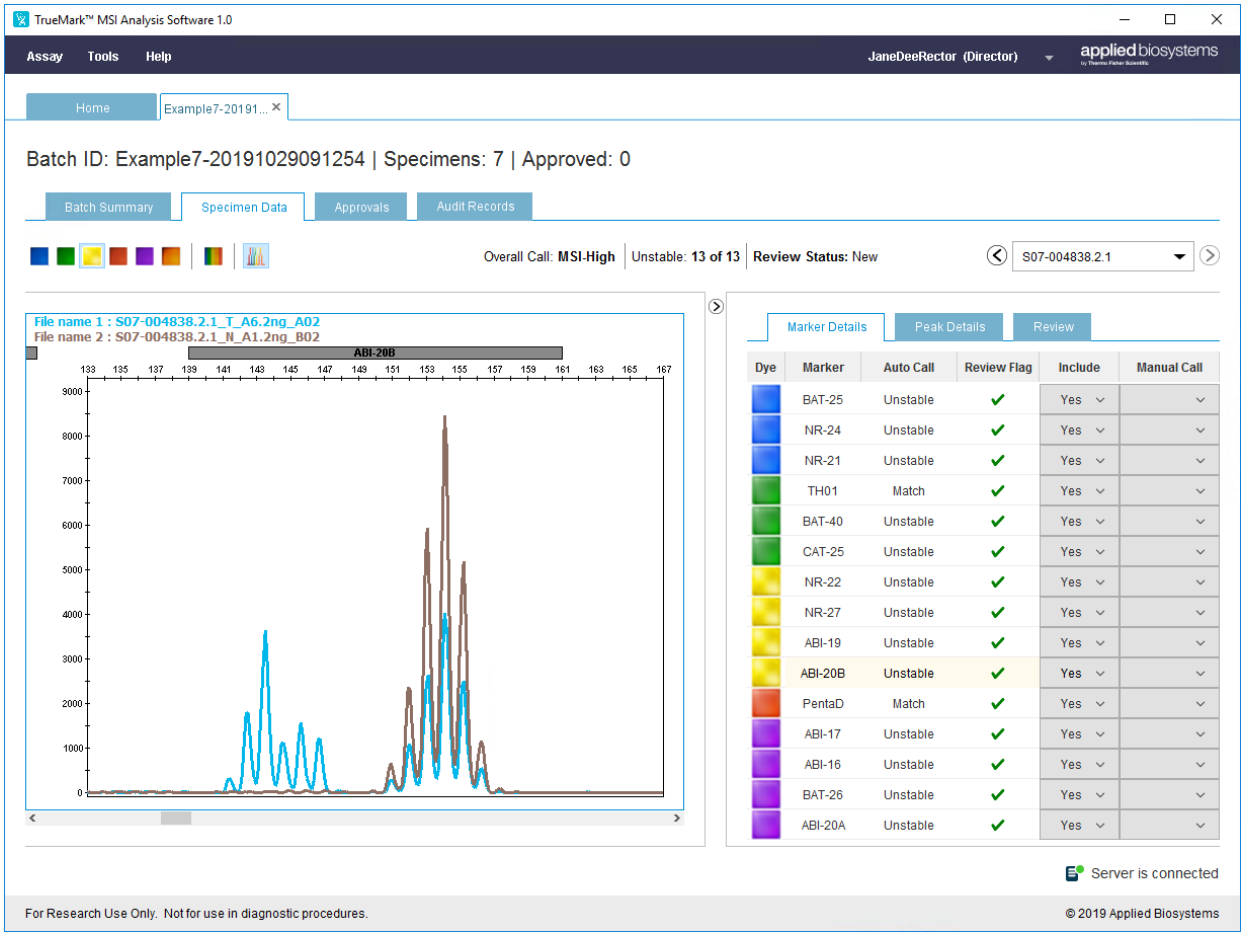


Figure 4 Alternative view: tumor and normal traces are overlaid to more easily evaluate smaller differences in fragment size distribution.

Automatic calling of low frequency small deletions in synthetic constructs

Instability in colon tumor tissue microsatellites are readily identified by large base pair deletions. However, extra-colonic tumors can feature small deletions that are more difficult to distinguish. To accurately report microsatellite instability in colon and other tumor tissue types, it is important to identify these small deletions.

We designed synthetic constructs with varying lengths of small deletions for microsatellite markers in the TrueMark™ MSI Assay, then evaluated them at several allele frequencies. Examples of these small deletions are shown in Figure 5 and Figure 6.

IMPORTANT! Run the tumor and normal samples on the same plate. The software can give inaccurate results if you use tumor and normal sample files from different runs. Additionally, the signal strength of tumor and normal samples should match as closely as possible in order to detect small deletions or low allele frequencies.

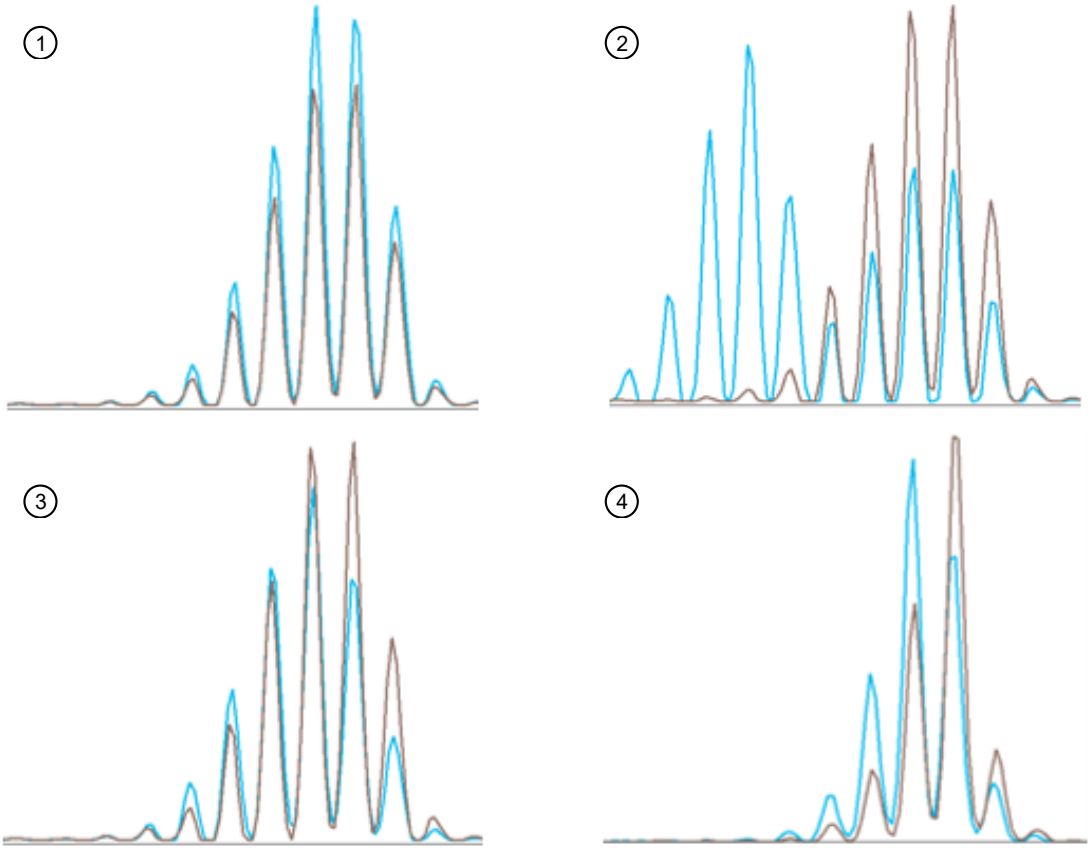


Figure 5 Examples of microsatellite stability and instability in synthetic constructs. The synthetic construct containing the wild-type allele is displayed in brown. The synthetic construct containing the mutant allele is displayed in blue.

① Marker BAT-25—Stable microsatellite with no deletions
② Marker BAT-25—Large unstable microsatellite with a 5 bp deletion at 50% allele frequency
③ Marker BAT-25—Unstable microsatellite with a 1 bp deletion at 50% allele frequency
④ Marker ABI-20A—Unstable microsatellite with a 1 bp deletion at 50% allele frequency

It is possible to detect small deletions (2–5 bp) at lower allele frequencies by visual inspection. However, single base pair deletions at 20% allele frequency are difficult to discern (Figure 6 and Figure 7).

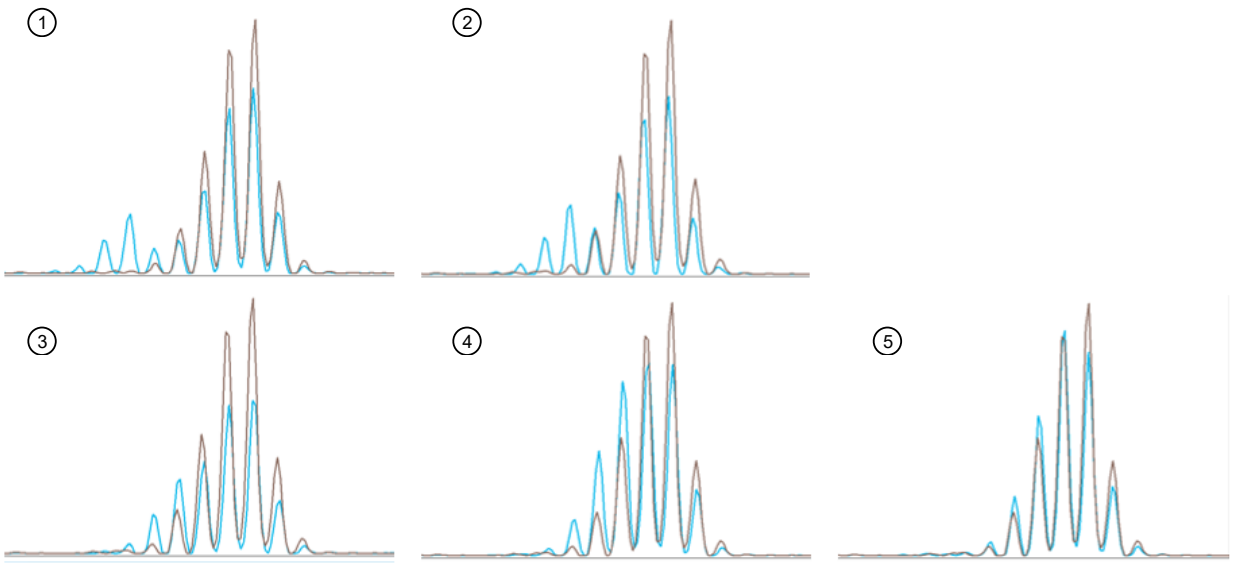


Figure 6 Examples of small low frequency deletions in synthetic constructs. The synthetic construct containing the wild-type NR-24 allele is displayed in brown. The synthetic construct containing the mutant NR-24 allele is displayed in blue.

1. Unstable microsatellite with a 5 bp deletion at 20% allele frequency
2. Unstable microsatellite with a 4 bp deletion at 20% allele frequency
3. Unstable microsatellite with a 3 bp deletion at 20% allele frequency
4. Unstable microsatellite with a 2 bp deletion at 20% allele frequency
5. Unstable microsatellite with a 1 bp deletion at 20% allele frequency

Automatic calling of low frequency small deletions in an endometrial tumor tissue sample

As an example of how the TrueMark™ MSI Analysis Software interprets low frequency small deletions, results from an endometrial tumor tissue sample with a relatively low fraction of tumor content (~25%) is shown in Figure 7.

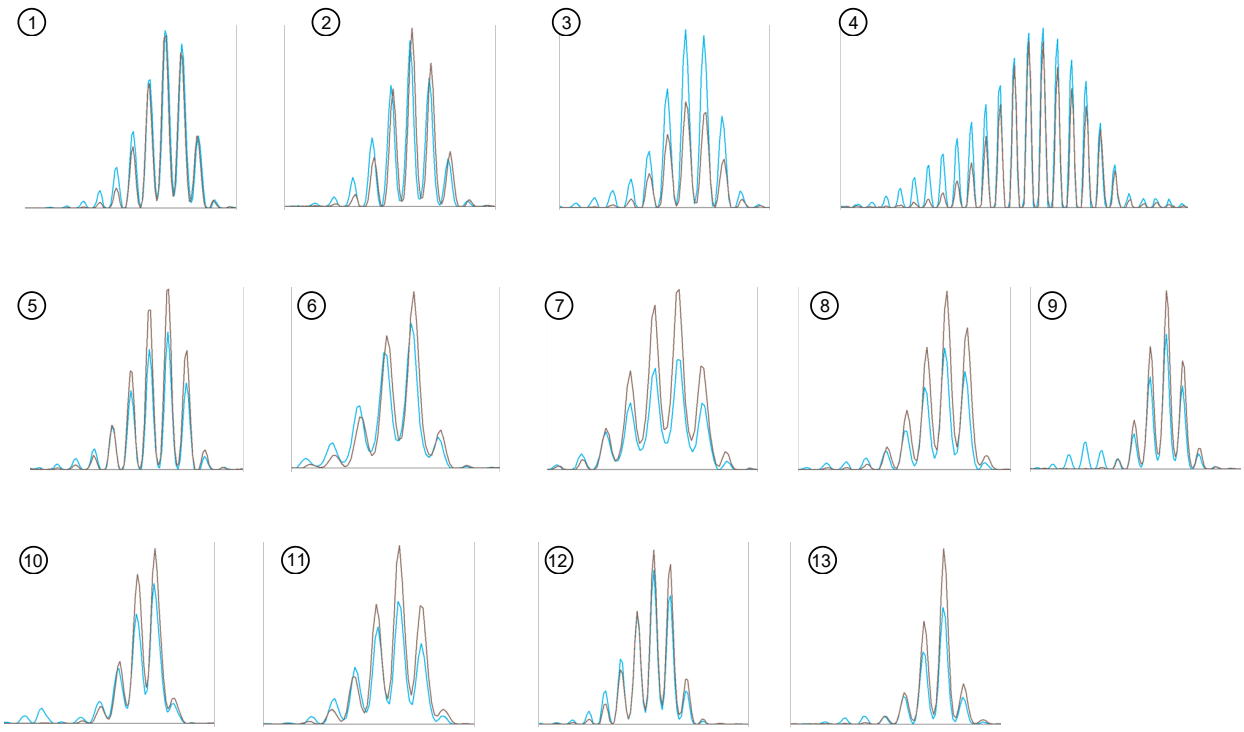
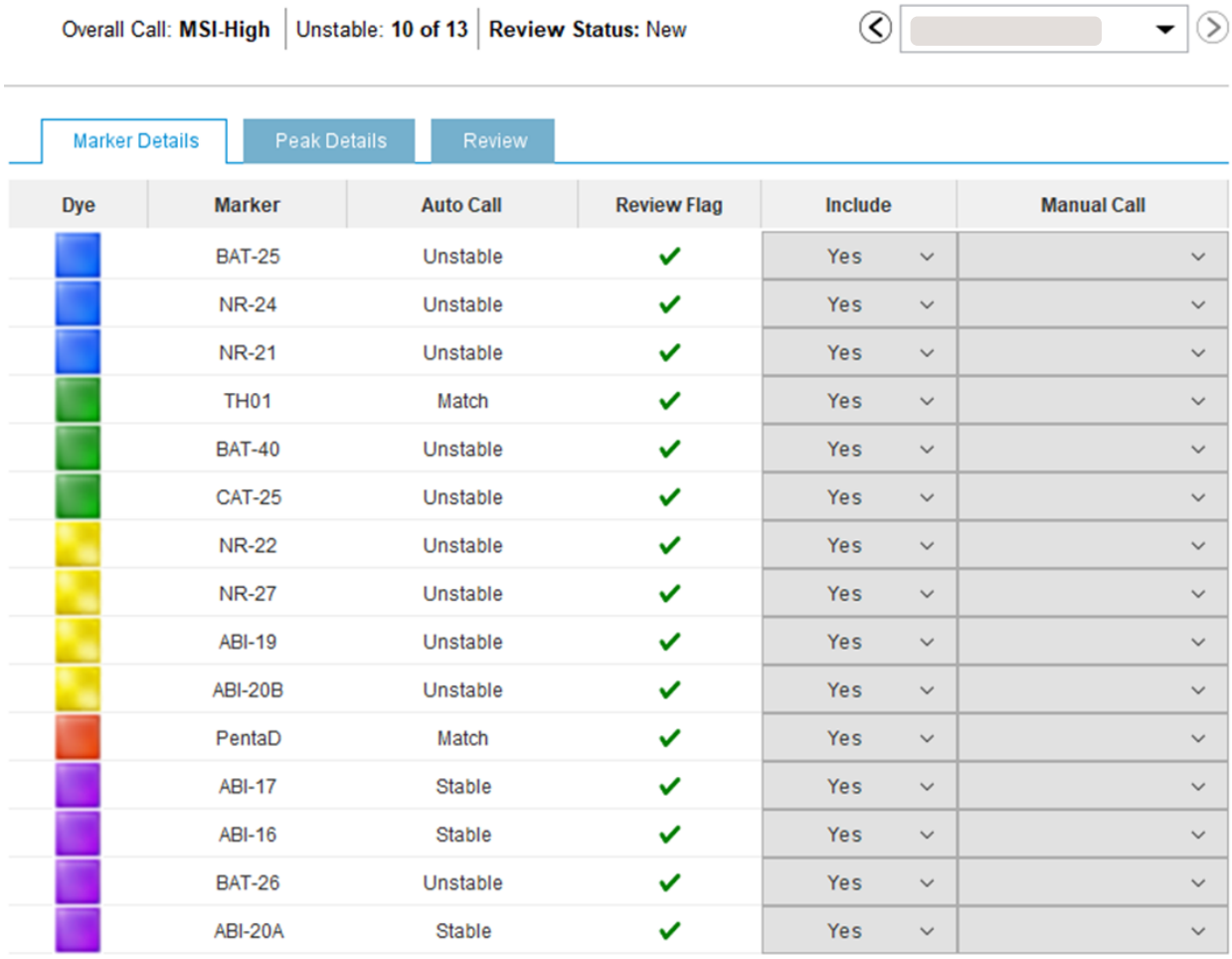


Figure 7 Example of small low frequency deletions in a mismatch repair (MSH2 and MSH6) deficient endometrial tumor tissue sample. The normal sample is displayed in brown. The tumor sample is displayed in blue. The tumor content of this specimen was 25%.

1. BAT-25
2. NR-24
3. NR-21
4. BAT-40
5. CAT-25
6. NR-22
7. NR-27
8. ABI-19
9. ABI-20B
10. ABI-17
11. ABI-16
12. BAT-26
13. ABI-20A

The TrueMark™ MSI Analysis Software called 10 of 13 markers in the endometrial tumor tissue sample as unstable, with an overall call of **MSI-High** (Figure 8).



Dye	Marker	Auto Call	Review Flag	Include	Manual Call
	BAT-25	Unstable	✔	Yes	
	NR-24	Unstable	✔	Yes	
	NR-21	Unstable	✔	Yes	
	TH01	Match	✔	Yes	
	BAT-40	Unstable	✔	Yes	
	CAT-25	Unstable	✔	Yes	
	NR-22	Unstable	✔	Yes	
	NR-27	Unstable	✔	Yes	
	ABI-19	Unstable	✔	Yes	
	ABI-20B	Unstable	✔	Yes	
	PentaD	Match	✔	Yes	
	ABI-17	Stable	✔	Yes	
	ABI-16	Stable	✔	Yes	
	BAT-26	Unstable	✔	Yes	
	ABI-20A	Stable	✔	Yes	

Figure 8 Marker calls in the TrueMark™ MSI Analysis Software

(*Optional*) Accept and approve a specimen

You must have permissions in the TrueMark™ MSI Analysis Software to accept (**Initial Review** and **Final Review**) and approve (**Approve Sample**) a specimen. For more information on set up and use of permissions, see the *TrueMark™ MSI Analysis Software User Guide* (Pub. No. MAN0018874).

1. In the **Specimen Data** tab, click the **Review** tab.
2. Click **Accept** to acknowledge that you have reviewed the specimen.

   Note: The comment **Accepted** is displayed, and the **Review Status** changes to **Under Review**.

3. Select the **Ready for Approval** checkbox.

   Note: The **Review Status** changes to **Ready for Approval**.

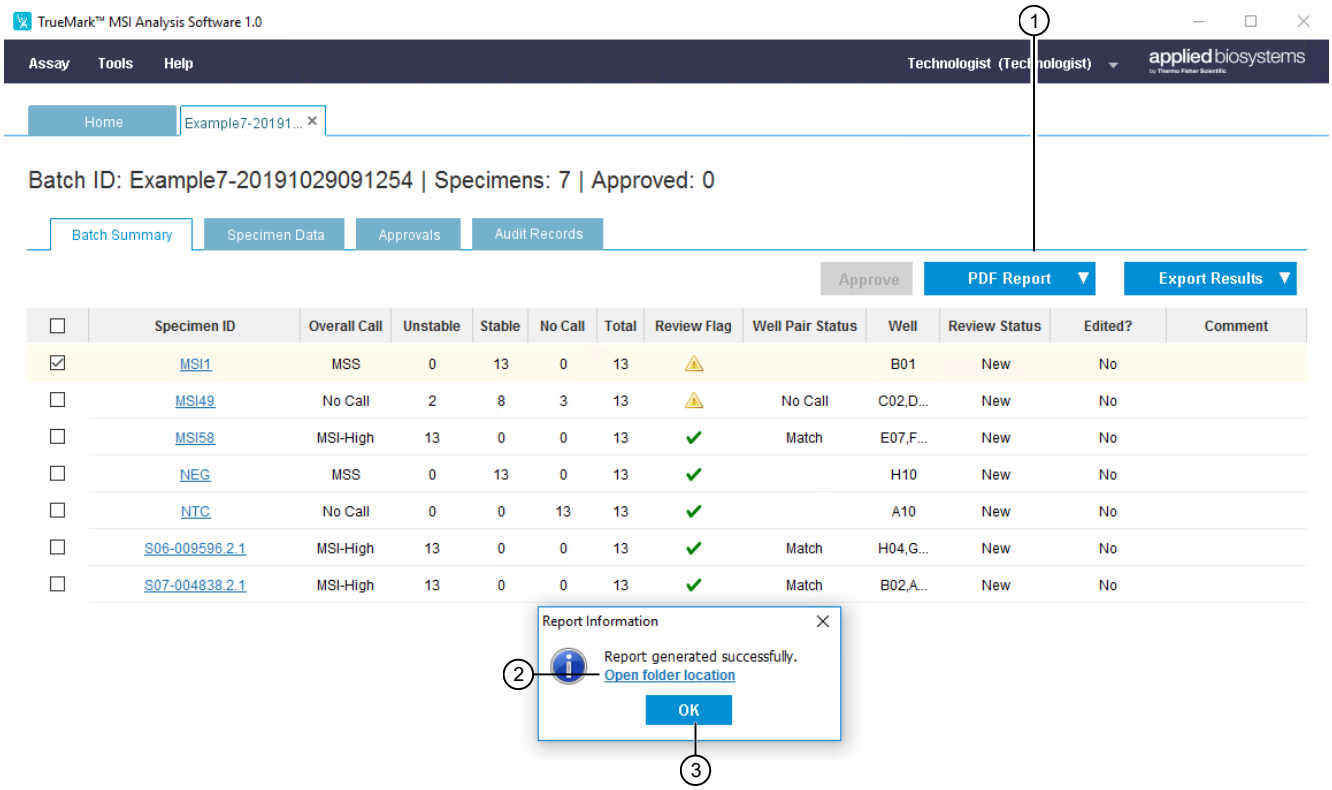
4. Open the **Batch Summary**.
5. Click the **Approve** button, then enter your user name and password.

   Note: Users without **Approve** permissions will not be allowed to sign.

If a specimen is approved, the approval history of the specimen appears in the Specimen PDF report. See the *TrueMark™ MSI Analysis Software User Guide* (Pub. No. MAN0018874) for more information.

Generate a report

1. In the **Batch Summary** pane of the batch of interest, click **PDF Report**.
2. Select **Batch Summary** or **Specimen**.
   A message is generated stating **Report generated successfully**. Click **Open folder location** to see where the report was saved, then click **OK**.



① **PDF Report** button
② **Open folder location** link
③ **OK** button

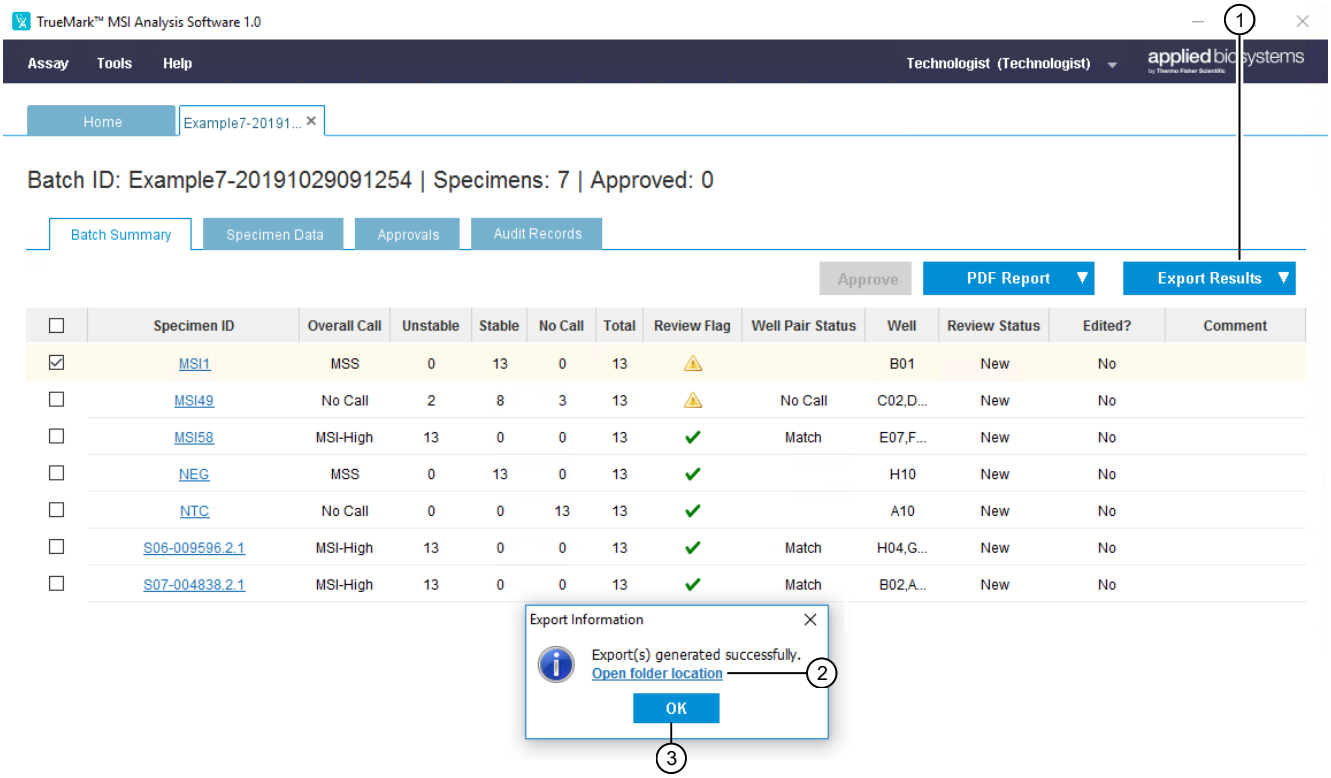
Export results

1. In the **Batch Summary** pane of the batch of interest, click **Export Results**.

2. Select the results format to export (**Batch Summary** or **Specimen**).

   Batch Summary results is available in CSV format. Specimen results are available in CSV or VCF formats.

   A message is generated stating **Export(s) generated successfully**. Click **Open folder location** to see where the results were saved, then click **OK**.



① **Export Results** button
② **Open folder location** link
③ **OK** button

A

Troubleshooting

Observation	Possible cause	Recommended action
Faint or no signal from both the TrueMark™ MSI Assay Amplification Control and the DNA test samples for all markers	The incorrect volume of Master Mix or Primer Set was used.	Use the correct volume of Master Mix or Primer Set.
	The Master Mix was not vortexed thoroughly before aliquoting.	Vortex the Master Mix thoroughly.
	The Primer Set was exposed to too much light.	Replace the Primer Set and store it protected from light.
	Evaporation.	Ensure that the adhesive seal or optical caps are properly sealed.
	The thermal cycler malfunctioned.	See the thermal cycler user guide and check the instrument calibration.
	Incorrect thermal cycler conditions were used.	Use correct thermal cycler conditions.
	The wrong PCR reaction tubes or plate were used.	Use MicroAmp™ Optical 96-Well Reaction Plate with Barcode with MicroAmp™ Clear Adhesive Film for the ProFlex™ Thermal Cycler.
	Insufficient PCR product was electrokinetically injected.	Use the correct genetic analyzer settings.
	Degraded formamide was used.	Check the storage of formamide. Do not thaw and refreeze multiple times. Use fresh Hi-Di™ Formamide.
Signal from the TrueMark™ MSI Assay Amplification Control but partial or no signal from DNA test samples	The test sample was diluted in the wrong buffer (for example, a TE buffer with an incorrect EDTA concentration).	Redilute the DNA using low-TE buffer (with 0.1 mM EDTA).
	Less than the recommended amount of DNA was added to the PCR reaction.	Quantitate the DNA sample using a Qubit™ dsDNA HS Assay Kit.
		Increase the injection time to boost the signal of the sample.
		Increase the number of PCR cycles to boost the signal of the sample.

Observation	Possible cause	Recommended action
More than the expected number of peaks present for a sample identification marker (TH01 and PentaD)	Exogenous DNA is present in the sample.	Use appropriate techniques to avoid introducing foreign DNA during laboratory handling.
	Incomplete 3' A base addition (n-1 nt position) occured.	Include the final extension step of 60°C for 25 minutes in the PCR.
		Incubate the amplification products at 60°C for an additional 10 minutes.
	The signal exceeds the dynamic range of the instrument and is causing signal "pull-up" into adjacent channels.	Ensure the cycle number is optimized. User fewer PCR cycles or interpret the off-scale data according to your laboratory procedure.
		Decrease the injection time in the run module.
		Load less DNA in the PCR reaction.
	Poor spectral separation occurred.	Perform a spectral calibration.
		Confirm that Filter Set J6 modules are installed and used for analysis.
	The double-stranded DNA was not completely denatured.	Use the recommended amount of Hi-Di™ Formamide and heat the sample plate at 95°C for 3 minutes.
	Contamination was carried over from the DNA extraction.	Perform the DNA extraction again.
	MSI present in the sample is reflected in the tumor tissue sample identification markers.	Assess the level of instability is the overall sample to assist in determining whether the extra peak(s) are due to contamination or instability.
Some but not all markers visible on electropherogram of DNA Test Samples	The PCR reaction volume used is lower than the volume that is required for the amplification.	Use the correct PCR reaction volume: 10 µL
	There are fewer large DNA fragments due to FFPE fragmentation.	Use more PCR cycles, increase the injection time of the run module, or load more DNA into the PCR reaction to boost signal in the sample.

Observation	Possible cause	Recommended action
Marker profiles contain many off-scale peaks	The PCR cycle number used was too high.	Perform a sensitivity experiment to determine the optimal PCR cycle number based on the sample type.
		Decrease the injection time in the run module.

Extra peaks in the electropherogram

Causes of extra peaks

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

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 (minus stutter), or less frequently, one repeat larger (plus stutter). 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.

The level of stutter in this kit is normal and as expected for STR chemistries that are designed to overcome inhibitors and obtain robust performance with single source reference samples.

Artifact 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).

Artifact observation

Due to improvements in PCR primer manufacturing processes, the incidence of artifacts has been greatly reduced in the TrueMark™ MSI Assay. Electropherograms are free of reproducible dye artifacts in the kit read region of 80–215 nt for commonly used analytical thresholds.

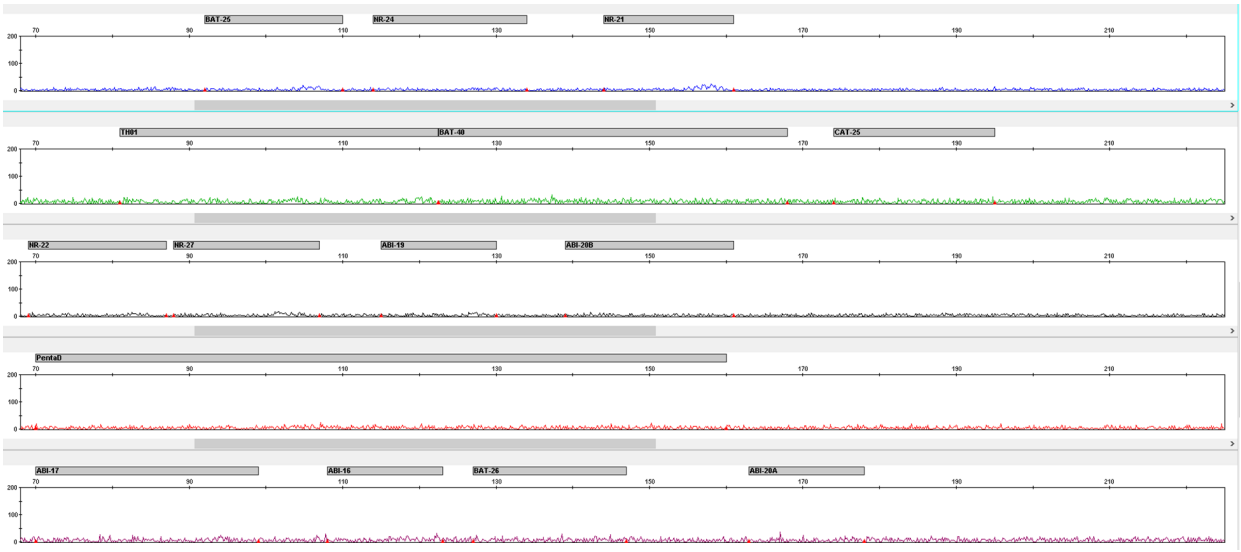


Figure 9 Example of low baseline-level fluorescence in a typical no template control (NTC) PCR. Data produced on a 3500xL Genetic Analyzer (Y-axis scale 0 to 200 RFU).

Table 9 Marker-specific artifacts observed with the TrueMark™ MSI Assay

Artifact	Color	Size	Type
NR–22	Black	75–90 nt	Incomplete amplification
TH01	Green	80–100 nt	Incomplete amplification of BAT–40
SID208–213	Purple	208–213 nt	Non-specific amplification. This set of non-specific peaks is much larger than the nearest MSI allele, ABI-20A, and should not be ignored.

Marker-specific artifacts commonly observed in the TrueMark™ MSI Assay are shown in Figure 10 to Figure 12.

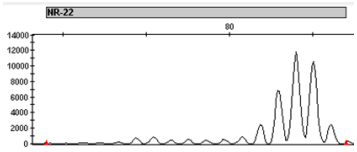


Figure 10 NR-22

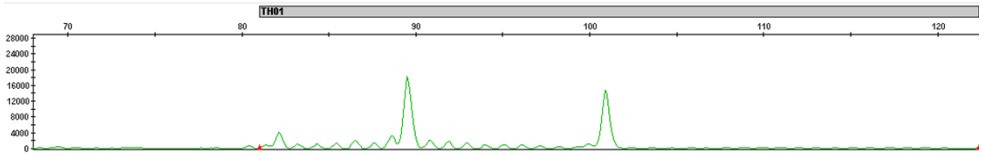


Figure 11 TH01

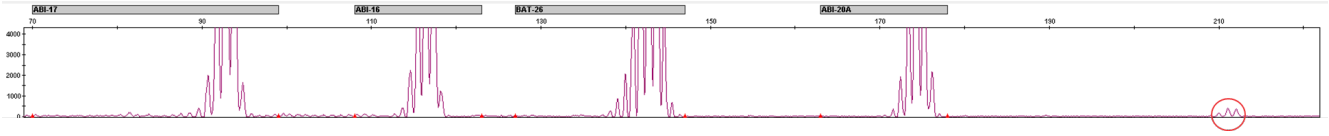


Figure 12 Non-specific amplification in SID channel

Additional reproducible DNA-dependent artifacts have been characterized and documented. It is important to consider noise and other amplification-related artifacts when interpreting data.

B Analyze the data with GeneMapper™ Software

Overview of the 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.

Set up the GeneMapper™ Software (v5.0 or later) for analysis (before first use of the kit)

Define custom display and plot settings

1. Navigate to the **Plot Settings Editor**.
2. Select **Display Settings**, then enter the following display settings.

   For Sample and Genotype plots

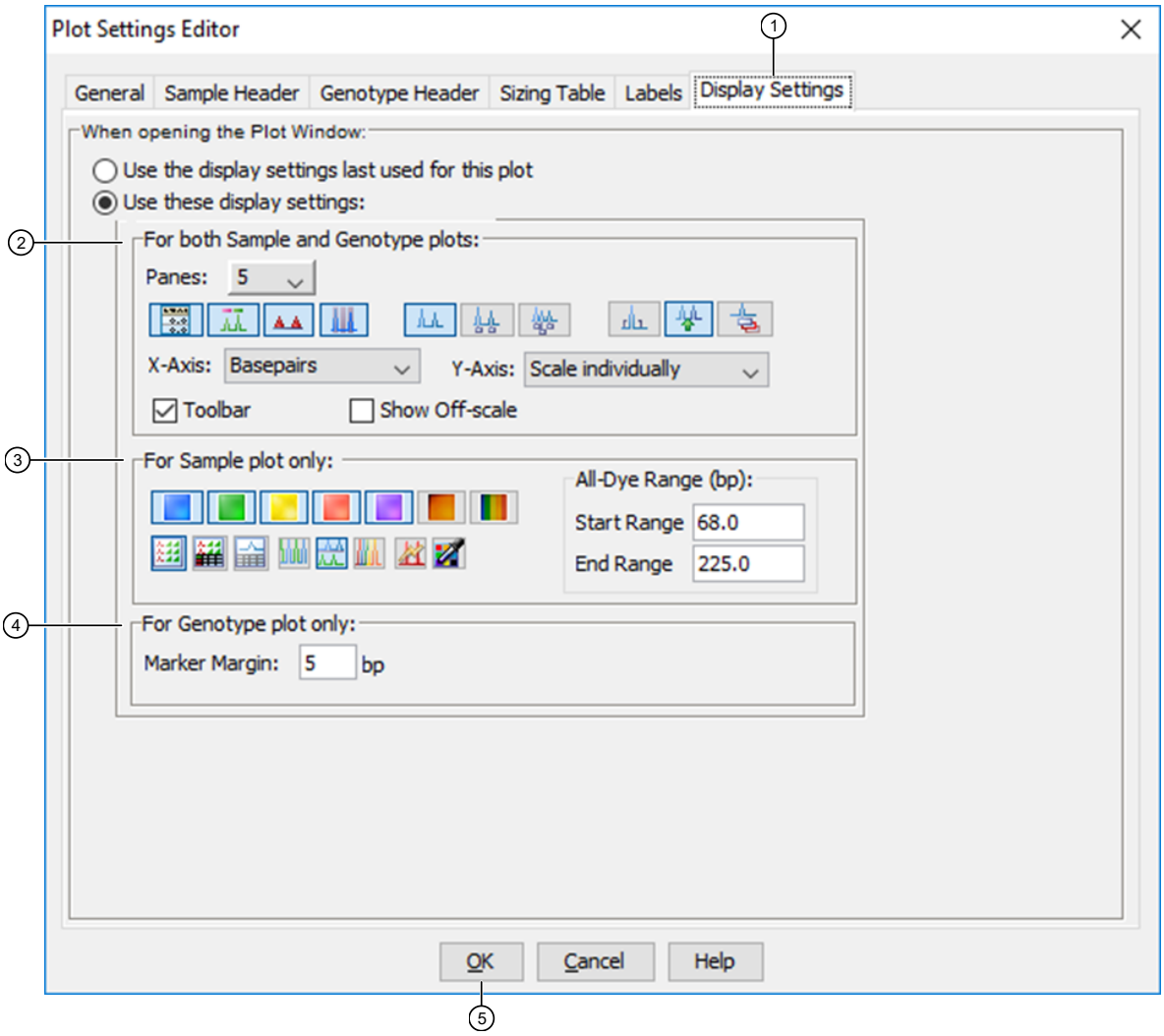
   - 5 panes
   - Select the graphs highlighted in blue
   - X-Axis—Basepairs
   - Y-Axis—Scale individually
   - Select **Toolbar**

   For Sample plot

   - Select the first five colors, then the graphs highlighted in blue
   - Start Range—68.0 bp
   - End Range—225.0 bp

For Genotype plot

- Marker Margin—5 bp



① Display Settings tab
② **For both Sample and Genotype plots** pane
③ **For Sample plot only** pane
④ **For Genotype plot only** pane
⑤ **OK** button

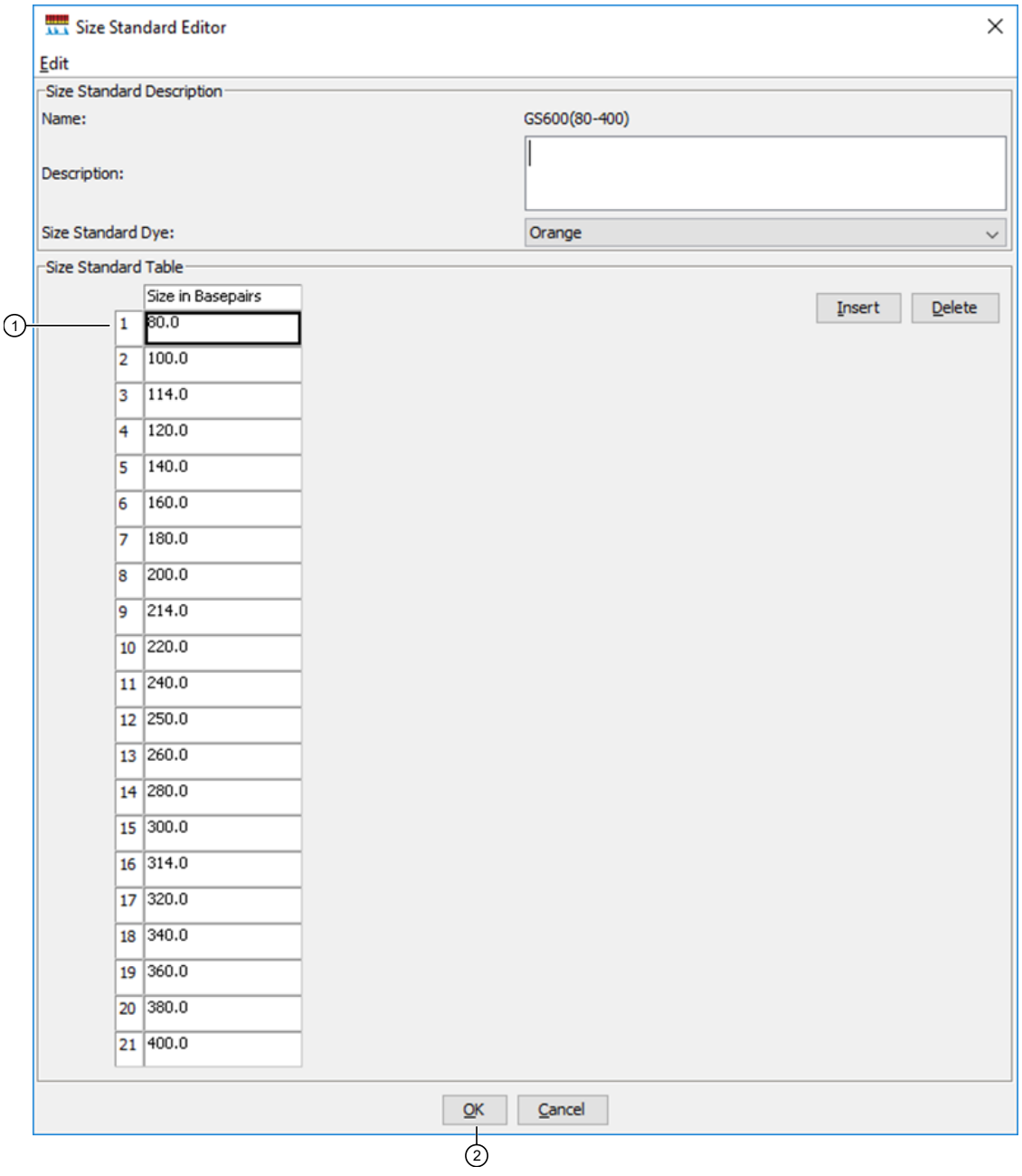
3. Click **OK**.

Define the Size Standard

1. Navigate to the **Size Standard Editor**, then click **New**.
2. *(Optional)* Enter a description of the Size Standard.

3. Specify settings in the Size Standard Editor.
   a. Enter a name as shown in the following figure [GS600(80-400)] or enter a new name.
   b. In the **Security Group** field, select the **Security Group** appropriate for your software configuration.
   c. In the **Size Standard Dye** field, select **Orange**.

d. In the **Size Standard Table**, use sizes between **80.0** and **400.0** bp (copied from the GS600LIZ default size standard).



① **Size Standard Table**
② **OK** button

4. Click **OK**.

Create an analysis method

Create an analysis method

IMPORTANT! Analysis methods are version-specific, so you must create an analysis method for each version of the software.

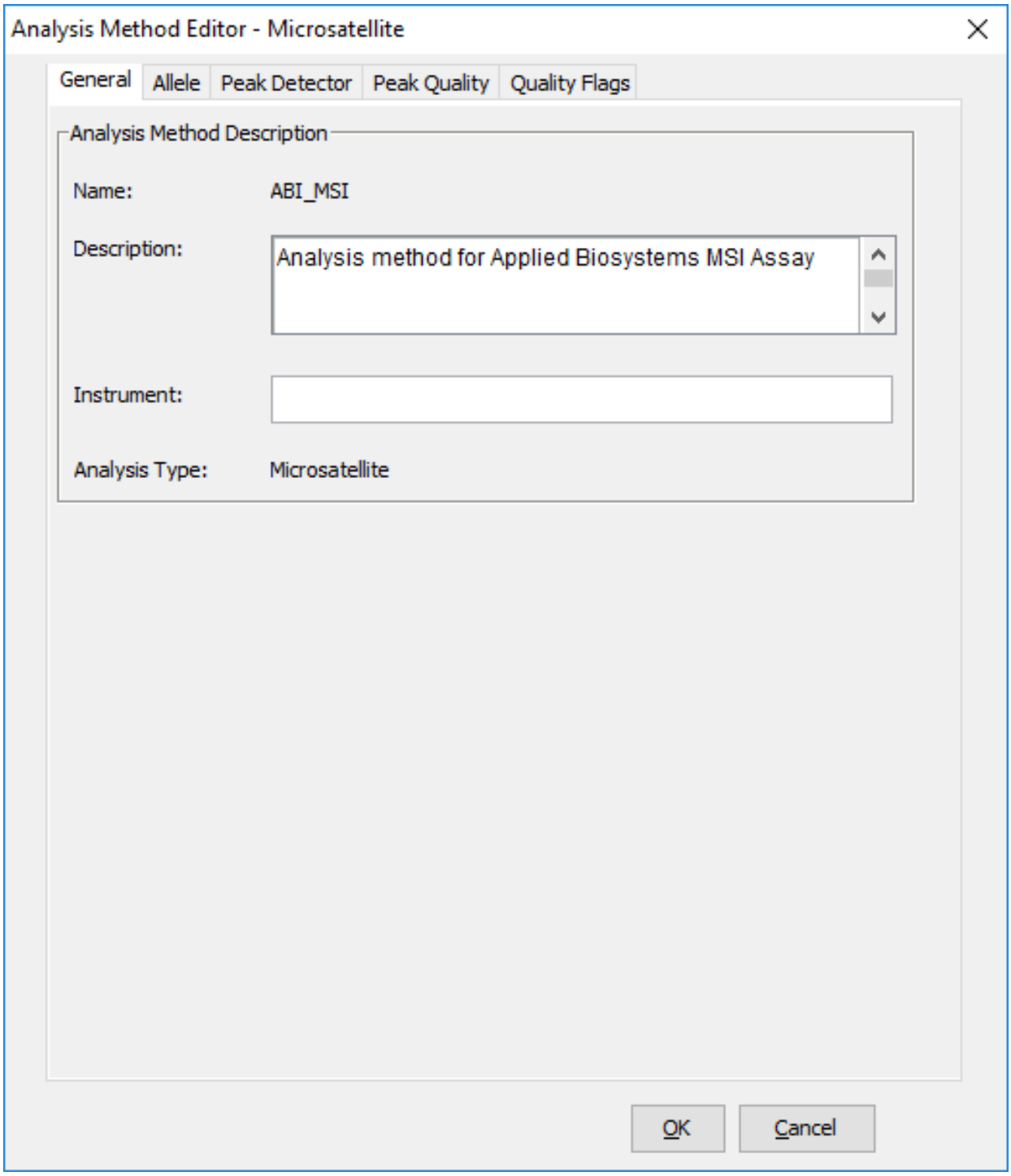
1. Select **Tools ▸ GeneMapper Manager** to open the **GeneMapper Manager**.
2. Click the **Analysis Methods** tab, then click **New** to open the **Analysis Method Editor** with the **General** tab selected.
3. Enter the settings shown in the figures on the following pages.

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

4. After you enter the settings on all tabs, click **Save**.

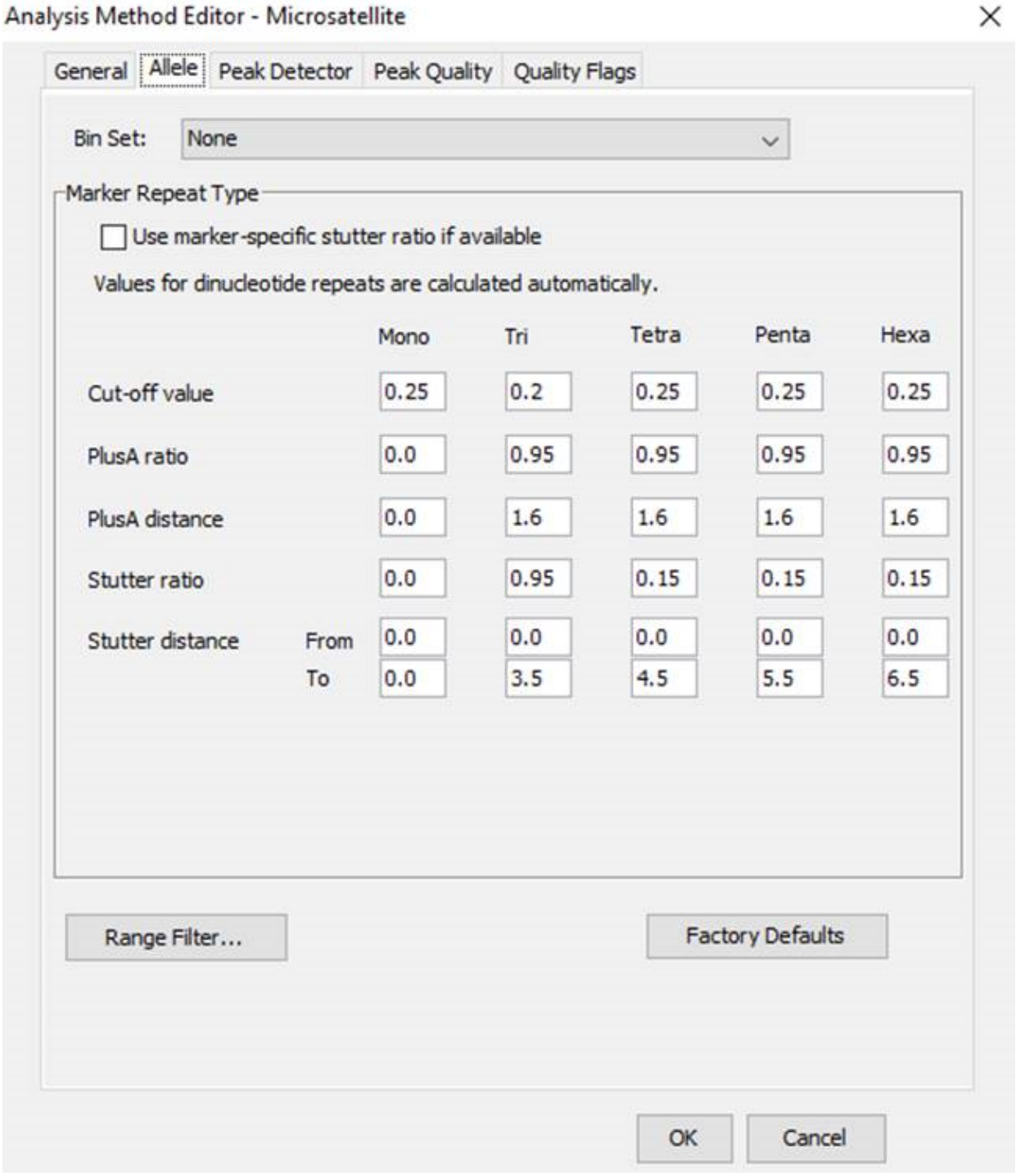
Analysis method settings

General tab settings



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

Allele tab settings

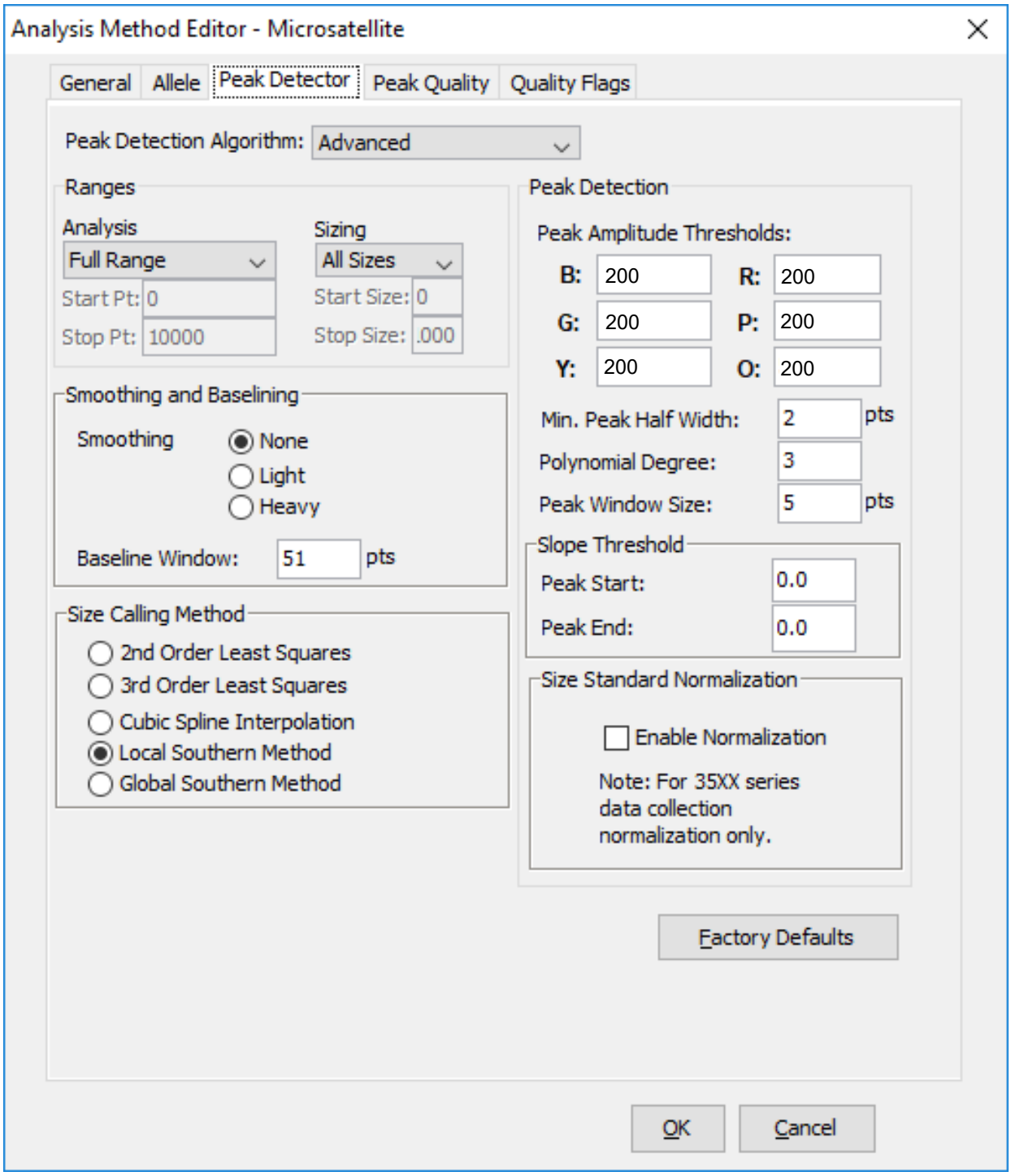


The following settings were used during development of the TrueMark™ MSI Assay:

- In the **Bin Set** field, select **None**.
- GeneMapper™ Software allows you to specify 4 types of marker repeat motifs: tri, tetra, penta, and hexa. You can enter parameter values for each type of repeat in the appropriate column.
- Deselect the **Use marker specific stutter ratio if available** checkbox (selected by default).

Perform appropriate internal studies to determine the appropriate filter setting to use.

Peak Detector tab settings

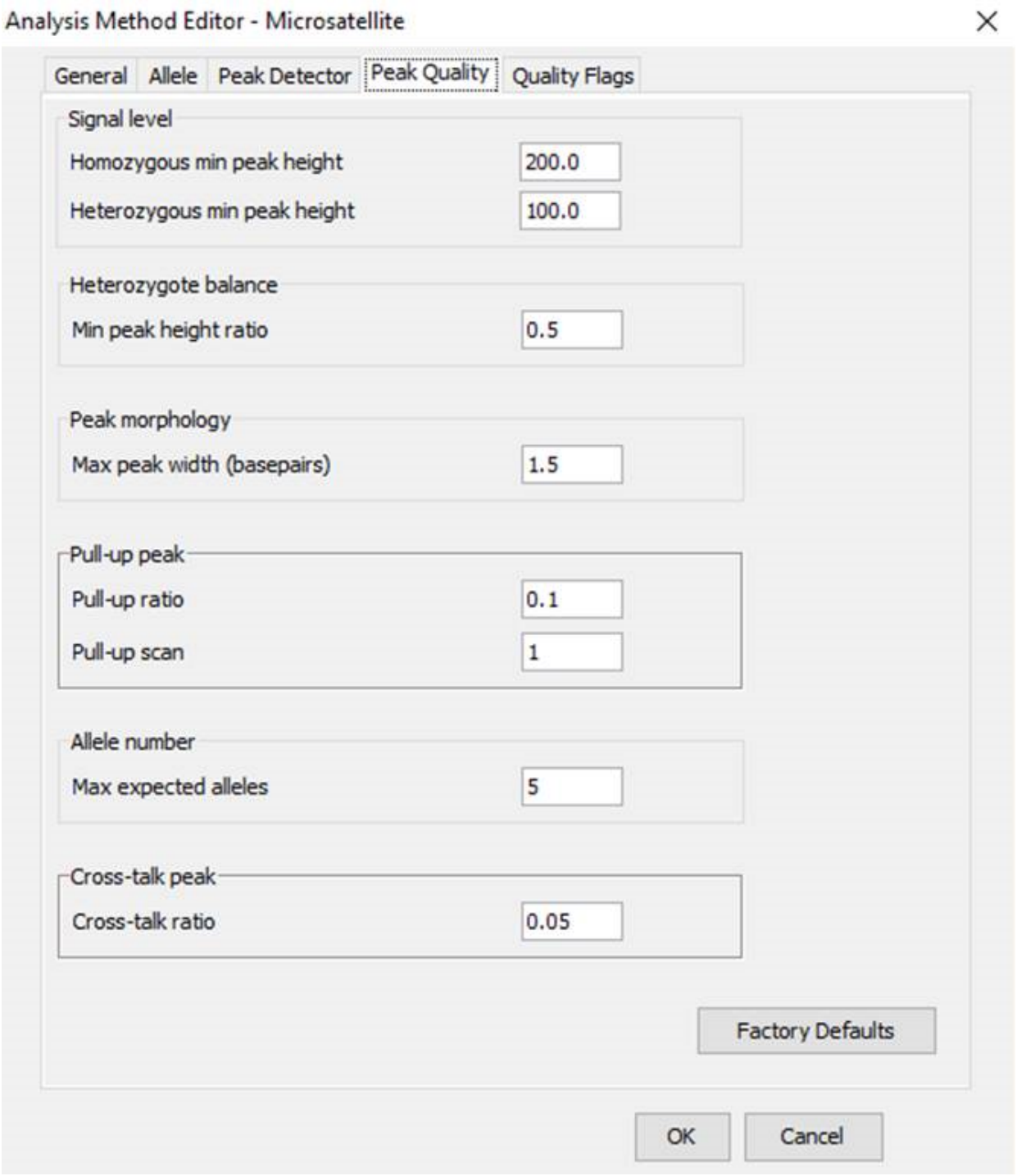


IMPORTANT! Perform the appropriate internal studies to determine the appropriate peak amplitude thresholds for interpretation of data.

Fill in the field information:

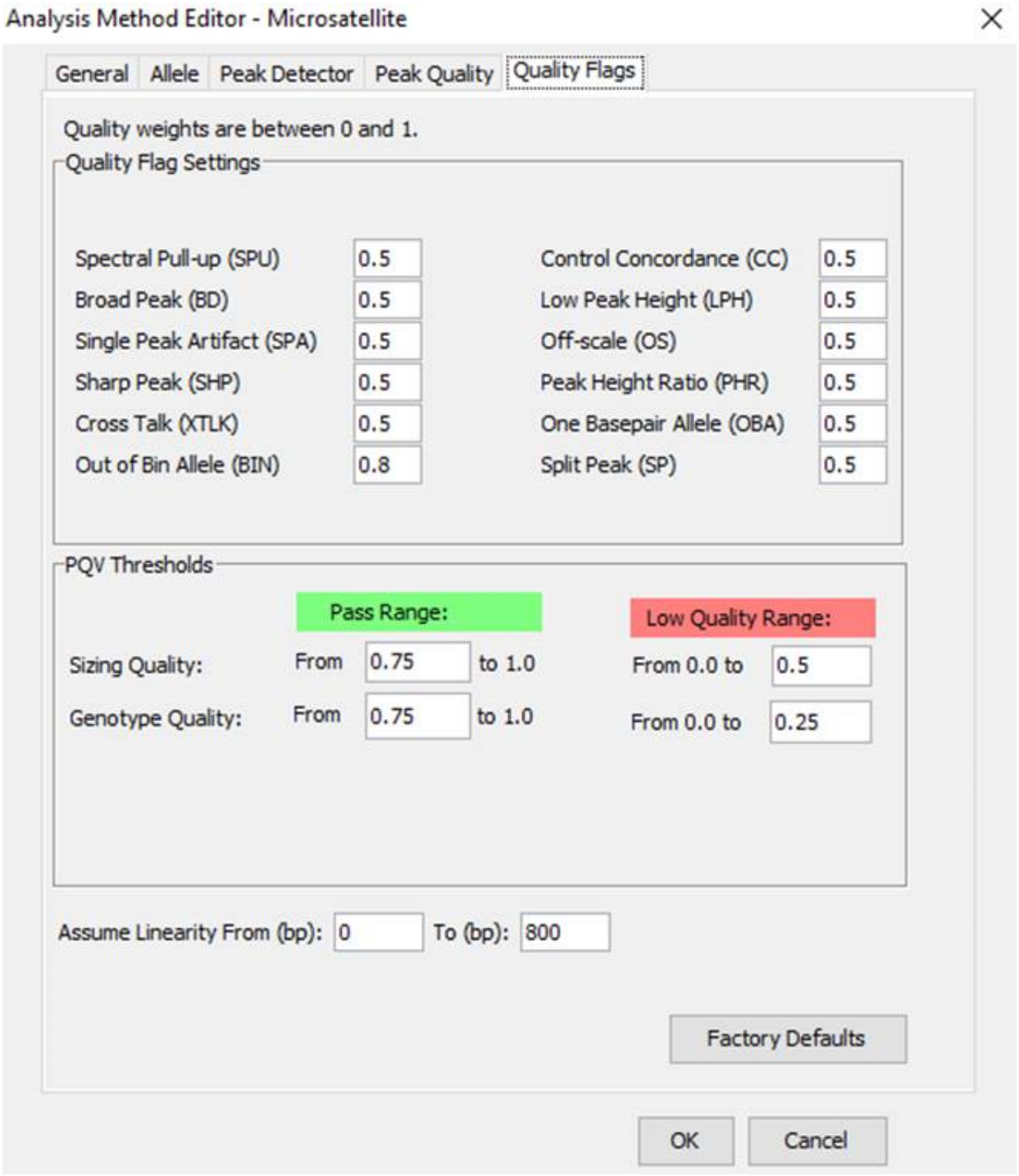
- **Peak Detection Algorithm**—Basic.
- **Minimum Peak Height**—Automatic.

Peak Quality tab settings



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

Quality Flags tab settings



IMPORTANT! The values that are shown are the values used during assay development. Perform appropriate internal studies to determine the appropriate values to use.

Analyze and edit sample files with GeneMapper™ Software

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

2. Apply analysis settings to the samples in the project.

Parameter	Settings
Sample Type	Select the sample type.
Analysis Method	Select the **ABI MSI Assay** analysis method.
Panel	Select the **ABI MSI Panel**.
Size Standard	Use **GS600(80-400)** with a size range of 80–400 bp for the Local Southern size calling method.

3. Click **Analyze**, enter a name for the project (in the **Save Project** dialog box), then click **OK** to start analysis.
   - The status bar displays the progress of analysis as a completion bar.
   - The table displays the row of the sample currently being analyzed in green (or red if analysis failed for the sample).

C

Safety

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

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

Chemical safety

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

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

Biological hazard safety

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

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. 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:
  cdc.gov/labs/bmbl
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Document	Publication number
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v3.3 User Guide	100079380
DS-36 Matrix Standard Kit (Dye Set J6) Product Information Sheet	4426042
GeneMapper™ Software v4.1 Quick Reference Guide	4403615
RecoverAll™ Total Nucleic Acid Isolation Kit Protocol	1975M
SeqStudio™ Genetic Analyzer Instrument and Software User Guide	MAN0018646
SeqStudio™ Flex Series Genetic Analyzer with Instrument Software v1.1.1 User Guide	100104689
TrueMark™ MSI Assay Quick Reference Guide	MAN0018869
TrueMark™ MSI Analysis Software User Guide	MAN0018874

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support

- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.