applied biosystems

CarrierMax™ FMR1 Reagent Kit

USER GUIDE

for use with:
3500/3500xL Genetic Analyzer
SeqStudio™ Genetic Analyzer
CarrierMax™ Software
GeneMapper™ Software

Catalog Number 952362
Publication Number MAN0018881
Revision B.0

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

ThermoFisher SCIENTIFIC

Manufactured by Microread Genetics Co. Ltd. for Life Technologies Holdings Pte Ltd. 33 Marsiling Industrial Estate Road 3 #07-06 Singapore.

For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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

Revision history: Pub. No. MAN0018881

Revision	Date	Description
B.0	15 March 2022	Updated the control DNA for the kit.
A.0	22 September 2020	New user guide for the CarrierMax™ FMR1 Reagent Kit.

Contents

1 Product information

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

Product description

The Applied Biosystems™ CarrierMax™ FMR1 Reagent Kit is used to detect the number of CGG repeats in the Fragile X Mental Retardation (FMR1) gene. Expansion of these repeats is known to lead to gene silencing. This kit uses a dual PCR system combining full length and triplet primed PCR amplification (TP-PCR), followed by fragment analysis on the Applied Biosystems™ 3500/3500xL Genetic Analyzer or the Applied Biosystems™ SeqStudio™ Genetic Analyzer, to accurately determine CGG repeats up to 200 copies and detect alleles that are > 200 CGG repeats.

Contents and storage

Table 1 CarrierMax™ FMR1 Reagent Kit

Contents	Cat. No. 952362 (48 reactions)	Storage
CarrierMax™ FMR1 Reagent PCR Reaction Buffer	1104 µL	–25°C to –15°C. Protect from light[1].
CarrierMax™ FMR1 Reagent Enzyme Mix	32 µL	
CarrierMax™ FMR1 Reagent Full Length Primers Mix	30 µL	
CarrierMax™ FMR1 Reagent Repeat Primers Mix	30 µL	
CarrierMax™ FMR1 Reagent Nuclease-free water	400 µL	
CarrierMax™ FMR1 Reagent QD1200 Size Standard	50 µL	

[1] The kit is stable for one year when stored at –25°C to –15°C. Do not freeze, then thaw more than 5 times.

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 2 Materials for generating PCR products

Item	Source
Instruments and equipment	
One of the following thermocyclers:[1] • Veriti™ Thermal Cycler • ProFlex™ 96-well PCR System • GeneAmp™ PCR System 9700	Contact your local sales office.
Benchtop microcentrifuge	MLS
Vortex mixer	MLS
Adjustable micropipettors	MLS
Plates and other consumables	
PureLink™ Genomic DNA Mini Kit or equivalent[2]	K1820-00
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4306737
MicroAmp™ Clear Adhesive Film, or equivalent	4306311
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	AM12450
Aerosol-resistant pipette tips	MLS
Other plastic consumables	**thermofisher.com/plastics**
Controls	
CarrierMax™ FMR1 Control DNA Kit	942414

[1] You can use an equivalent thermal cycler. Optimize the protocols for other thermal cyclers.
[2] The MagMAX™ DNA Multi-Sample Ultra 2.0 Kit (Cat. No. A36570) can be used for automated sample preparation.

Table 3 Materials for capillary electrophoresis

Item	Source
Instruments and equipment	
One of the following genetic analyzers: • 3500/3500xL Genetic Analyzer with: – 3500 Data Collection Software (v3.3 or later; Windows™ 10 operating system) • SeqStudio™ Genetic Analyzer with: – (Recommended) SeqStudio™ Plate Manager	Contact your local sales office.
Biosafety cabinet	MLS
Benchtop microcentrifuge	MLS
Vortex mixer	MLS
Adjustable micropipettors	MLS
General reagents	
Hi-Di™ Formamide	4311320
CarrierMax™ A5D Matrix Standard Kit	952364
Consumables and reagents for the 3500/3500xL Genetic Analyzer	
Septa Cathode Buffer Container (for the 3500 series Genetic analyzers)	4410715
Septa for 3500/3500xL Genetic Analyzers, 96 well	4412614
Anode Buffer Container	4393927
Cathode Buffer Container	4408256
Polymer, POP-7™ (96)	A26073
Conditioning reagent	4393718
Capillary array, 8-Capillary, 50-cm	4404685
Capillary array, 24-Capillary, 50-cm	4404689
Retainer & Base Set (Standard) for 3500/3500xL Genetic Analyzers, 96 well	4410228
Retainer & Base Set (Standard) for 3500/3500xL Genetic Analyzers, 8 tube	4410231
Consumables and reagents for the SeqStudio™ Genetic Analyzer	
Septa for SeqStudio™ Genetic Analyzer, 96 well	A36541
Septa for SeqStudio™ Genetic Analyzer, 8 strip	A36543

Table 3 Materials for capillary electrophoresis *(continued)*

Item	Source
SeqStudio™ Cartridge or SeqStudio™ Cartridge v2	A33671 or A41331
Cathode Buffer Container	A33401
Reservoir Septa	A35640
Plates and other consumables	
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4306737
MicroAmp™ Optical 96-Well Reaction Plate	N8010560
MicroAmp™ Clear Adhesive Film, or equivalent	4306311
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	AM12450

Table 4 Materials to analyze data

Item	Source
GeneMapper™ Software v5.0 or later	Contact your local sales office.
CarrierMax™ Software	Complimentary download from **thermofisher.com/carriermax-fmr1**.

Workflow

System workflow

Chapter 2, Prepare for capillary electrophoresis

Chapter 3, Prepare samples and run the PCR

Chapter 4, Analyze the results

2 Prepare for capillary electrophoresis

3500/3500xL Genetic Analyzer

Perform spectral calibration

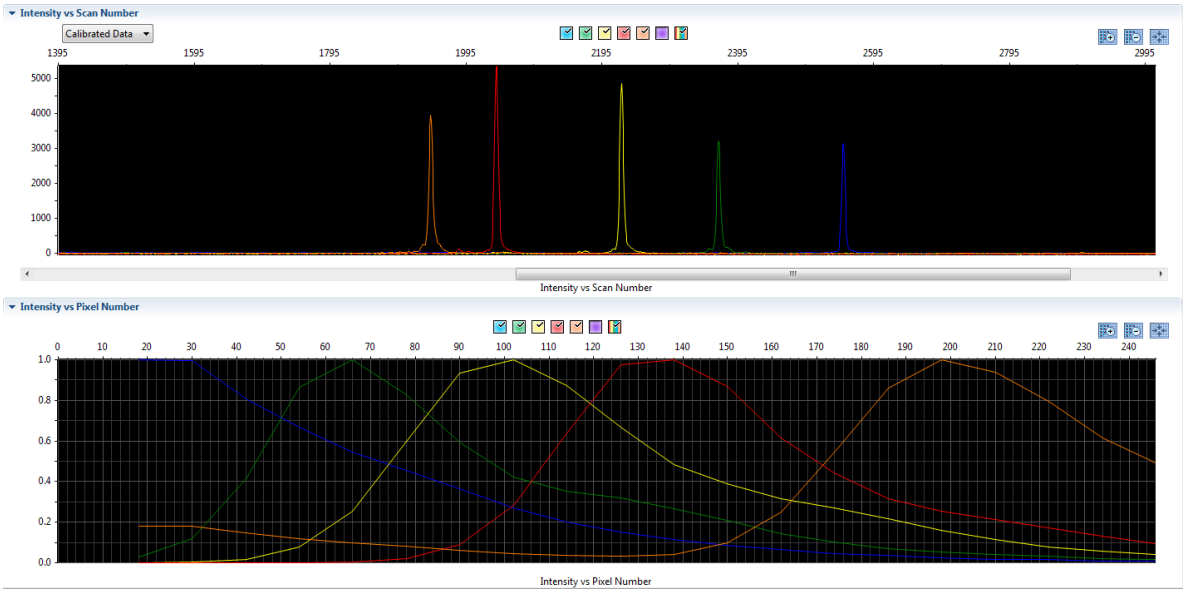
Before using the CarrierMax™ FMR1 Reagent Kit for the first time, perform a spectral calibration with the CarrierMax™ A5D Matrix Standard.

Spectral calibration is required each time a new array is installed.

1. (First time only) Create an **A5D Matrix Standard** dye set in the 3500 Series Data Collection Software.
   a. Go to **Library**, select **Dye Set**, then click **Create**.
   b. Go to the **Dye Set Name**, then enter **A5D**. Choose **Matrix Standard** in **Chemistry**.
   c. Go to **Dye Set Template**, then select **E5 Template**.
   d. Click **Save**.
2. Perform a spectral calibration using the CarrierMax™ A5D Matrix Standard Kit (Cat. No. 952364).

   IMPORTANT! A spectral calibration must be performed for the A5D Dye Set to run the assay.

   See the *CarrierMax™ A5D Matrix Standard Kit Product Information Sheet* (Pub. No. MAN0018893).
   The following figure is an example of a passing 5-dye spectral calibration.



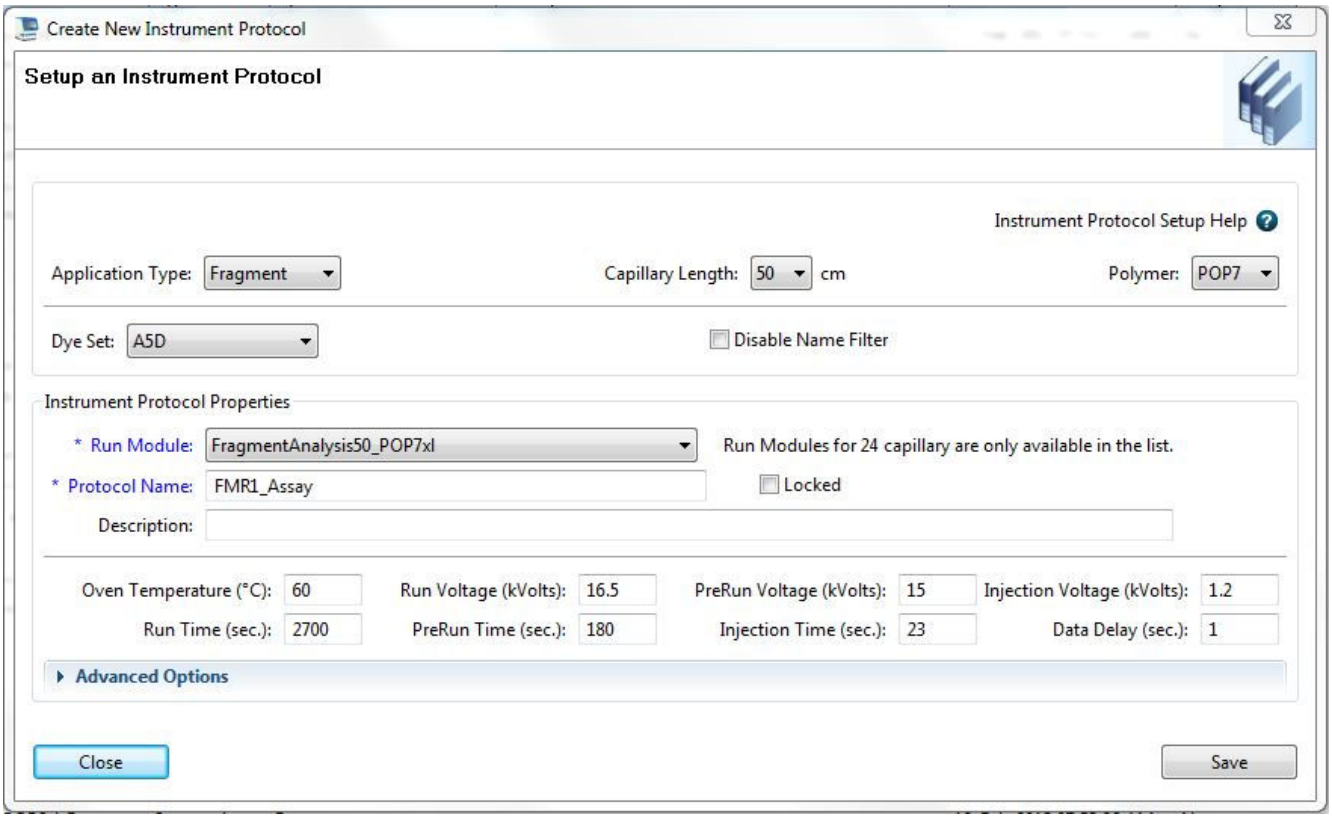
Electrophoresis software setup

(Recommended) Ensure that your genetic analyzer is running on the Windows™ operating system with 3500 Data Collection Software v3.3 or v4.

The following instructions describe setting up an assay. For more detailed information on the instrument procedures, see the documents listed in Appendix C, “Documentation and support”.

Create an instrument protocol

1. Navigate to the **Instrument Protocols** library.
2. Click **Create**.
3. Specify the settings listed below.
   - **Capillary length—50cm**
   - **Polymer—POP7**
   - **Dye Set—A5D**
   - **Run Module—FragmentAnalysis50_POP7xl**
   - **Protocol Name—FMR1_Assay**
   - **Oven Temperature (°C)—60**
   - **Run Time (sec)—2,700**
   - **Run Voltage (kVolts)—16.5**
   - **PreRun Time (sec)—180**
   - **PreRun Voltage (kVolts)—15**
   - **Injection Time (sec)—23**
   - **Injection Voltage (kVolts)—1.2**
   - **Data Delay (sec)—1**

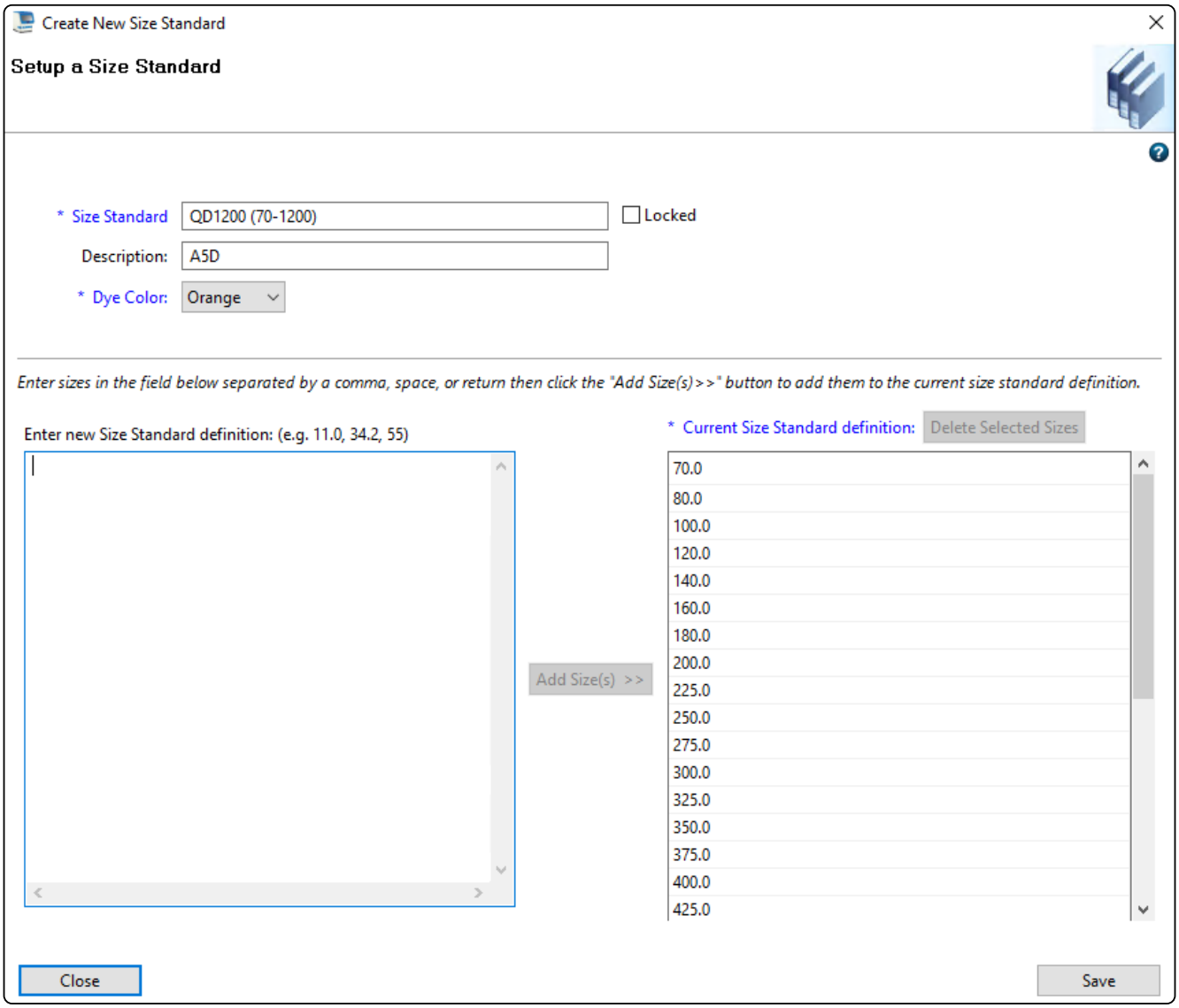


4. Click **Save**.

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 sizecalling (fragment analysis) protocol, you add a size standard to the protocol.

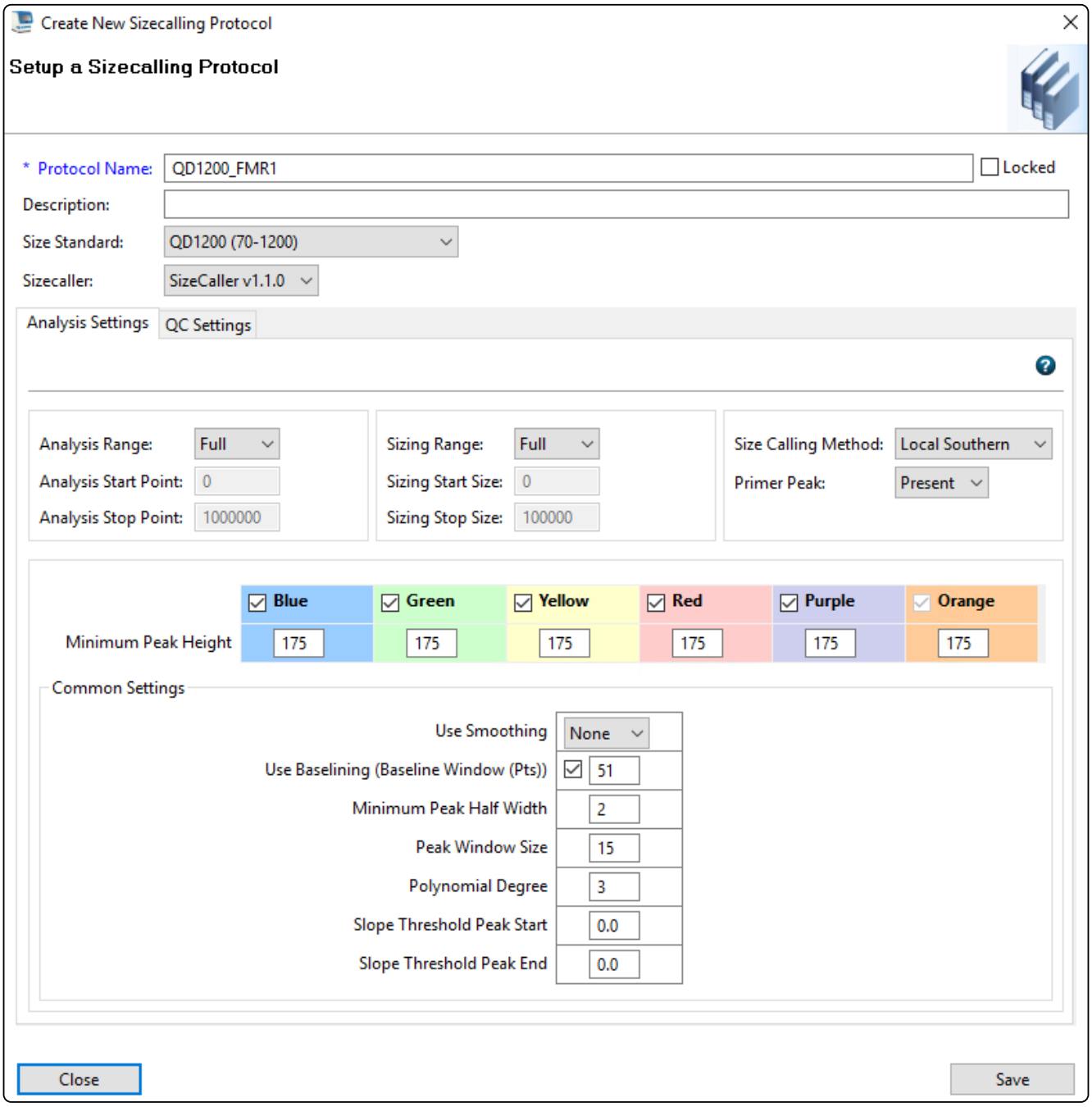
1. Navigate to the **Size Standards** library.
2. Click **Create**.
3. Specify the settings listed below.
   - **Size Standard name**—**QD1200 (70-1200)**; Select **Locked**.
   - **Description**—**A5D**
   - **Dye Color**—**Orange**
   - **Size Standards** (list in left text box)—**70, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 600, 650, 700, 750, 800, 850, 900, 950 1000, 1100, 1200**


Create New Size Standard
Setup a Size Standard
* Size Standard
QD1200 (70-1200)
Locked
Description:
A5D
* Dye Color:
Orange
Enter sizes in the field below separated by a comma, space, or return then click the "Add Size(s) >>" button to add them to the current size standard definition.
Enter new Size Standard definition: (e.g. 11.0, 34.2, 55)
Add Size(s) >>
* Current Size Standard definition:
Delete Selected Sizes
70.0
80.0
100.0
120.0
140.0
160.0
180.0
200.0
225.0
250.0
275.0
300.0
325.0
350.0
375.0
400.0
425.0
Close
Save

4. Click **Save**.

Create the sizecalling protocol

1. Navigate to the **Sizecalling Protocols** library.
2. Click **Create**.
3. Specify the settings listed below.
   - **Protocol Name**—**QD1200_FMR1**
   - **Sizecaller**—**SizeCaller v1.1.0**
   - **Analysis Range and Sizing Range**—**Full**
   - **Size Calling Method**—**Local Southern**
   - **Primer Peak**—**Present**
   - **Minimum Peak Height**—175 (all)
   - **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)


Create New Sizecalling Protocol
Setup a Sizecalling Protocol
* Protocol Name:
QD1200_FMR1
Locked
Description:
Size Standard:
QD1200 (70-1200)
Sizecaller:
SizeCaller v1.1.0
Analysis Settings
QC Settings
Analysis Range:
Full
Analysis Start Point:
0
Analysis Stop Point:
1000000
Sizing Range:
Full
Sizing Start Size:
0
Sizing Stop Size:
100000
Size Calling Method:
Local Southern
Primer Peak:
Present
Blue
Green
Yellow
Red
Purple
Orange
Minimum Peak Height
175
175
175
175
175
175
Common Settings
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
0.0
Slope Threshold Peak End
0.0
Close
Save

4. Click **Save**.

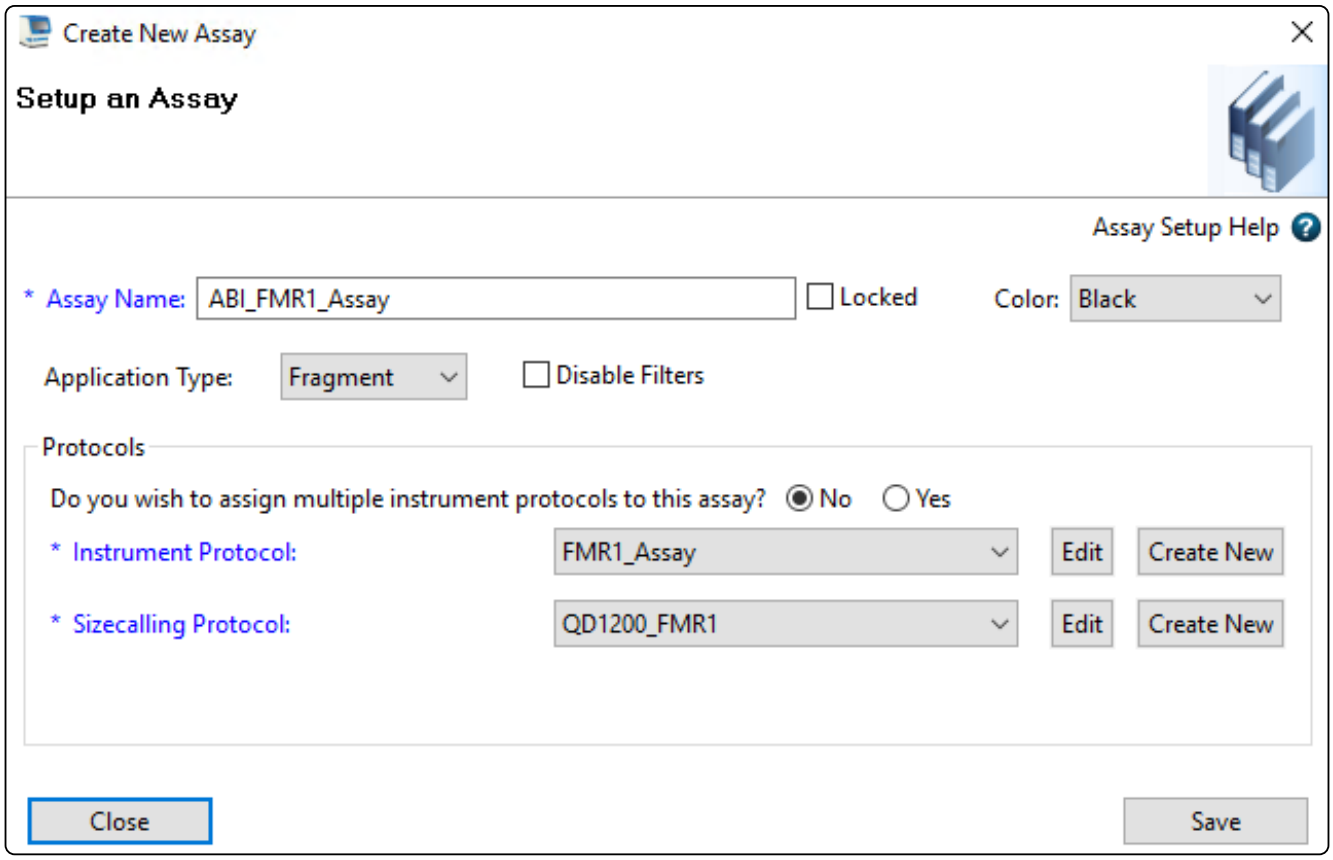
Create the assay

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 **Create**.

3. Specify the settings.
   - **Assay Name**—ABI_FMR1_Assay
   - **Color**—Black
   - **Application Type**—Fragment
   - **Do you wish to assign multiple protocols to this assay?**—No
   - **Instrument Protocol**—**FMR1_Assay** (select from the dropdown list)
   - **Sizecalling Protocol**—**QD1200_FMR1** (select from the dropdown list)



4. Click **Save**.

5. To run the **ABI_FMR1_Assay** on the instrument, create a **Plate Record**. The **Plate Record** will use the **ABI_FMR1_Assay** and include sample names. See the *3500/3500xL Genetic Analyzer User Guide* (Pub. No. 100079380) for information on creating a **Plate Record**.

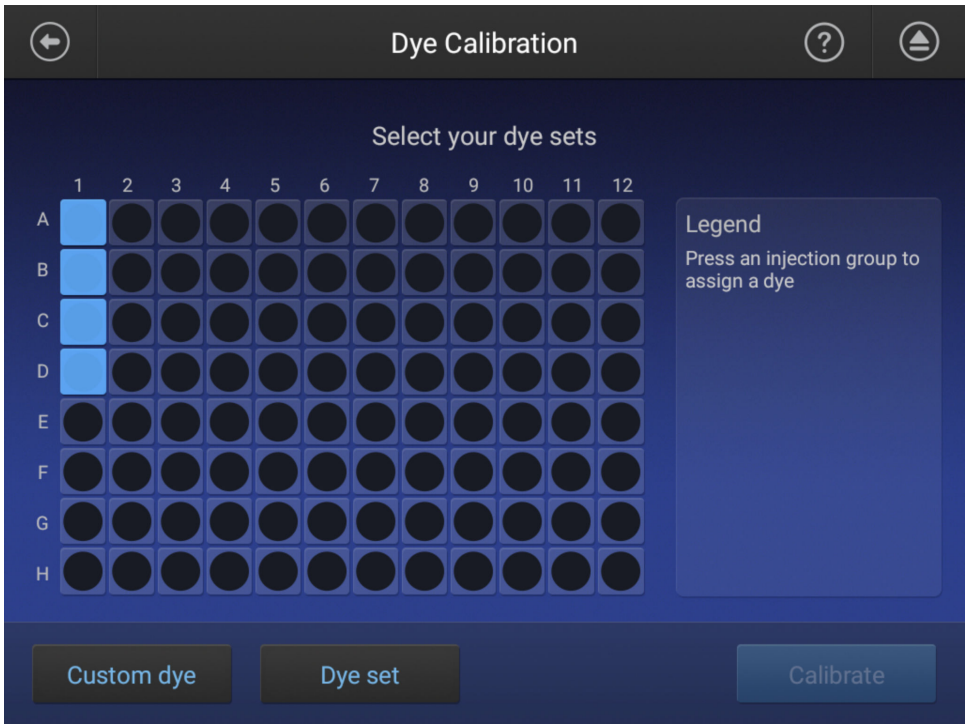
SeqStudio™ Genetic Analyzer

Perform a system dye calibration

A system dye calibration requires ~30 minutes to complete.

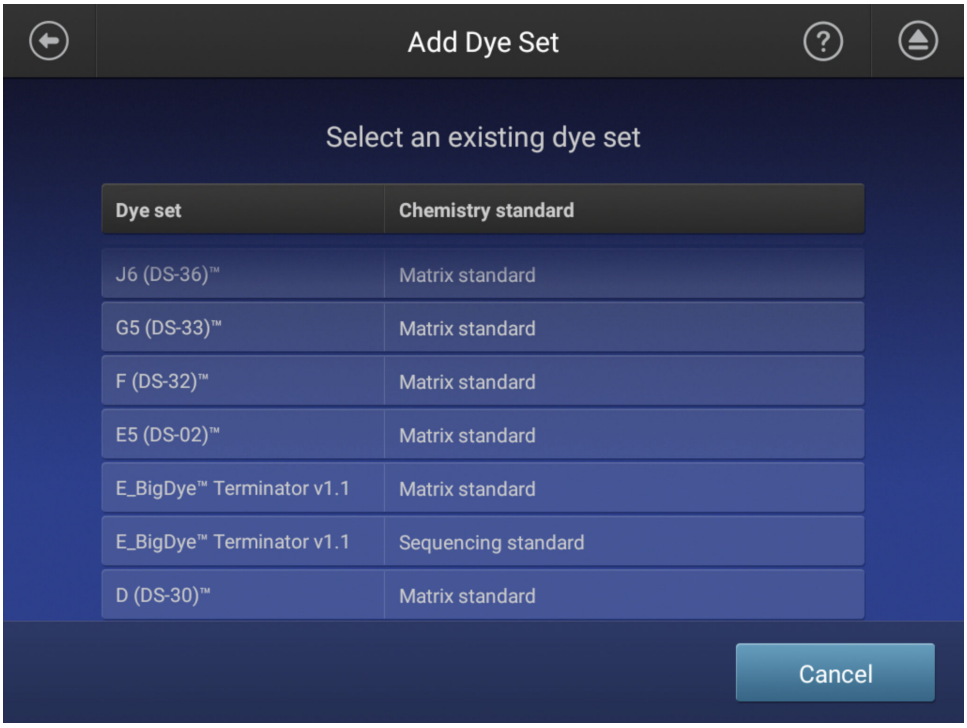
Prepare the dye set calibration standards and plate as described in the *CarrierMax™ A5D Matrix Standard Kit Product Information Sheet* (Pub. No. MAN0018893).

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

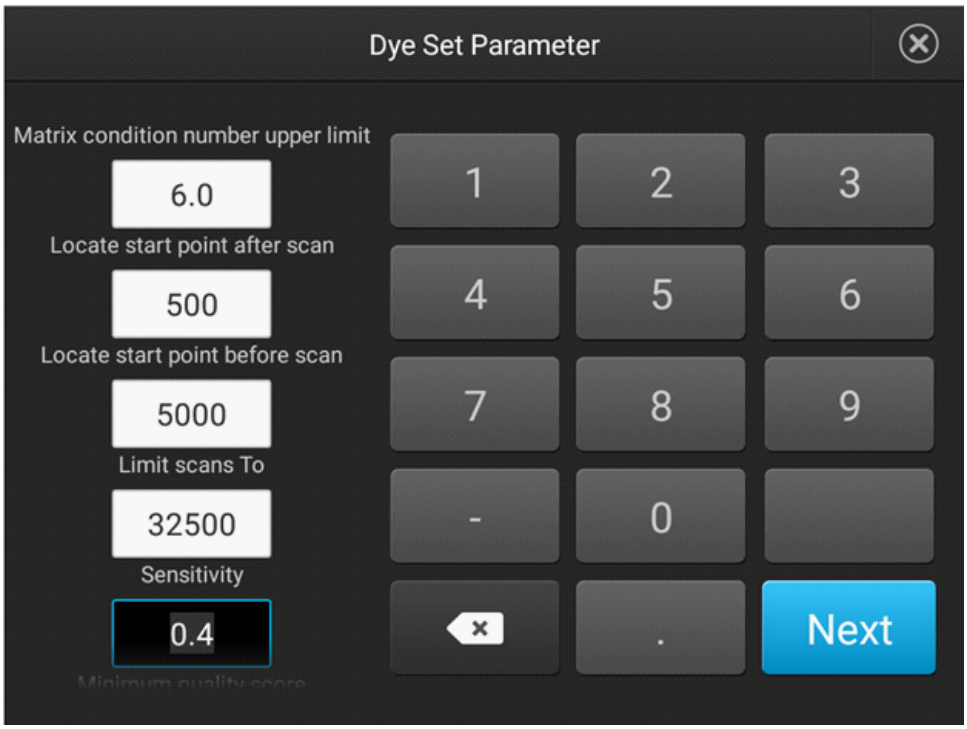


3. In the **Dye Set** screen, tap **Add**.

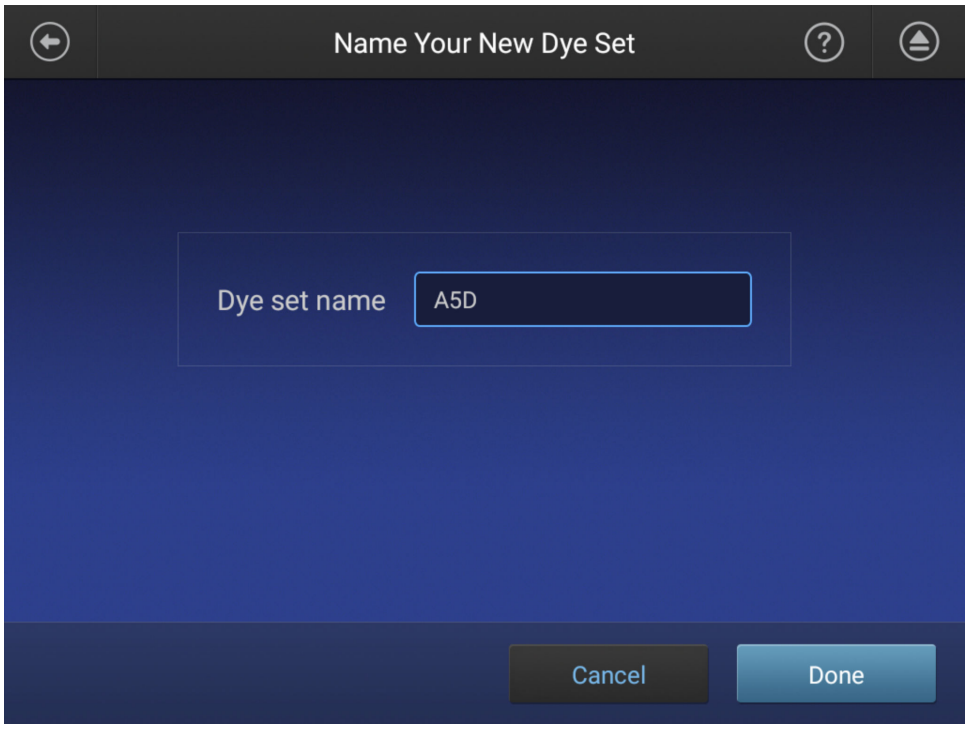
4. Select E5 (DS-02) as a starting template.



5. In the **Dye Set Parameter** screen, tap **Next**.



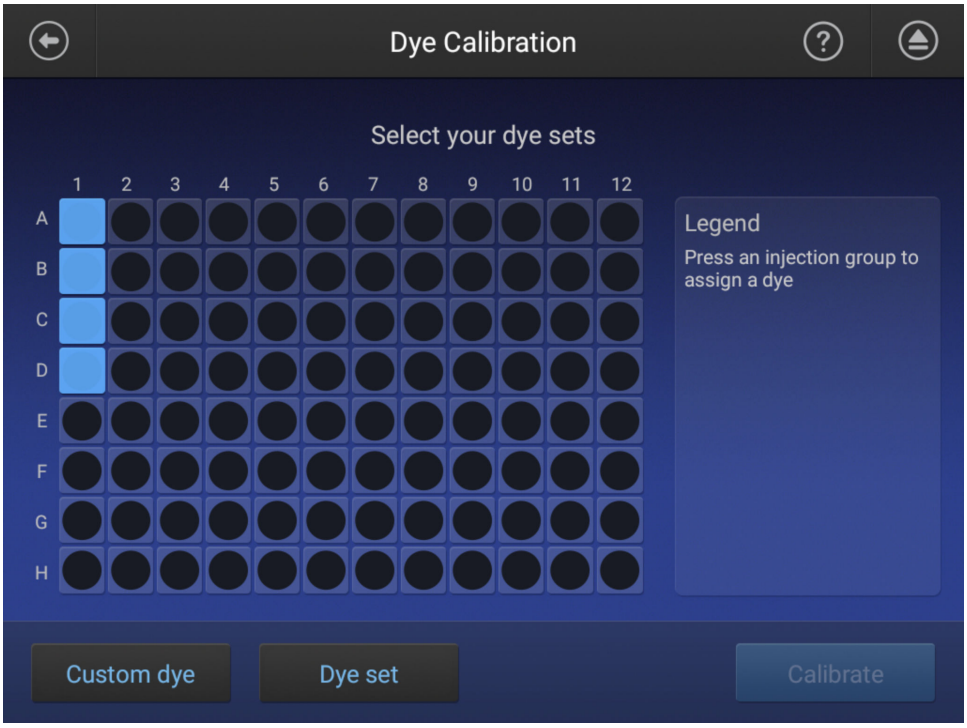
6. Set the **Dye set name** to **A5D**, then click **Done**.



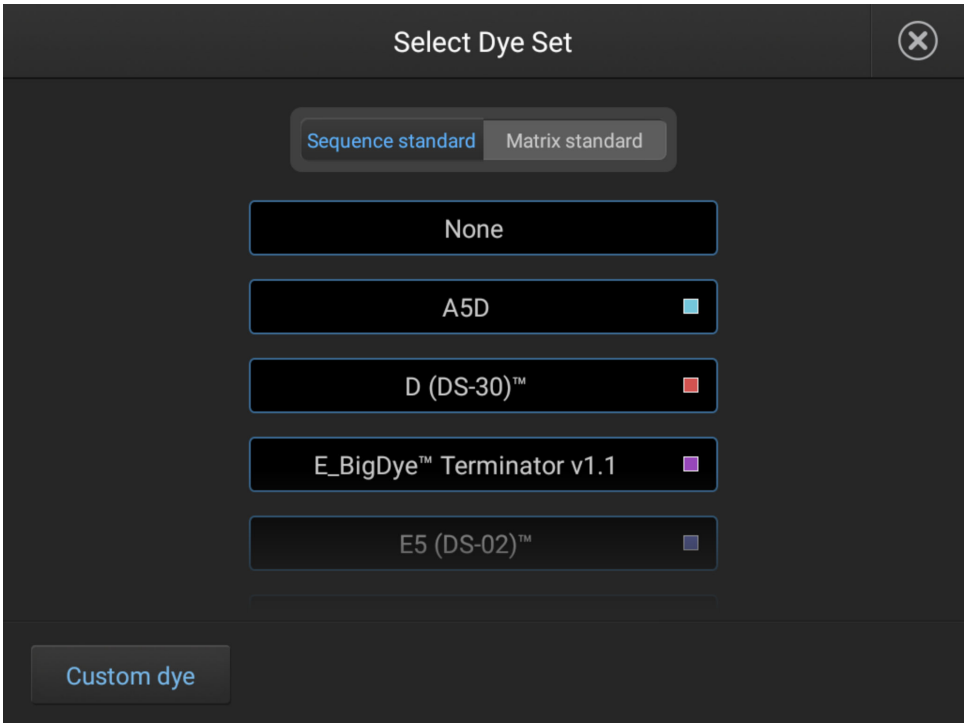
A5D is then listed in the **Dye Set** list.

7. Return to the home screen, tap **Settings** ▸ **Maintenance and Service** ▸ **Calibration** ▸ **Dye Calibration**.

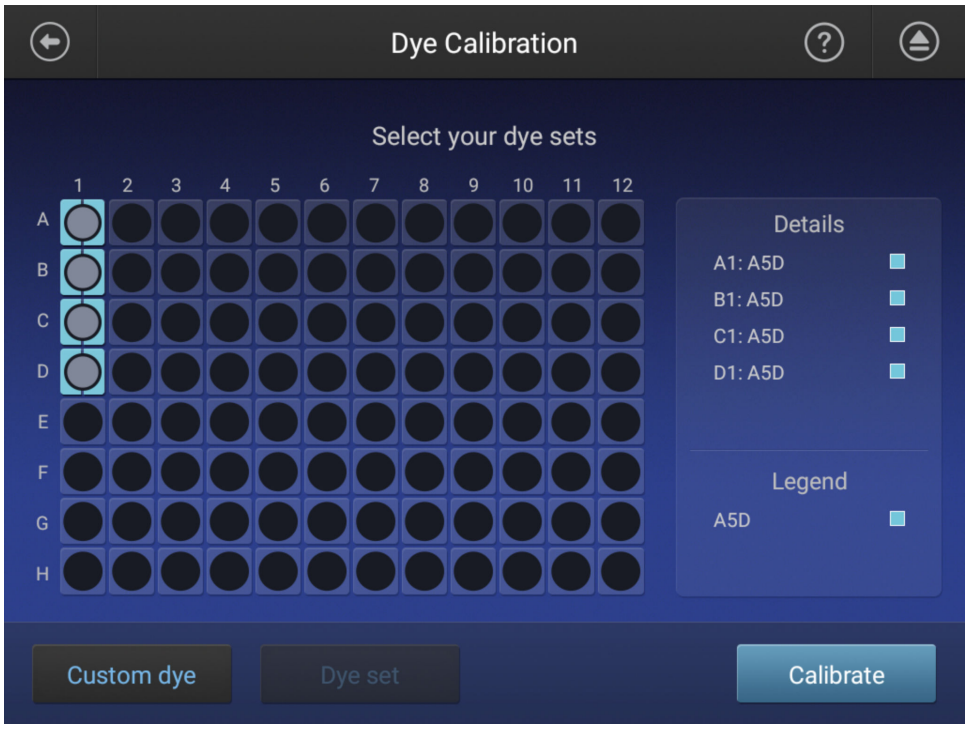
8. Highlight the injection group to assign a dye, then tap **Dye set**.



9. Tap **Matrix Standard**, then select the **A5D** matrix standard.



10. Tap **Calibrate**.



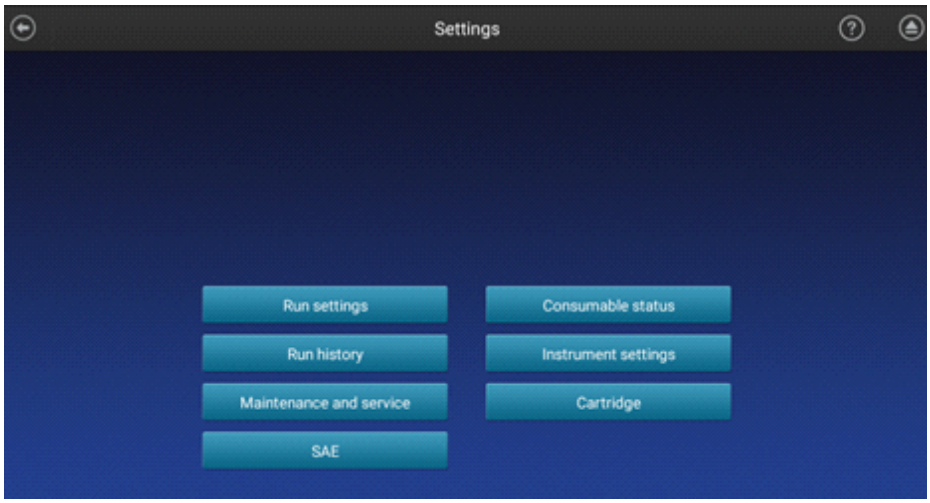
The calibration run starts.

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.

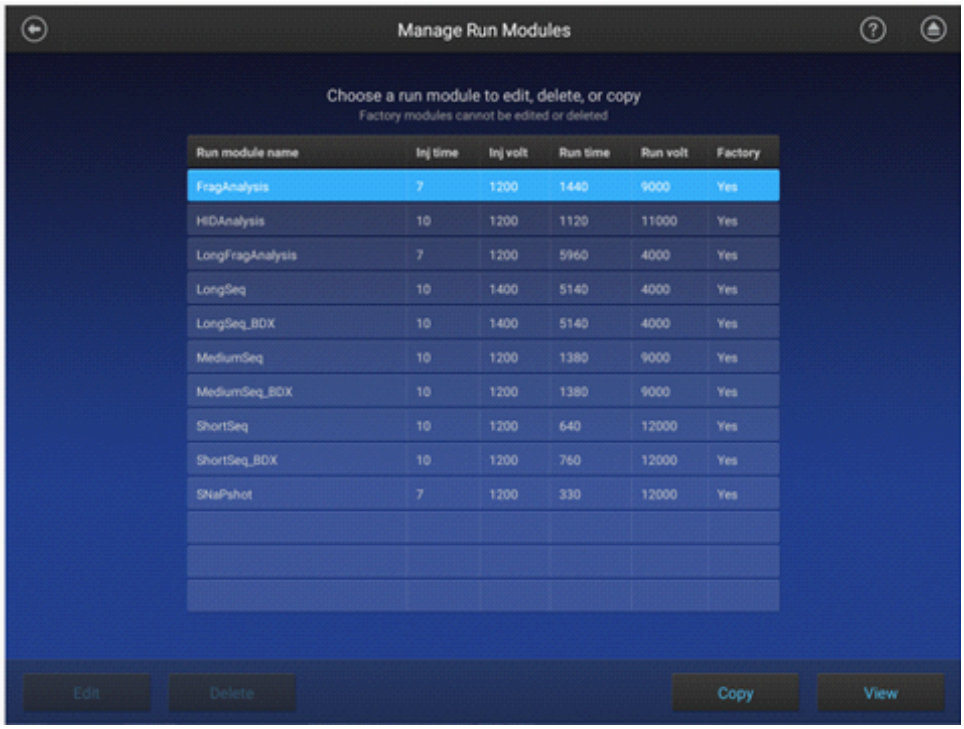
Create the CarrierMax™ FMR1 Reagent Kit run module

1. On the SeqStudio™ Genetic Analyzer, go to **Settings** ▸ **Run Settings**.



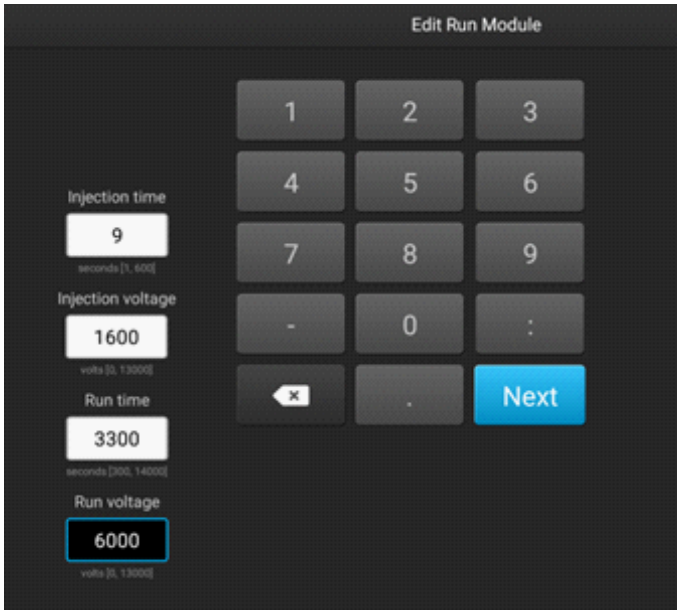
2. Select **Run modules** ▸ **FragAnalysis**, then tap **Copy**.

3. Select the default **FragAnalysis** run module (or a user-created run module), then tap **Copy**.



An editable copy of the **FragAnalysis** run module is created.

4. In the **Edit Run Module** screen enter the following parameters.
   - Injection Time—9 seconds
   - Injection Voltage—1,600 volts
   - Separation (Run) Time—3,300 seconds
   - Separation (Run) Voltage—6,000 volts

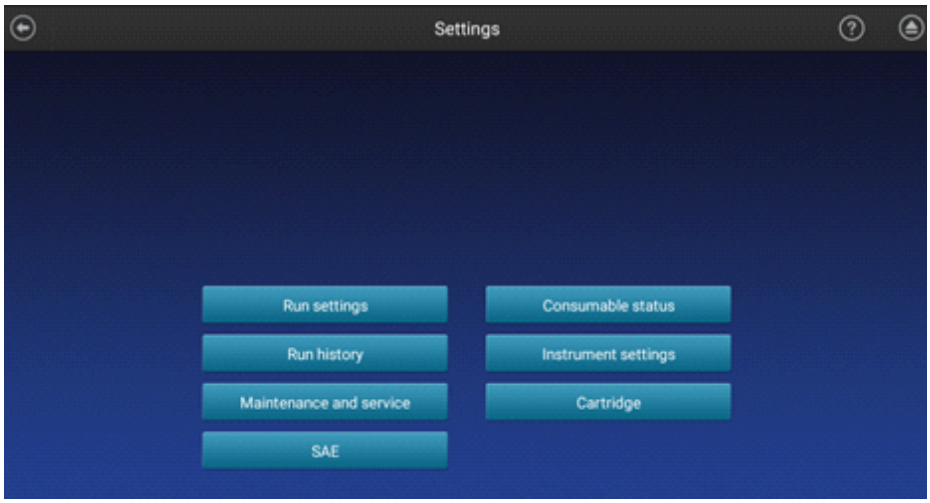


5. Tap **Next**, then change the **Run Module Name** to **ABI_FMR1_Assay**.

6. Tap **Done**.

Create the size standards

1. On the SeqStudio™ Genetic Analyzer, go to **Settings** ▸ **Run Settings**.



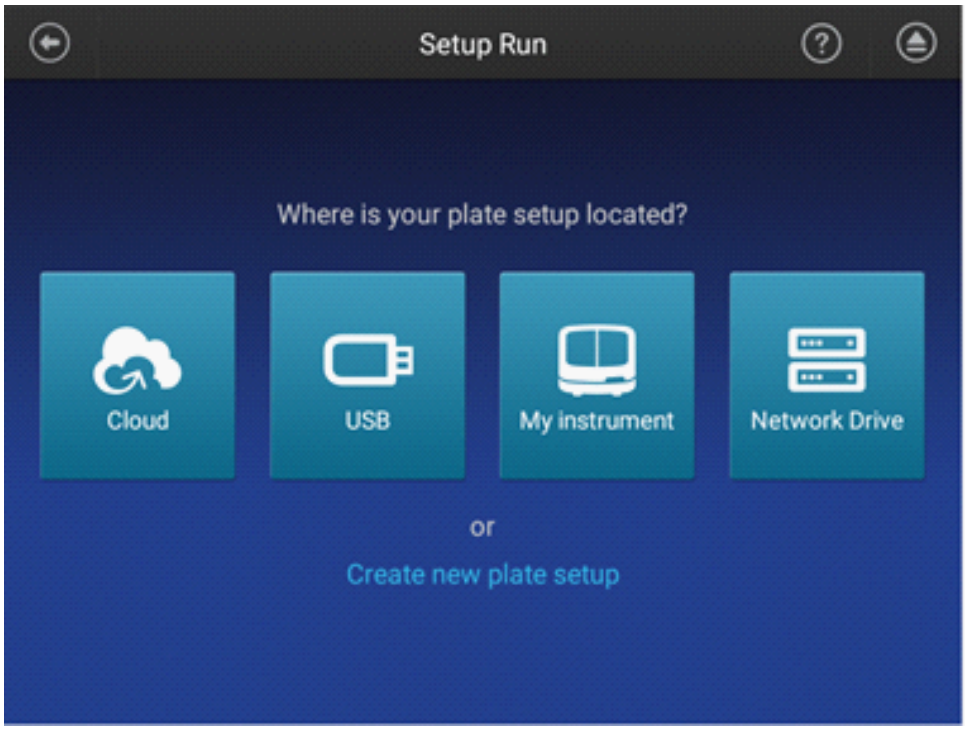
2. Select **Size standard** ▸ **GS1200LIZ**, then tap **Copy**.
   An editable copy of the size stanadard is created.

3. In the **Edit Size Standard** screen enter the following parameters.
   - **Name—QD1200**
   - **Dye (Color)—Orange**
   - **Fragment sizes—70, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200**

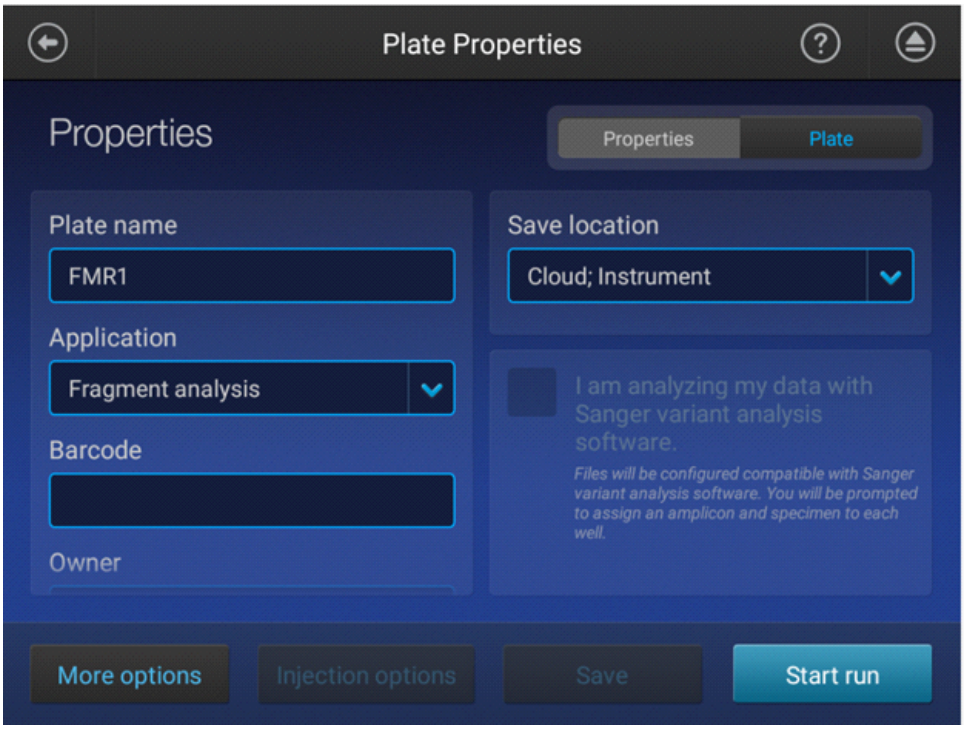
4. Tap **Done**.

Create a new plate setup

1. On the SeqStudio™ Genetic Analyzer, tap **Setup run** ▸ **Create new plate setup**.

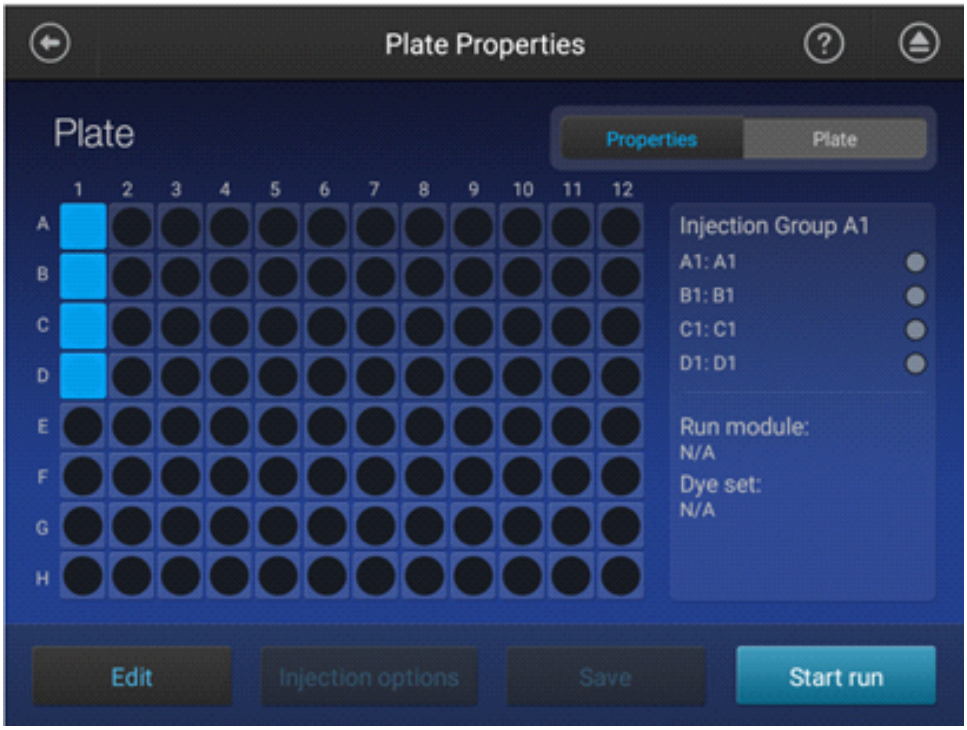


2. In the **Properties** tab of the **Plate Properties** screen, enter the following settings.
   - **Plate Name—FMR1**
   - **Application—Fragment analysis**
   - **Save location—Cloud; Instrument**

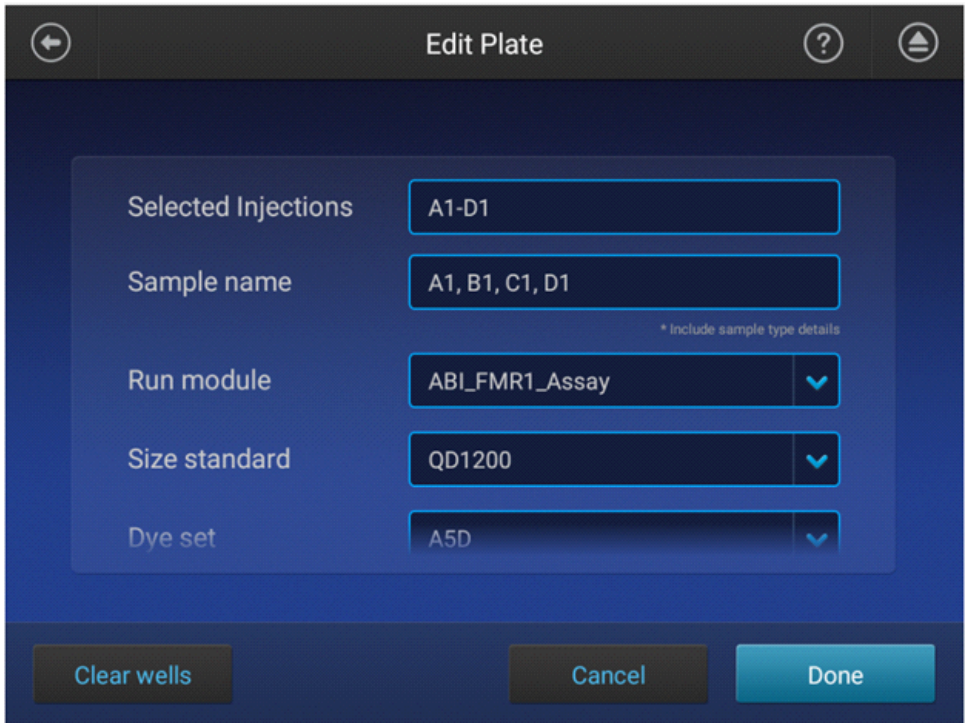


3. Tap the **Plate** tab.

4. Highlight the **Injection Groups**, then tap **Edit**.

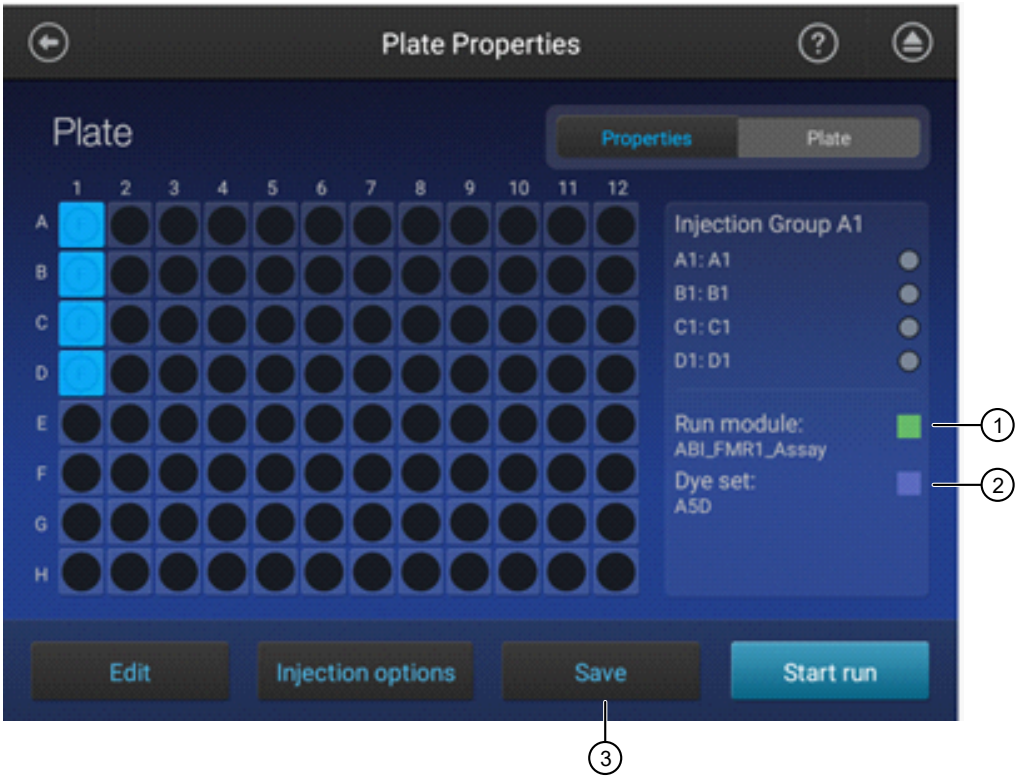


5. In the **Edit Plate** screen, make the following selections.
   - **Run module—ABI_FMR1_Assay**
   - **Size standard—QD1200**
   - **Dye set—A5D**



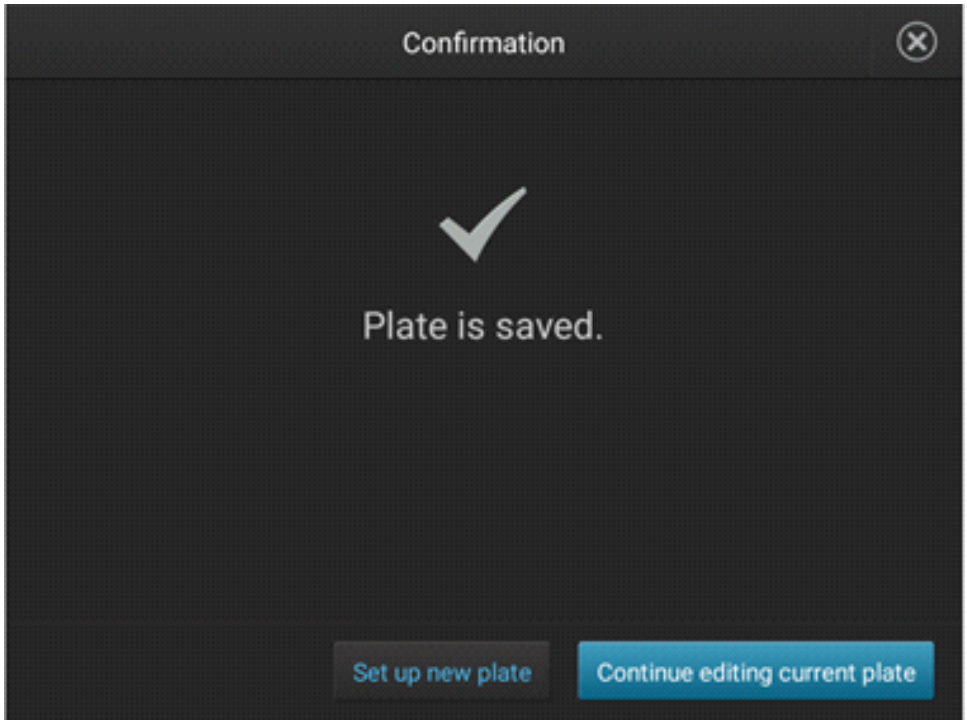
6. Tap **Done**.

7. Highlight the injection groups to confirm the correct **Run module** and **Dye set** have been selected, then tap **Save**.



① **Run module**
② **Dye set**
③ **Save**

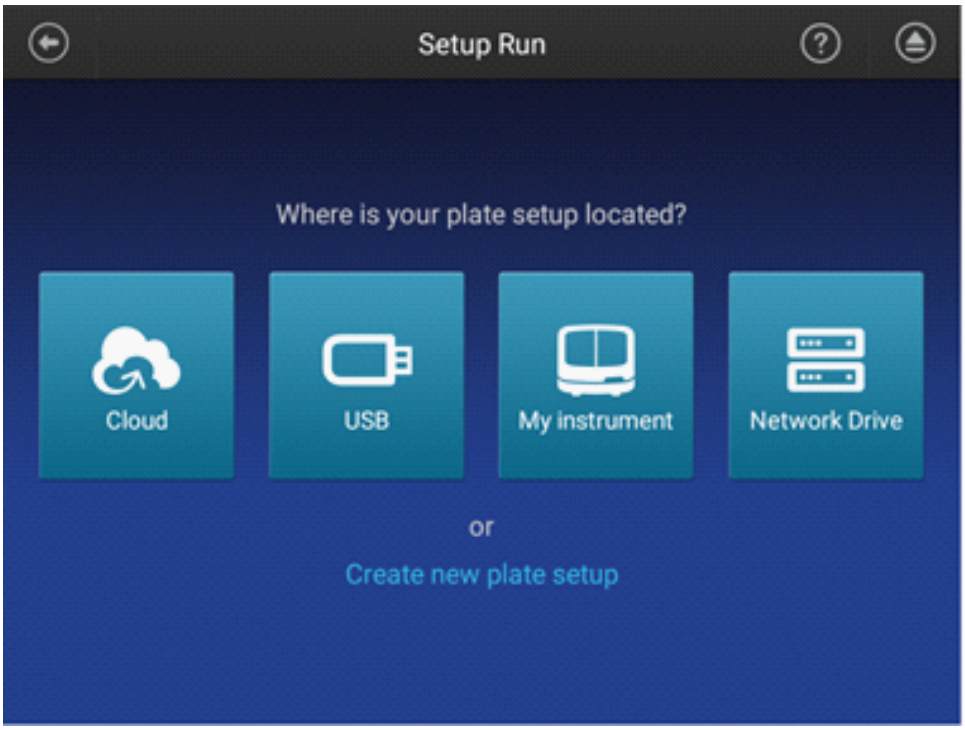
The plate is saved on the instrument.


Confirmation
Plate is saved.
Set up new plate
Continue editing current plate

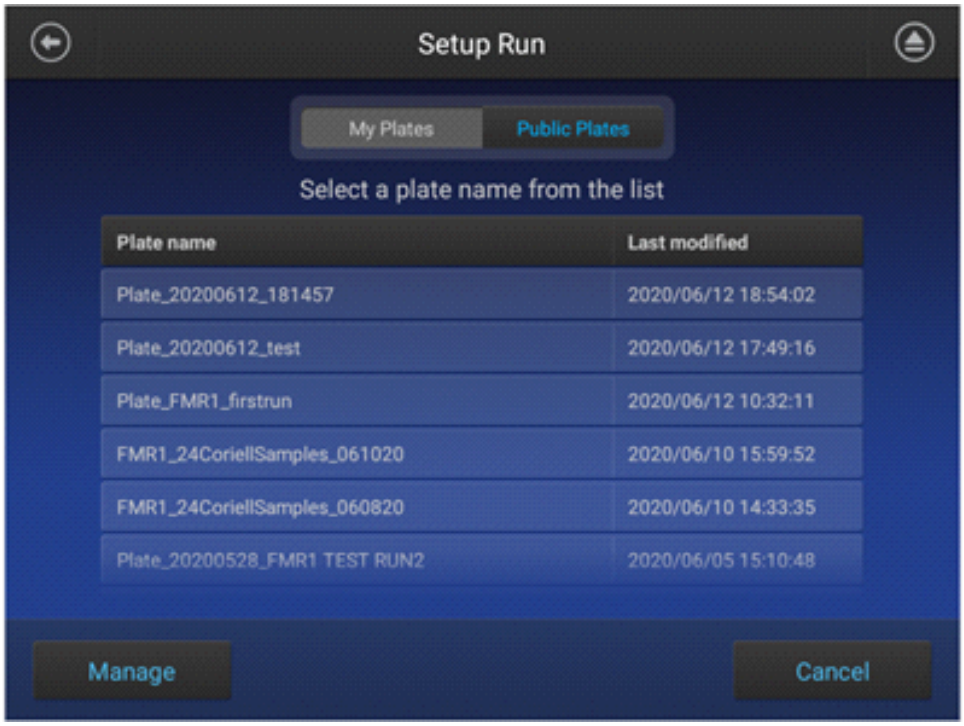
Export the SeqStudio™ Genetic Analyzer plate setup to the Cloud

The plate setup created on the SeqStudio™ Genetic Analyzer can be exported to the Cloud for future experiment setup in SeqStudio™ Plate Manager. SeqStudio™ Plate Manager allows for easier import of samples and for plate setup off the instrument.

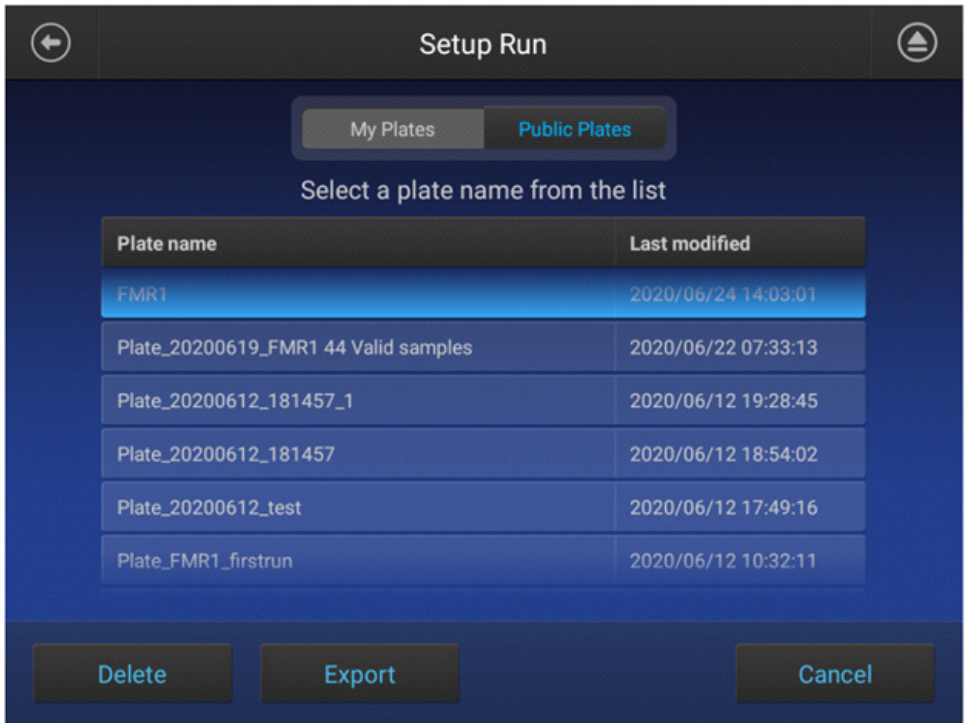
1. On the SeqStudio™ Genetic Analyzer homescreen, tap **Setup run** ▸ **My instrument**.



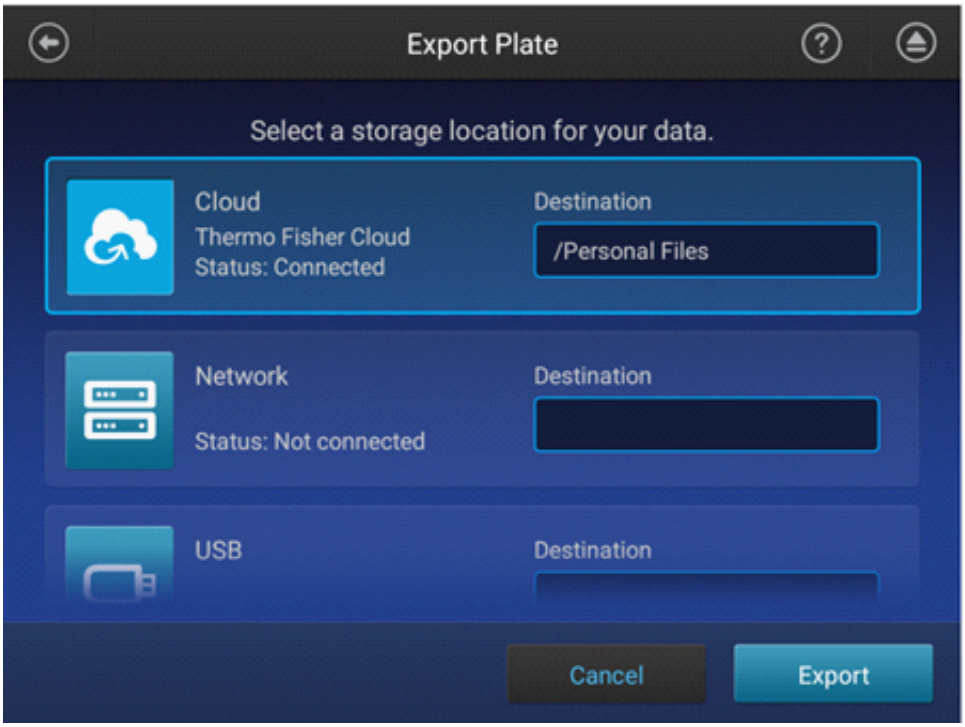
2. Tap **Manage**.



3. Select the **Plate name** to export, then tap **Export**.

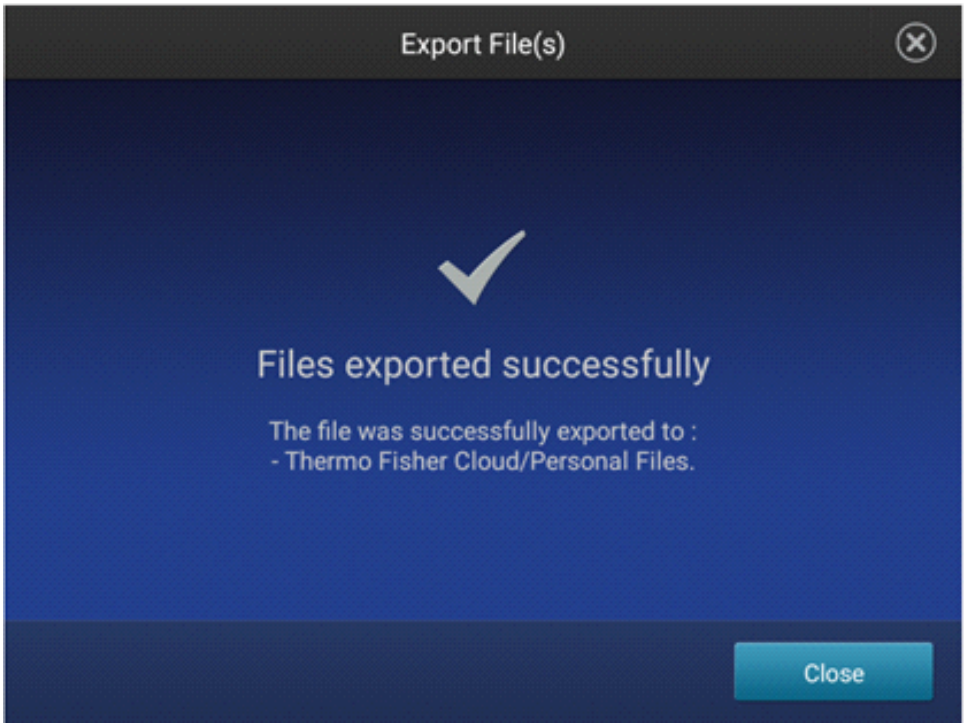


4. Select the **Cloud** storage destination, then tap **Export**.

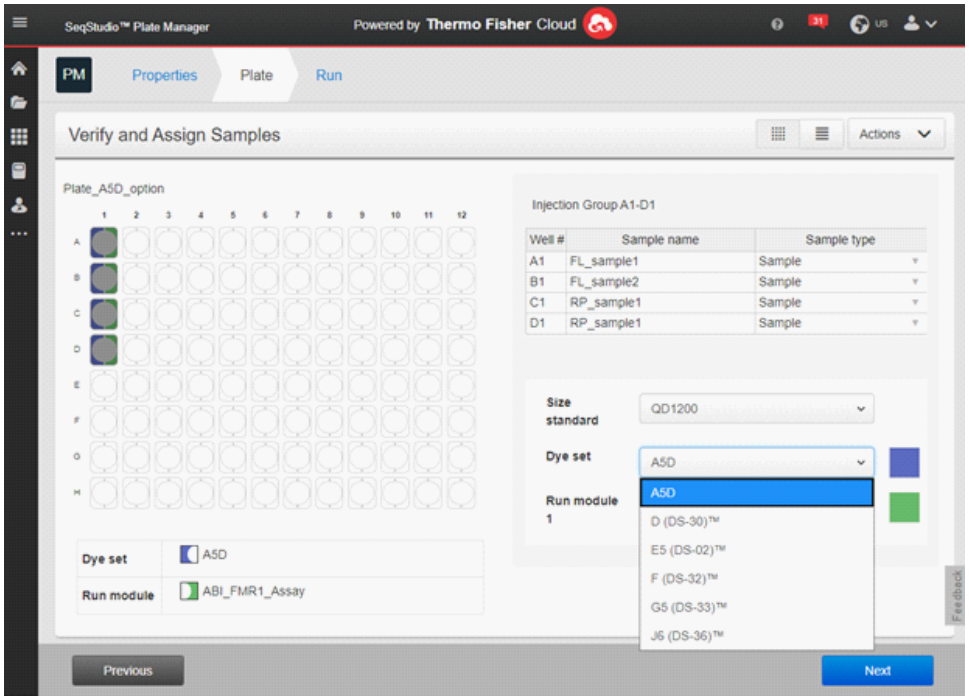


The plate setup is exported to the **Thermo Fisher Cloud** (PSM file).

5. Tap **Close**.



Note: The **A5D** dye set is now availabe for new plate setup in SeqStudio™ Plate Manager.



The plate setup is now saved and available for editing in the **Thermo Fisher Cloud**.

3 Prepare samples and run the PCR

DNA sample preparation

This kit is optimized for use with genomic DNA. We recommend extraction with the PureLink™ Genomic DNA Mini Kit (Cat. No. K1820-00) and fluorometric methods for DNA quantification such as the Qubit™ Fluorometer and the Qubit™ dsDNA HS (High Sensitivity) Assay Kit (Cat No. Q32851). Alternatively, dsDNA can also be quantitated using Quant-iT™ PicoGreen™ dsDNA Assay Kit (Cat. No. P7589) on a microplate reader. The optimal and recommended DNA concentration is 40 ng/ µL. Store at 2–8°C for up to one week, or at –20°C for up to 6 months.

Note: We do not recommend methods such as densitometry for this assay (for example, a NanoDrop™ Spectrophotometer). These methods do not discriminate between DNA and RNA and are sensitive to small RNA fragments leading to over-estimation of dsDNA yield.

Optimize all protocols with your standard laboratory procedures.

Set up the PCR reactions

- Thaw all components (except the CarrierMax™ FMR1 Reagent Enzyme Mix) to room temperature, then vortex and briefly centrifuge to mix. Place the tubes on ice.
- Remove the tube of CarrierMax™ FMR1 Reagent Enzyme Mix from the freezer immediately before use, gently flick the tube 3 times to mix, then centrifuge briefly. Place the tube on ice.

IMPORTANT! Perform all steps on ice.

1. In a 1.5 mL microfuge tube (Nonstick, RNase-Free Microfuge Tubes, 1.5 mL), prepare the **PCR Reaction Mix**.

Component	Volume per reaction[1]	
	Full length	CGG Repeat
CarrierMax™ FMR1 Reagent PCR Reaction Buffer	11.5 µL	11.5 µL
CarrierMax™ FMR1 Reagent Full Length Primers Mix	0.6 µL	—
CarrierMax™ FMR1 Reagent Repeat Primers Mix	—	0.6 µL
CarrierMax™ FMR1 Reagent Enzyme Mix	0.3 µL	0.3 µL
CarrierMax™ FMR1 Reagent Nuclease-free water	1.6 µL	1.6 µL
Total volume	**14 µL**	**14 µL**

[1] Scale reaction volume according to the number of reactions, including overage for pipetting.

2. Vortex the **PCR Reaction Mix**, then centrifuge briefly before use.
3. To the labeled PCR reaction plate, add the following components.
   a. Add 14 µL of PCR reaction mix to each sample or no template control (NTC) well.
   b. Add one of the following to sample wells:
      - 1 µL of sample DNA (40 ng recommended)
      - 1 µL of CarrierMax™ FMR1 Reagent Nuclease-free water (NTC)
      - (*Optional*) 40 ng of control from the CarrierMax™ FMR1 Control DNA Kit (see "Required materials not supplied" on page 6)
4. Seal the plate tightly with adhesive film. Vortex briefly to mix, then centrifuge briefly to bring the mixture to the bottom of the tube and eliminate air bubbles.
5. Immediately proceed to "Run the PCR" on page 36.

Run the PCR

1. Program the thermal cycling conditions.

Step	Temperature	Time	Cycles
Hot start	95°C	5 minutes	1
Denature	97°C	35 seconds	10
Anneal	65 to 55°C (–1°C/cycle)	35 seconds	
Extend	68°C	4 minutes	
Denature	97°C	35 seconds	20
Anneal	60°C	35 seconds	
Extend	68°C	4 minutes to 10 minutes 40 seconds (+20 seconds/cycle)	
Extend	68°C	10 minutes	1
Hold	15°C	∞	

2. Set the appropriate reaction volume, 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 24 hours, or at –25°C to –15°C for up to 72 hours. Avoid multiple freeze-thaw steps.

3500/3500xL Genetic Analyzer

Dilute the PCR products

Dilute the PCR products before capillary electrophoresis.

- Dilute full length (FL) PCR products 1:40. For example, 1 µL PCR product + 39 µL of nuclease-free water.
- Dilute repeat primer (RP) PCR products 1:15. For example, 1 µL PCR product + 14 µL of nuclease-free water.

Note: If no amplification is observed after capillary electrophoresis, re-test the sample without dilution.

Prepare samples for capillary electrophoresis

Prepare the samples for capillary electrophoresis immediately before loading. This protocol is for 96-well plates, but MicroAmp™ 8-Tube Strips (0.2-mL) with MicroAmp™ 8-Cap Strips can also be used.

1. In a 1.5 mL microfuge tube, prepare the mix of Hi-Di™ Formamide and CarrierMax™ FMR1 Reagent QD1200 Size Standard for the required number of reactions.

Component	Volume per reaction
CarrierMax™ FMR1 Reagent QD1200 Size Standard	0.3 µL
Hi-Di™ Formamide	8.7 µL
Total volume	9 µL

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

2. Vortex the mix of Hi-Di™ Formamide and CarrierMax™ FMR1 Reagent QD1200 Size Standard well, then centrifuge briefly before use.

3. Prepare the fragment analysis reactions. To a MicroAmp™ Optical 96-Well Reaction Plate, add the following components.
   - 9 µL of the Hi-Di™ Formamide and CarrierMax™ FMR1 Reagent QD1200 Size Standard mix
   - 1 µL of diluted PCR product (see “Run the PCR” on page 36)

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

4. Seal the reaction plate with MicroAmp™ Clear Adhesive Film.

5. Vortex the reaction plate well, then centrifuge briefly to bring the contents to the bottom of the wells and eliminate air bubbles.

6. Denature the DNA fragments:
   a. Incubate the mixture at 95°C for 3 minutes.

   b. Incubate the mixture on ice for 3 minutes.

7. Centrifuge the plate briefly bring the contents to the bottom of the wells and eliminate air bubbles.
8. Remove the adhesive film, then cover the plate with a septa.
   a. Align the holes on the septa with the wells of the plate.
   b. Press firmly until the septa snaps into position.
9. Assemble the plate with the retainer and base.
10. Load the plate on the instrument.

Run the reactions on the 3500/3500xL Genetic Analyzer. See the instrument user guide for specifics on setting up the run.

SeqStudio™ Genetic Analyzer

Dilute the PCR products

Dilute the PCR products before capillary electrophoresis.

- Dilute full length (FL) PCR products 1:25. For example, 1 µL PCR product + 24 µL of nuclease-free water.
- Dilute repeat primer (RP) PCR products 1:5. For example, 2 µL PCR product + 8 µL of nuclease-free water.

Note: If no amplification is observed after capillary electrophoresis, re-test the sample without dilution.

Prepare samples for capillary electrophoresis

Prepare the samples for capillary electrophoresis immediately before loading. This protocol is for 96-well plates, but MicroAmp™ 8-Tube Strips (0.2-mL) with MicroAmp™ 8-Cap Strips can also be used.

1. In a 1.5 mL microfuge tube, dilute the CarrierMax™ FMR1 Reagent QD1200 Size Standard.

Component	Volume
CarrierMax™ FMR1 Reagent QD1200 Size Standard	2 µL
Hi-Di™ Formamide	1 mL
Total volume	1.002 mL

2. Vortex the mix of Hi-Di™ Formamide and CarrierMax™ FMR1 Reagent QD1200 Size Standard well, then centrifuge briefly before use.

3. Prepare the fragment analysis reactions. To a MicroAmp™ Optical 96-Well Reaction Plate, add the following components.
   - 9 µL of the Hi-Di™ Formamide and CarrierMax™ FMR1 Reagent QD1200 Size Standard mix
   - 1 µL of diluted PCR product (see “Run the PCR” on page 36)

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

4. Seal the reaction plate with MicroAmp™ Clear Adhesive Film.

5. Vortex the reaction plate well, then centrifuge briefly to bring the contents to the bottom of the wells and eliminate air bubbles.

6. Denature the DNA fragments:
   a. Incubate the mixture at 95°C for 3 minutes.
   b. Incubate the mixture on ice for 3 minutes.

7. Centrifuge the plate briefly bring the contents to the bottom of the wells and eliminate air bubbles.

8. Remove the adhesive film, then cover the plate with a septa.
   a. Align the holes on the septa with the wells of the plate.
   b. Press firmly until the septa snaps into position.
9. Assemble the plate with the retainer and base.
10. Load the plate on the instrument.

Run the reactions on the SeqStudio™ Genetic Analyzer. See the instrument user guide for specifics on setting up the run.

Naming conventions

IMPORTANT! Sample names must match exactly in the GeneMapper™ Softwareand the CSV export for the CarrierMax™ Software to recognize FL and RP paired reactions.

FL or RP must be added to the file name to be correctly imported.

Example sample names:

- FL-XXX
- RP-XXX

The XXX part of the name must match exactly in the CarrierMax™ Software in order for the FL and RP files to be analyzed as a paired result from the sample.

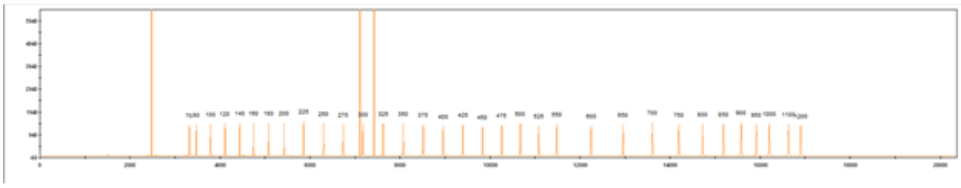
Analyze the results

Data analysis workflow

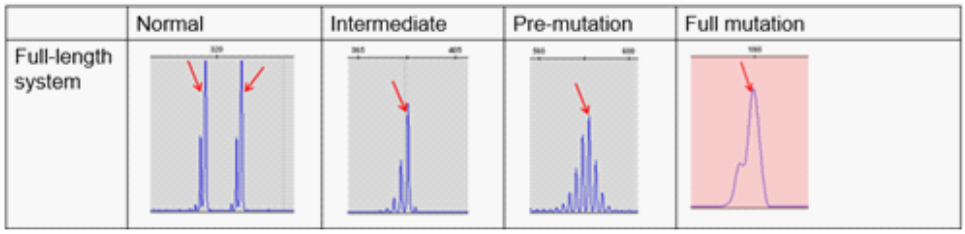
Create a project and analyze sample files with GeneMapper™ Software

Note: Before first use, the latest instrument-specific settings files must be imported. See "About importing files into the GeneMapper™ Software database" on page 43.

Determine sizing quality (SQ), then correct as required

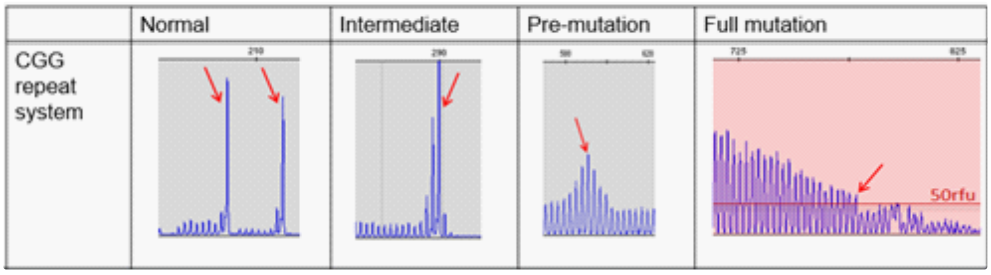


Review the full length (FL) peak results



For samples with possible full mutation alleles, see step 4 or go to the "Results troubleshooting" on page 71.

Review the repeat primers (RP) peak results



Export the GeneMapper™ Software genotypes table

Ensure that the sample naming conventions have been followed. See "Naming conventions" on page 40.

Analyze the data with CarrierMax™ Software

If there are **No call** results, see the "Results troubleshooting" on page 71.

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.

About importing files into the GeneMapper™ Software database

Before you use GeneMapper™ Software to analyze assay data for the first time, you must import the latest settings files.

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

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

File to import	File type	Description	See
Panel	TXT	Defines the markers (loci) that are being interrogated. **IMPORTANT!** Use only panel and bin TXT files that are provided by Thermo Fisher Scientific. If you use panel and bin files that you create in the GeneMapper™ Software, you may see off-ladder alleles.	"Import panel and bins" on page 44
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.	"Import analysis method" on page 46
Size Standard	XML	Defines the sizes of the fragments present in the size standard.	"Import the size standard" on page 45

(continued)

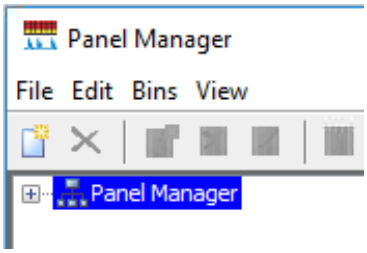
File to import	File type	Description	See
Table Settings	XML	Hide or show columns, filter results, sort order.	"Import table settings and plot settings" on page 47
Plot Settings	XML	Defines settings for electropherogram plot display including Sample Header Settings, Genotypes Header settings, Sizing Table settings, peak labels settings, and display settings for Sample and Genotype plots.	

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 out of bin alleles.

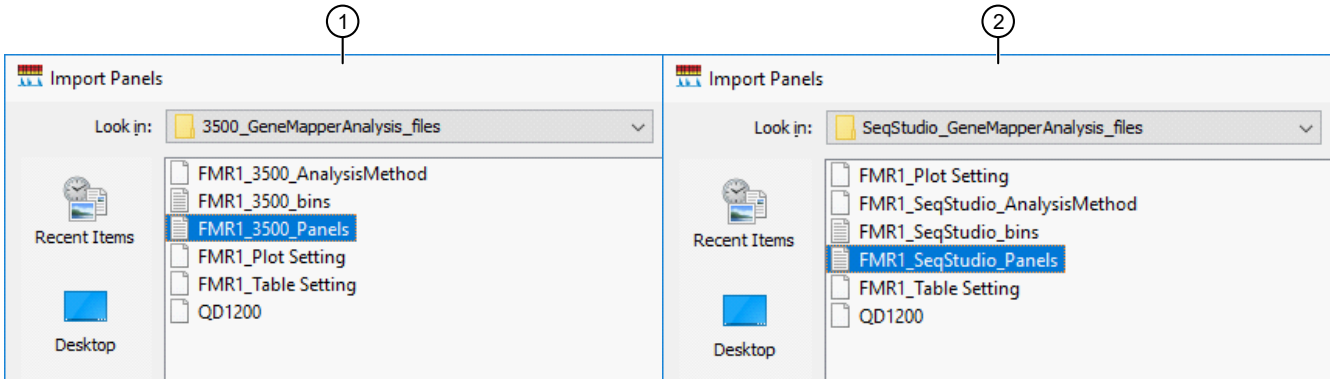
1. Start the GeneMapper™ Software, then log in with the appropriate user name and password.
2. Select **Tools** ▸ **Panel Manager**.
3. In the left pane, click **Panel Manager**.

   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 panel file for your instrument.

   Note: The files can be downloaded from thermofisher.com/carriermax-fmr1.

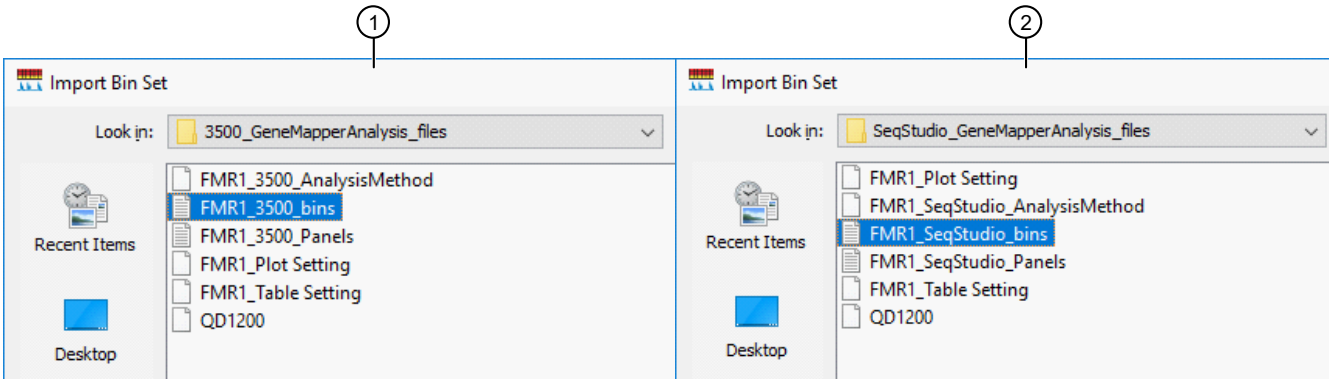


① 3500/3500xL Genetic Analyzer
② SeqStudio™ Genetic Analyzer

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 FMR1 panel.
   b. Select **File ▸ Import Bin Set** to open the **Import Bin Set** dialog box.
   c. Navigate to, then select, the appropriate bin file for your instrument.



① 3500/3500xL Genetic Analyzer
② SeqStudio™ Genetic Analyzer

6. *(Optional)* View the imported panels and bins in the navigation pane: Double-click the FMR1 folders in the left pane.
   The panel information is displayed in the right pane and the markers are displayed below it.

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

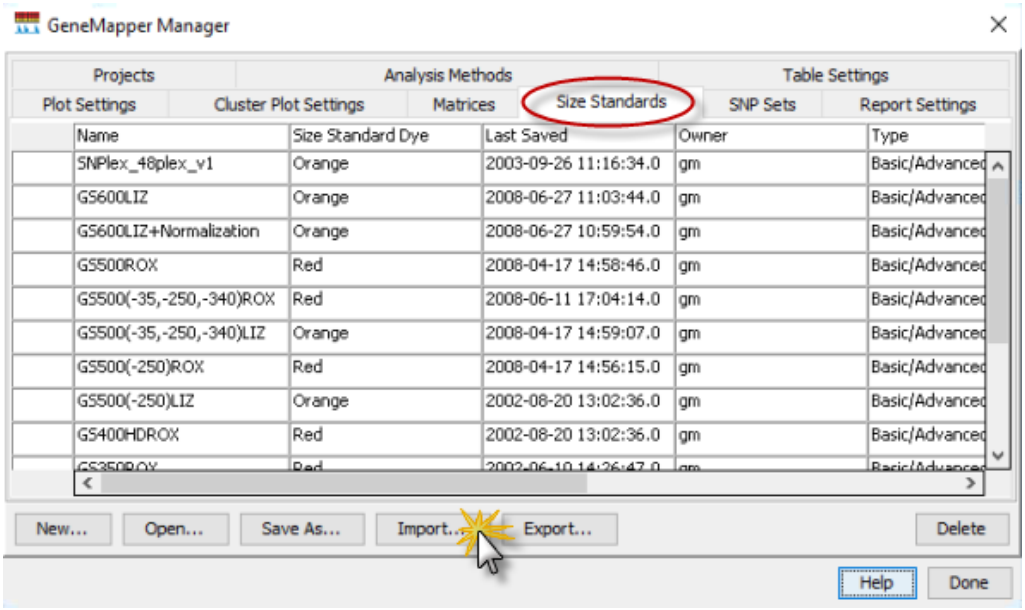
IMPORTANT! If you close the **Panel Manager** without clicking **Apply**, the panels, bin sets, and marker stutter are not imported into the software database.

Import the size standard

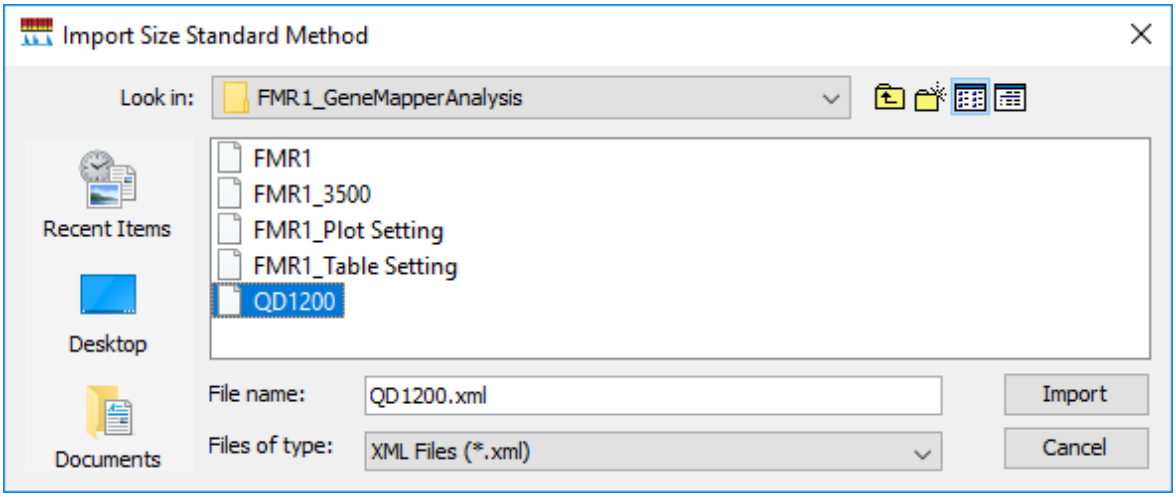
Import the latest size standard from the website into the GeneMapper™ Software database:

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

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



4. Navigate to, then select, the appropriate size standards file.



5. Click **Import**.

6. Click **Done**.

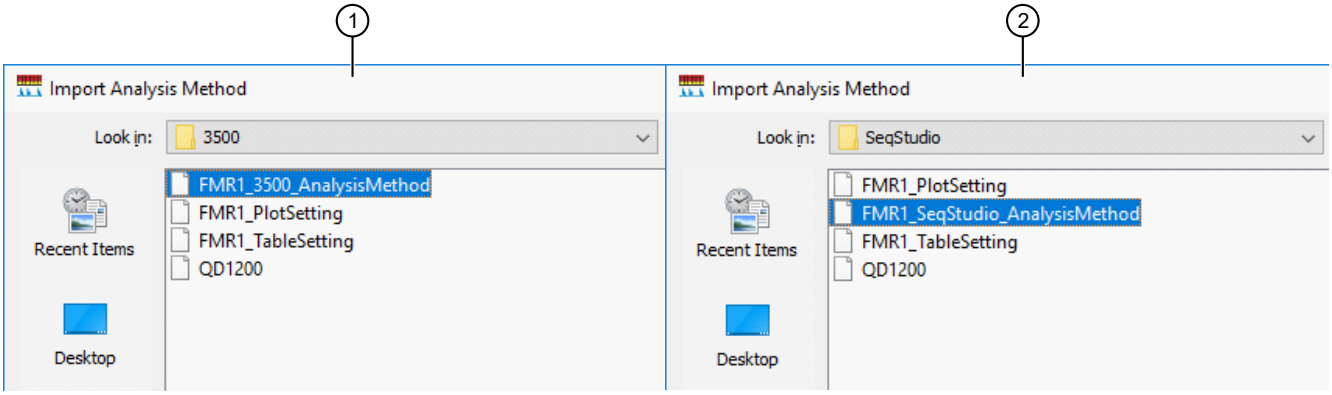
Import analysis method

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 analysis method file for your instrument type.



① 3500/3500xL Genetic Analyzer
② SeqStudio™ Genetic Analyzer

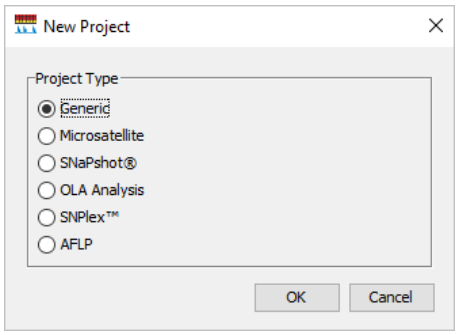
5. Click **Done**.

Import table settings and plot settings

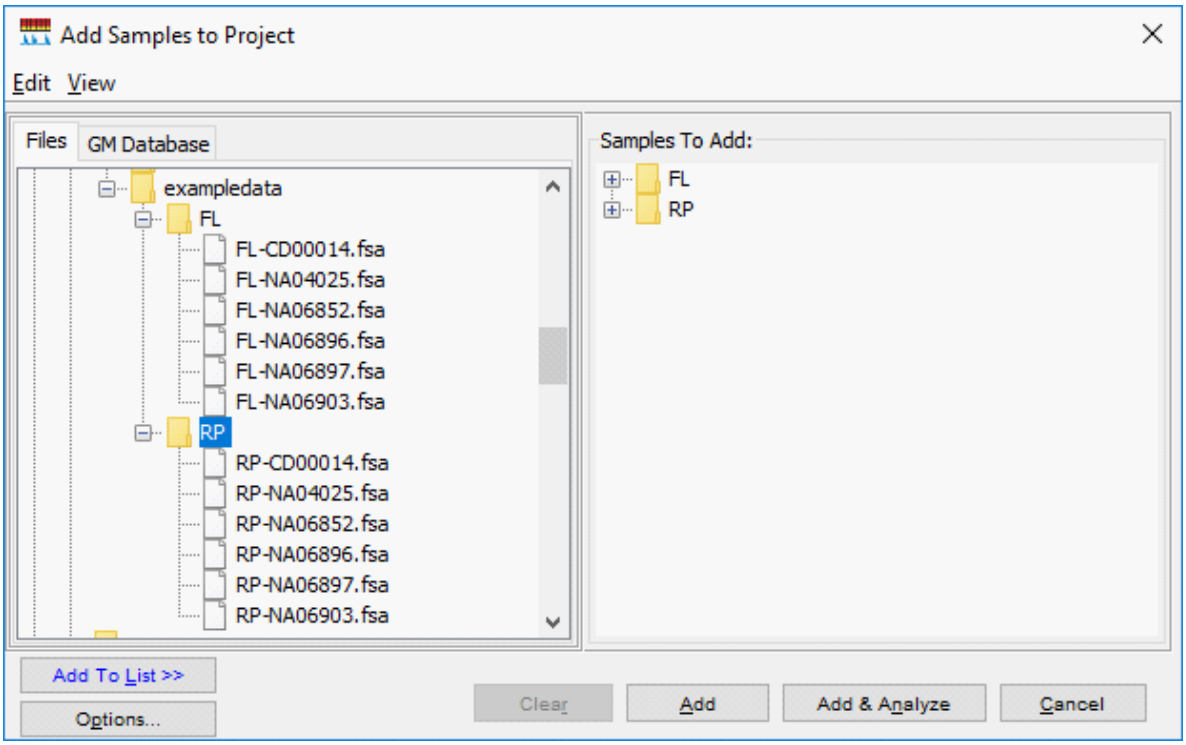
1. In the GeneMapper™ Software, select **Tools** ▸ **GeneMapper Manager**.
2. In the **Table Settings** tab, click **Import**.
3. Navigate to, then select, the appropriate table setting file.
4. Click **Import**.
5. In the **Plot Settings** tab, click **Import**.
6. Navigate to, then select, the appropriate plot setting file.
7. Click **Import**.
8. Click **Done**.

Create a project and analyze sample files with GeneMapper™ Software

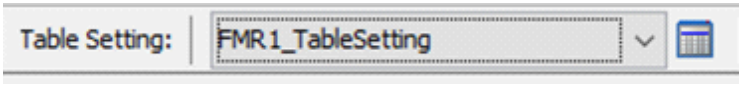
1. If a project is currently open, select **File** ▸ **New Project**, then select **Generic**.



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**.



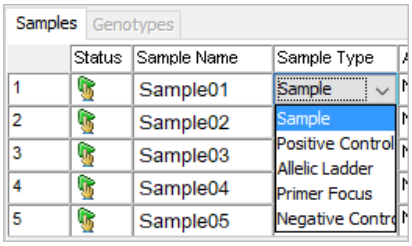
3. Select the table setting that determines the columns that are displayed in samples table and genotypes table: Click the arrow next to the **Table Setting** list, then select the setting.



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.



   b. Repeat for the **Analysis Method**, **Panel**, and **Size Standard** fields.

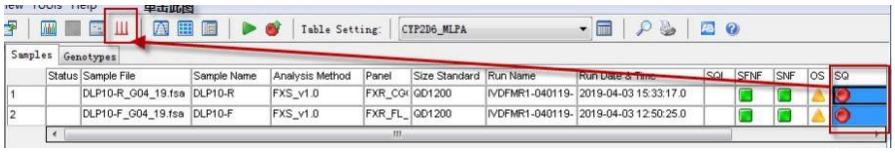
   IMPORTANT! Analysis method is instrument-specific. Ensure that you select the appropriate analysis method for the sample files in the project.

   c. *If this is the first time the analysis method is used*: Double-click the analysis method in the first row, then click the **Allele** tab. Select the bin set you imported.

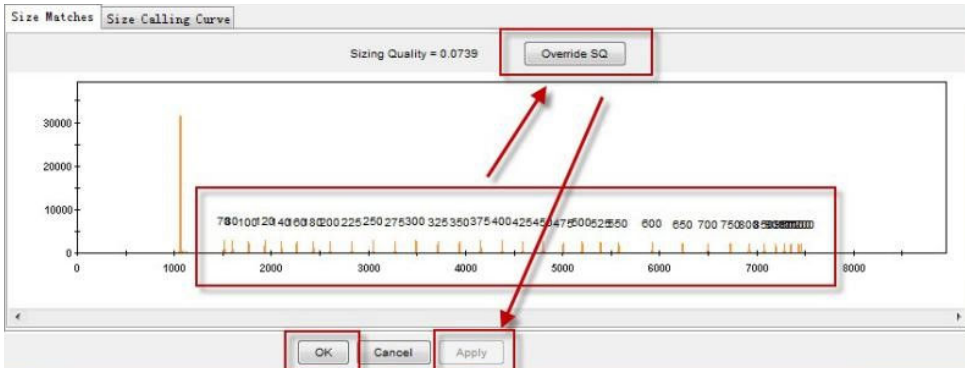
d. Fill in remaining rows.

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.

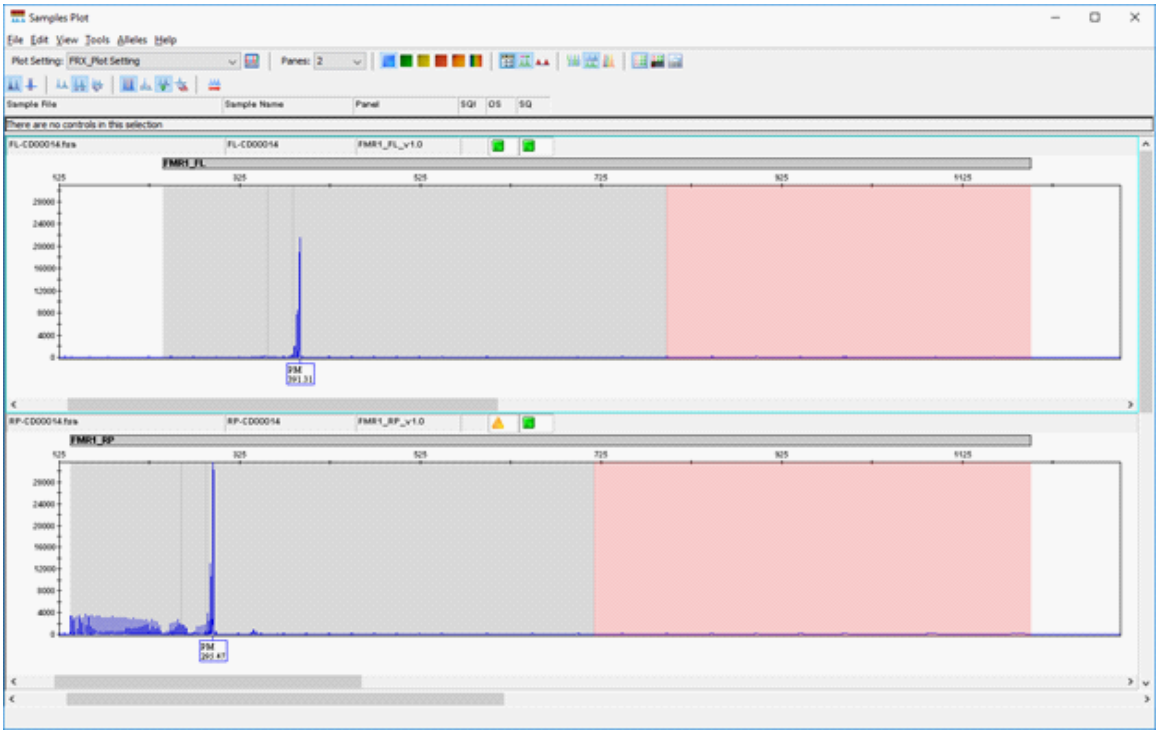


Note: If the SQ field displays a , determine that the size standard is normal. Correct the size standard manually, or if there are no abnormalities, click **Override SQ** ▸ **Apply** ▸ **OK**, then reanalyze.

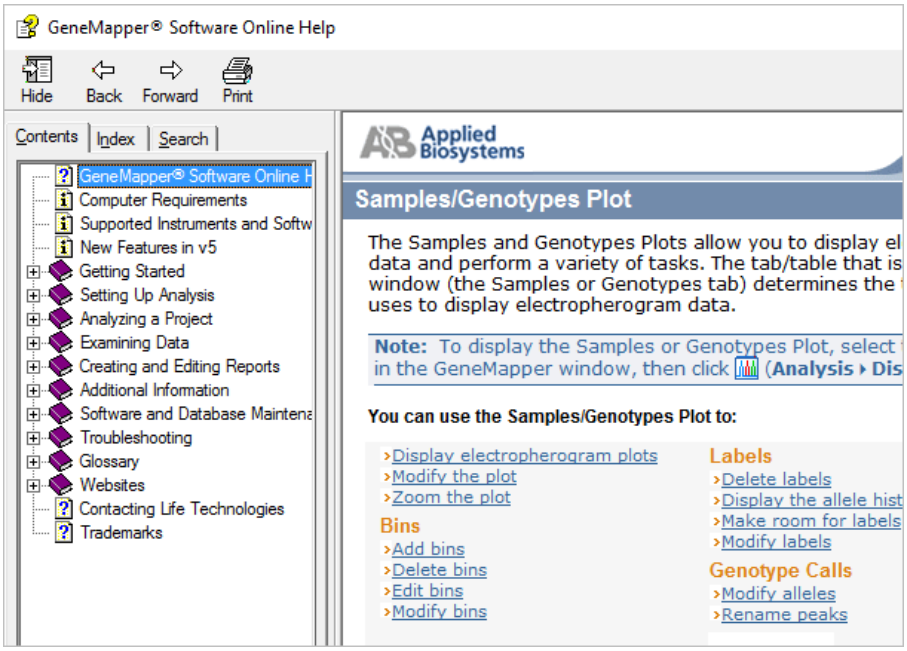


Display and zoom on sample plots

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



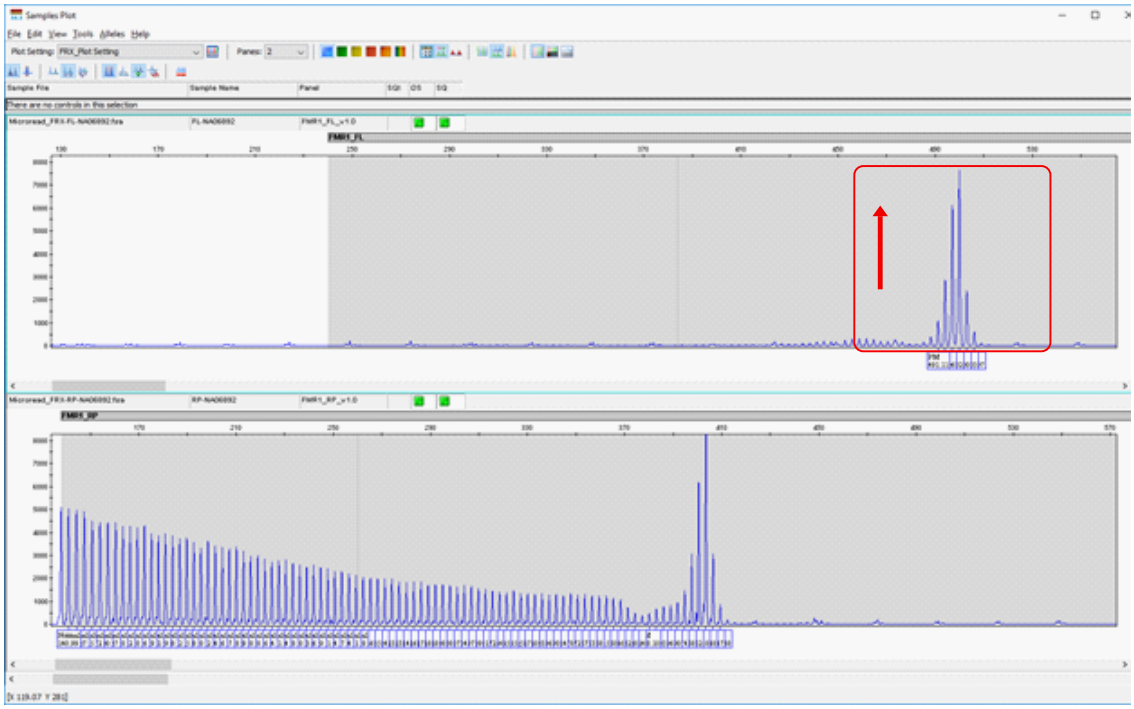
For more information on the samples plot, select **Help ▸ Contents and Index**.



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

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

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



- To unzoom double-click the x-axis.

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

Results guidelines

IMPORTANT! Negative and positive controls must be run with every sample run.

Expected results:

- Negative controls—Use nuclease-free water. Negative controls are normal if they do not show amplification products within the detection range of the full-length system (240-1200bp) or the repeat system (138-1200bp).
- Positive controls—Controls from the Coriell Institute are recommended. See the "Required materials not supplied" on page 6.
- Samples—peaks of each amplicon should be similar. Peaks should not be included if they display an abnormal peak shape or are < 50 rfu.

For more information see "Peak troubleshooting for the CarrierMax™ FMR1 Reagent Kit" on page 67.

Determine sizing quality (SQ), then correct as required

1. In the sample table, select a sample with a **SQ** label, then click **(Size Match Editor)**.
2. In the Size Match Editor, select **Add**, **Delete**, or **Change** for individual peaks as needed.
3. Repeat for each sample with a **SQ** label.

Review the full length (FL) peak results

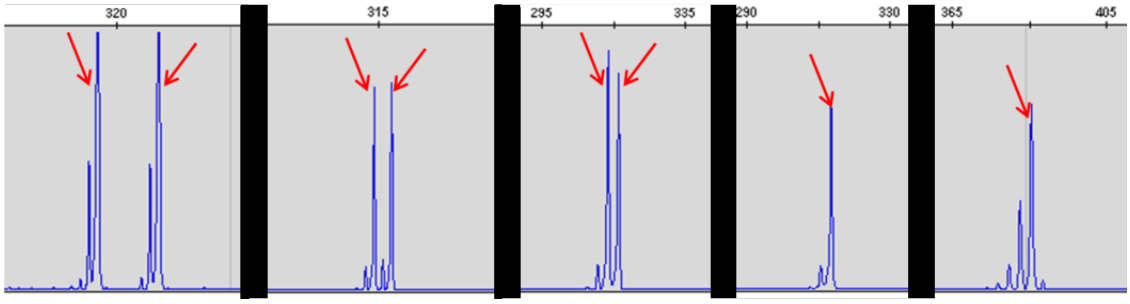
1. Perform a quality control check by following the guidelines in "Results guidelines" on page 52.

2. In the **Sample Plot** window, select **Alleles ▸ Delete Alleles ▸ Delete All Alleles**.

3. Select the valid full length (FL) target peaks. For more information, see "Peak troubleshooting for the CarrierMax™ FMR1 Reagent Kit" on page 67.

   a. Select full length (FL) peaks for normal alleles between 240.0-355.5 bp and intermediate alleles between 355.6-383.9 bp.

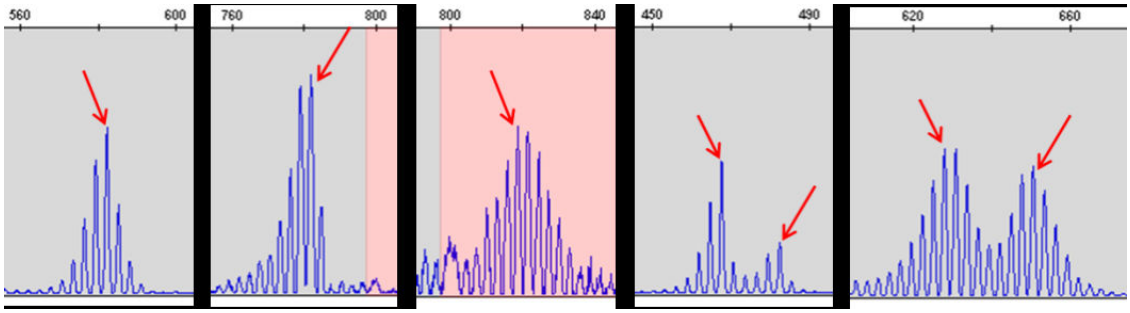
   Select the highest signal peak as the full length gene-specific target peak, generally the right-most peak as there may be multiple peaks in this range.



Example of FL peaks for normal to intermediate alleles between 240.0-355.5 bp and intermediate alleles between 355.6-383.9 bp.

   b. Select full length (FL) peaks for premutation alleles 384.0-797.9 bp.

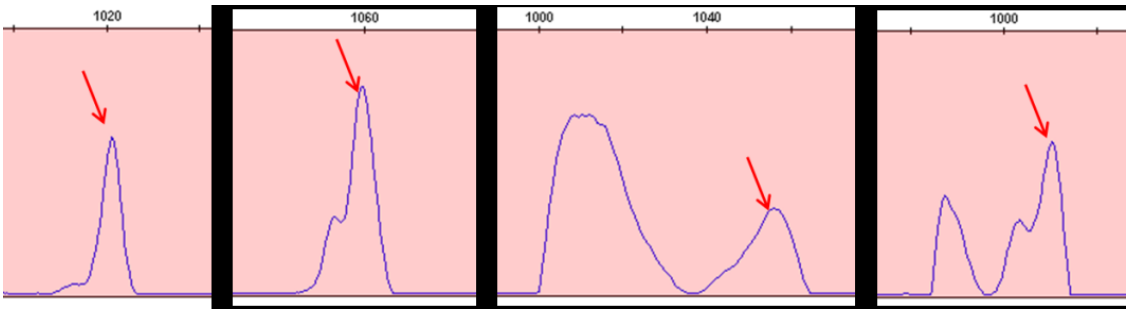
   Select the highest signal peaks, generally the center peak for multi-peak alleles or peaks in the same size range. Premutation or full mutation alleles can display a single peak population less than 8 peaks from center to end, or a complex distributions of peaks.



Example of FL peaks for premutation alleles between 384.0-797.9 bp.

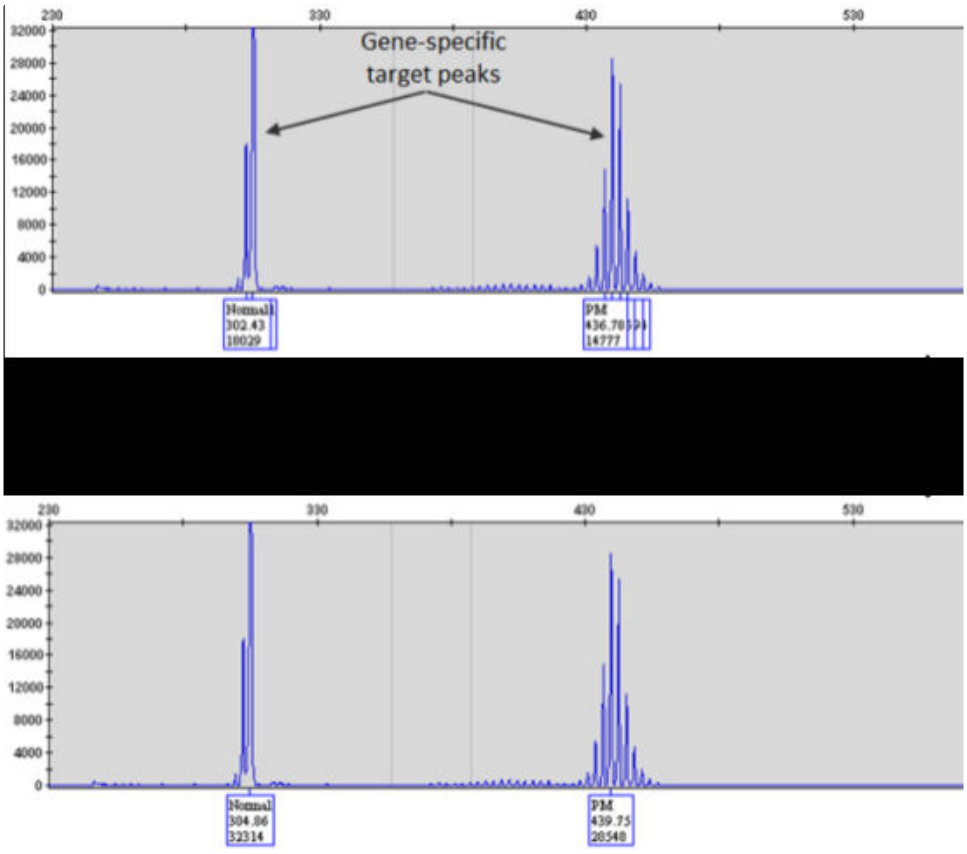
c. Select full length (FL) peaks for full mutation alleles >798 bp.

Note: When the full length peak size of the product is >798 bp, completely continuous peaks may be harder to obtain due to the limitation of the electrophoresis resolution. Select only the component of the peak group containing the highest peak, then identify the peak as >200.



Example of FL peaks for full mutation alleles >798 bp.

The following example shows electropherograms before and after peak selection.



Example electropherogram of Full Length (FL) PCR product with default analysis settings (top) and only gene-specific target peaks selected from the electropherogram (bottom).

4. Determine whether any samples need to be rerun (no FL peak above background threshold).
   a. Look for CGG expansion in or near the full mutation region in the repeat primers (RP) results.

b. If there is CGG expansion, re-run FL and RP reactions with undiluted sample, then check the FL results again.

Note: The FL and RP reactions can also be run with twice the input DNA if needed.

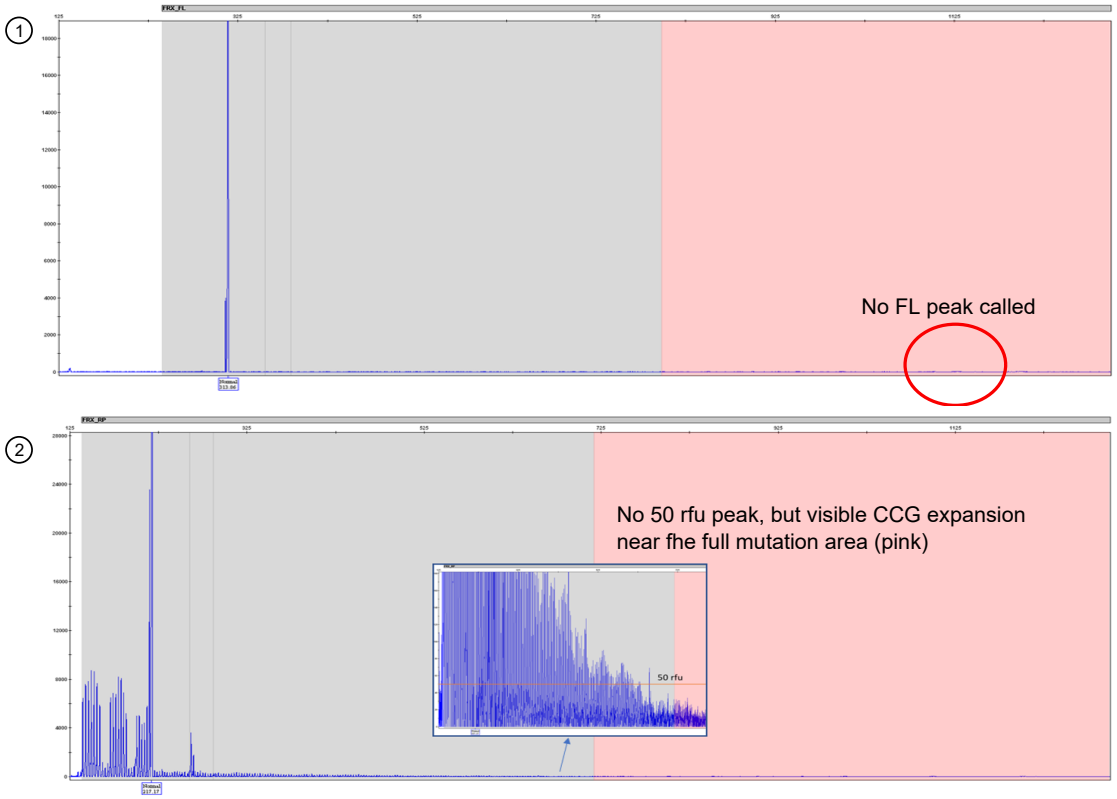


Figure 1 Diluted sample showing no FL peak above background, but CCG expansion with the RP primers near the full mutation region (pink)

1 Electropherogram of FL reactions (diluted)
2 Electropherogram of RP reactions (diluted)

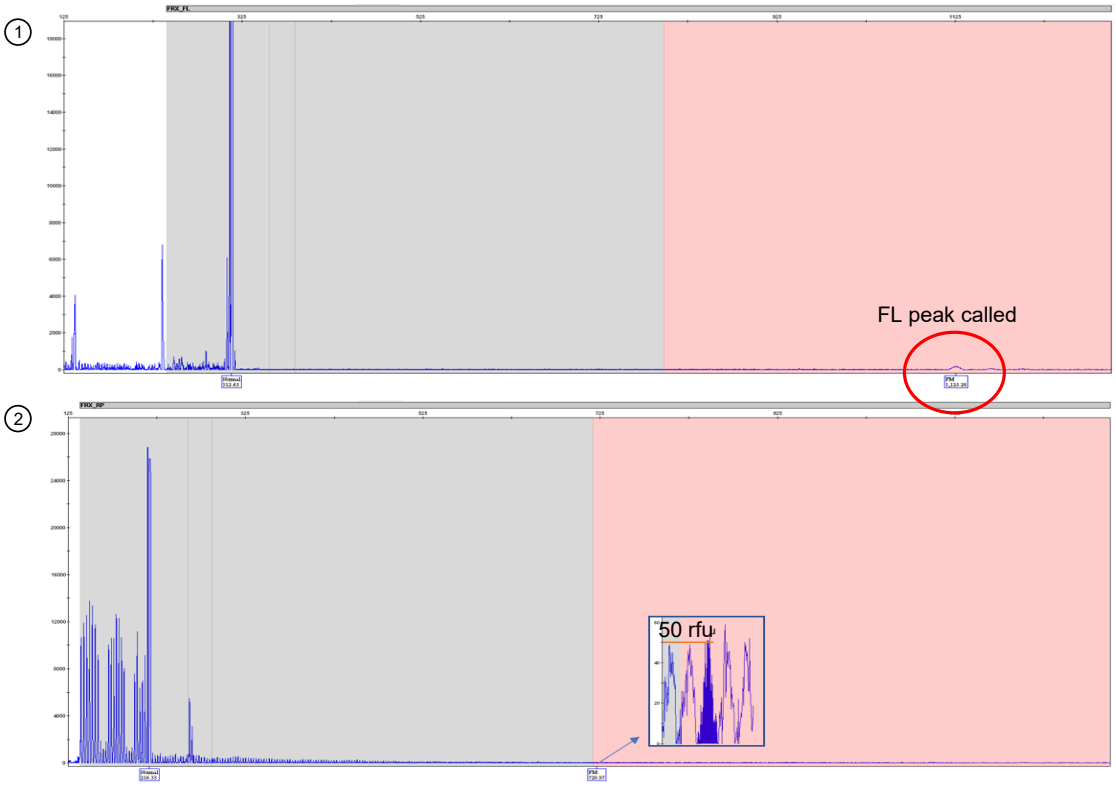


Figure 2 Undiluted sample showing a FL peak above background and 50 rfu peaks in the full mutation region with the RP primers

1 Electropherogram of FL reactions (undiluted)
2 Electropherogram of RP reactions (undiluted)

Review the repeat primers (RP) peak results

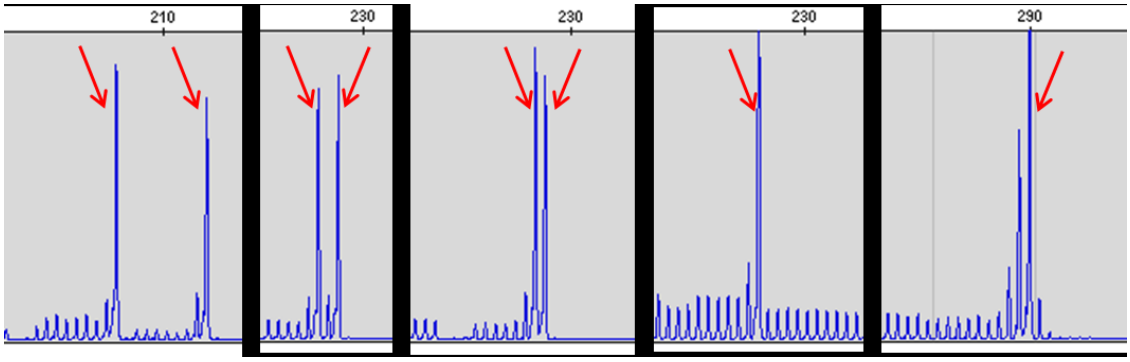
1. Perform a quality control check by following the guidelines in “Results guidelines” on page 52.

2. In the **Sample Plot** window, select **Alleles ▸ Delete Alleles ▸ Delete All Alleles**.

3. Select the valid repeat primers (RP) target peaks. For more information, see “Peak troubleshooting for the CarrierMax™ FMR1 Reagent Kit” on page 67.

   a. Select repeat primed (RP) peaks for normal to intermediate alleles between 138.0-259.9 bp and intermediate alleles between 260.0-287.0 bp.

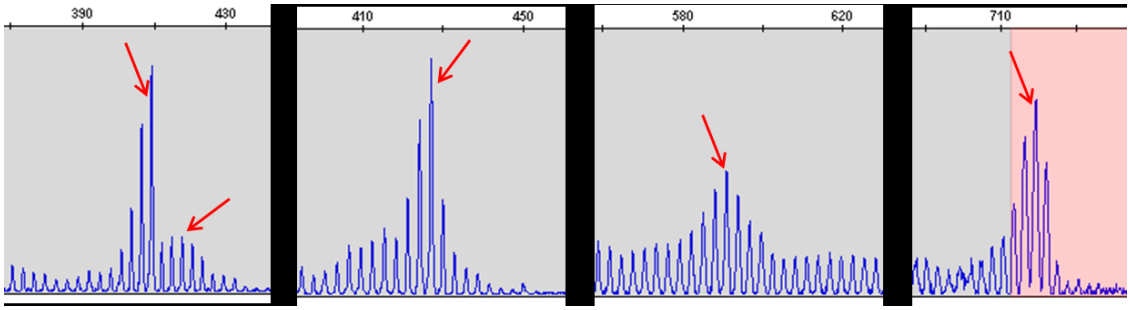
   Select the highest signal peak, generally the right-most peak as there may be multiple peaks in this range. "Step signal" peaks appear. These are peaks < 2 peaks from the highest peak to the right end, or the left continous peaks more than one times higher than the right.



Example of RP peaks for normal alleles between 138.0-259.9 bp and intermediate alleles between 260.0-287.0 bp

   b. Select repeat primed (RP) peaks for premutation alleles 287.1-716.9 bp.

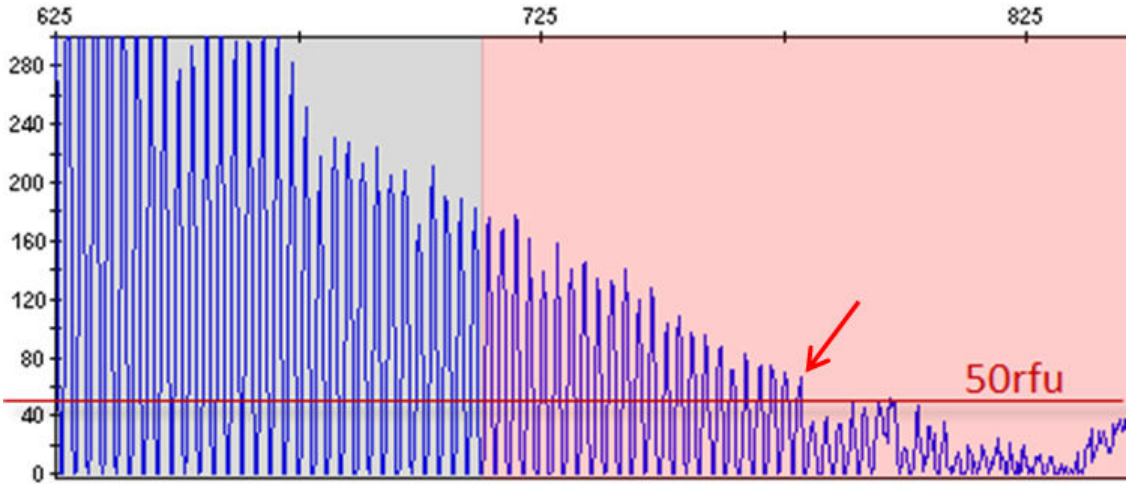
   Select the highest peak, generally the middle peak. "Five finger" peaks occur, with the highest signal peak >1 times larger than the surrounding peaks.



Example of RP peaks for premutation alleles between 287.1-716.9 bp.

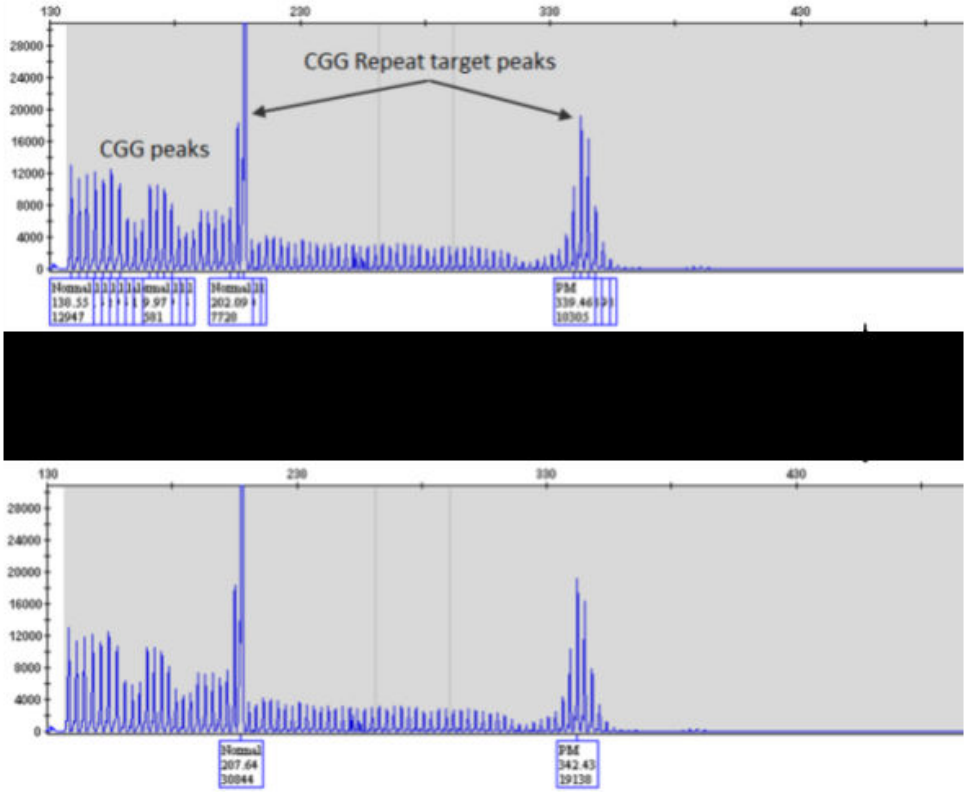
c. Select full length (RP) peaks for full mutation alleles >717 bp.

Note: When the repeat primed peak size is >717 bp, there is generally no obvious peaks, only a sloping product peak pattern. Select the maximum size peak (>50 rfu) and identify it as > 200.



Example of RP peaks for full mutations >717 bp.

The following example shows electropherograms before and after peak selection.

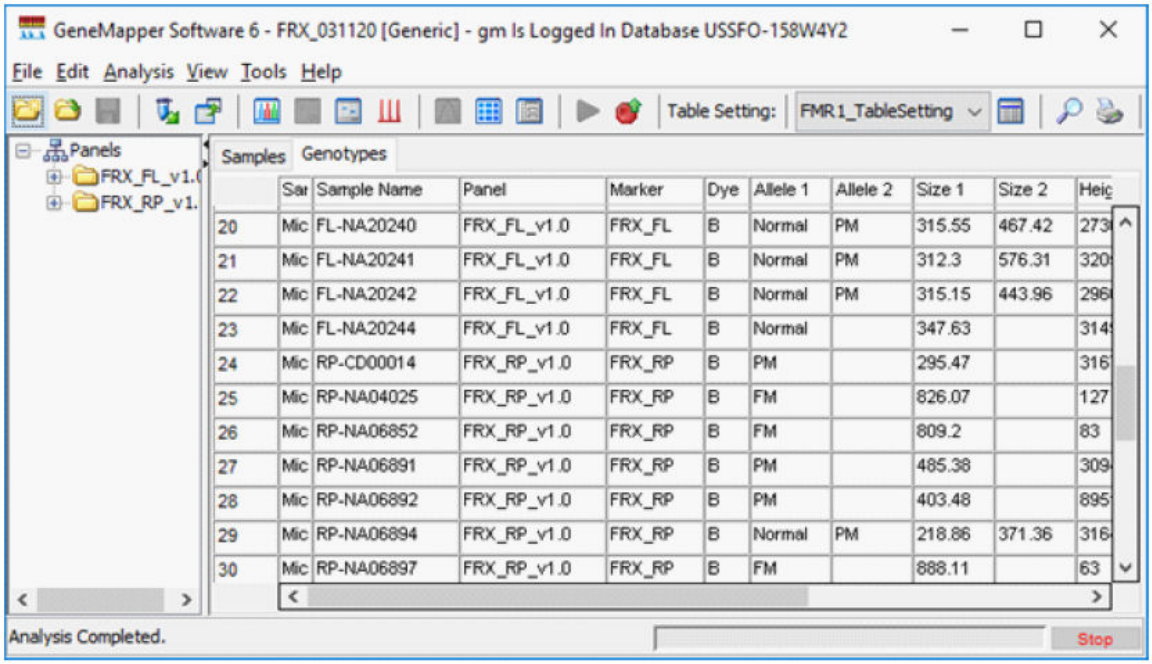


Example electropherogram of CGG Repeat Primed (RP) PCR product with default analysis settings (top) and only CGG Repeat target peaks selected from the electropherogram (bottom).

Note: In most cases, there are 1 or 2 target peaks for female samples and 1 peak for male samples. If more target peaks are identified, see “Results troubleshooting” on page 71.

Export the GeneMapper™ Software genotypes table

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



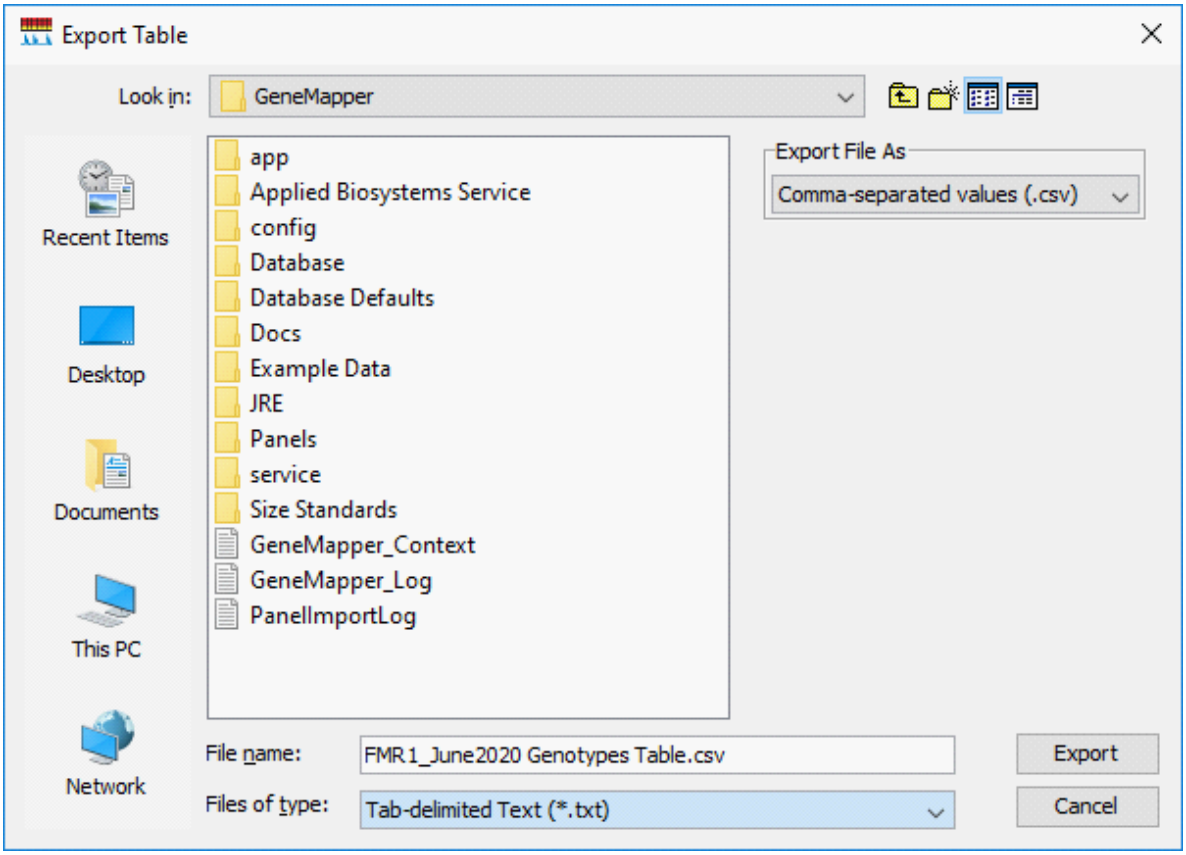
	Sar	Sample Name	Panel	Marker	Dye	Allele 1	Allele 2	Size 1	Size 2	Heig
20	Mic	FL-NA20240	FRX_FL_v1.0	FRX_FL	B	Normal	PM	315.55	467.42	273
21	Mic	FL-NA20241	FRX_FL_v1.0	FRX_FL	B	Normal	PM	312.3	576.31	320
22	Mic	FL-NA20242	FRX_FL_v1.0	FRX_FL	B	Normal	PM	315.15	443.96	296
23	Mic	FL-NA20244	FRX_FL_v1.0	FRX_FL	B	Normal		347.63		314
24	Mic	RP-CD00014	FRX_RP_v1.0	FRX_RP	B	PM		295.47		316
25	Mic	RP-NA04025	FRX_RP_v1.0	FRX_RP	B	FM		826.07		127
26	Mic	RP-NA06852	FRX_RP_v1.0	FRX_RP	B	FM		809.2		83
27	Mic	RP-NA06891	FRX_RP_v1.0	FRX_RP	B	PM		485.38		309
28	Mic	RP-NA06892	FRX_RP_v1.0	FRX_RP	B	PM		403.48		895
29	Mic	RP-NA06894	FRX_RP_v1.0	FRX_RP	B	Normal	PM	218.86	371.36	316
30	Mic	RP-NA06897	FRX_RP_v1.0	FRX_RP	B	FM		888.11		63

3. Display the columns that you want to export.

IMPORTANT! Full length (FL) and Repeat Primed (RP) genotype results must be exported as separte tables.

4. Highlight the FL panel, then select **File** ▸ **Export Table**.
5. Select **CSV** as the export file type (the default is TXT).

6. Click **Export**.



7. Highlight the RP panel, then select **File** ▸ **Export Table**.
8. Select **CSV** as the export file type (the default is TXT).
9. Click **Export**.

Analyze the data with CarrierMax™ Software

Download and install the CarrierMax™ Software

1. Navigate to **thermofisher.com/carriermax-fmr1**.
2. Download the **CMAXSetup.exe** file.

   Note: The CarrierMax™ Software is compatible with Windows™ 7 and Windows™ 10 operating systems.

3. Double-click the **CMAXSetup.exe** file to launch the InstallShield Wizard, then follow the prompts.

   Note: If a security warning is displayed, click **Run**.

4. Upon installation completion, click **Finish**.

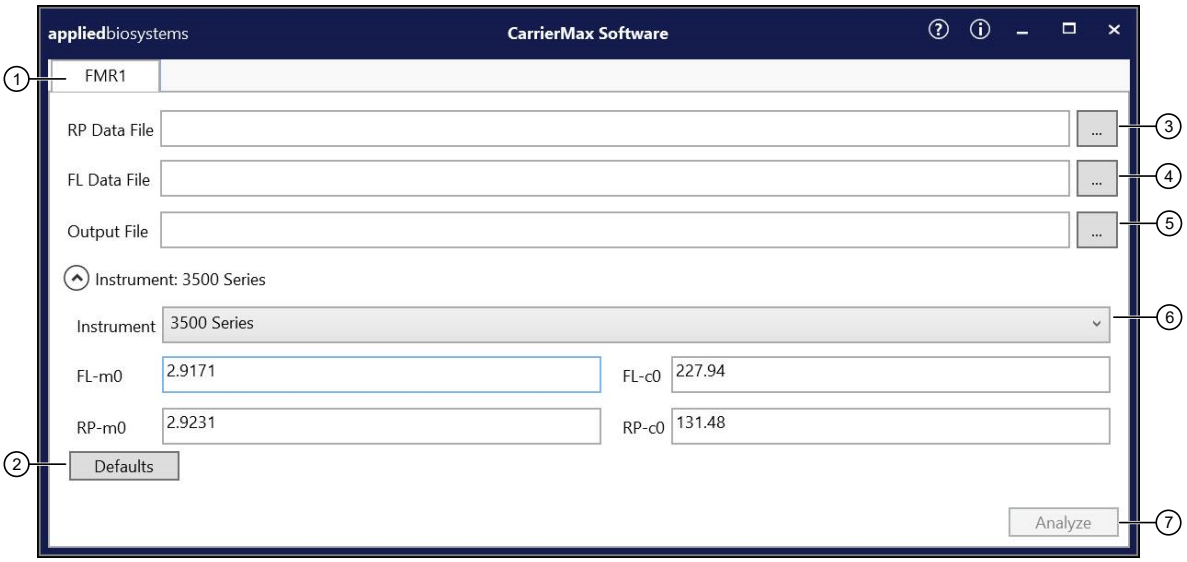
Import sample files

1. In the **Home** screen, click **... (Ellipses)** for the **RP Data File**.
2. Navigate to, then select the sample files (CSV) to import. Click **Open**.
3. In the **Home** screen, click **... (Ellipses)** for the **FL Data File**.
4. Navigate to, then select the sample files (CSV) to import. Click **Open**.
5. In the **Home** screen, click **... (Ellipses)** for the **Output File**. This is the file where your results will be stored. The file must also be in CSV format.
6. Navigate to, then select the output file (CSV) for export. Click **Open**.
7. Select the appropriate instrument from the dropdown list.

   Note: When an instrument is selected, the default factory parameter settings are displayed. This selection remains for future runs.

8. (*Optional*) Adjust the parameters if needed. Click **Defaults** to revert to default parameters.

9. Click **Analyze**.



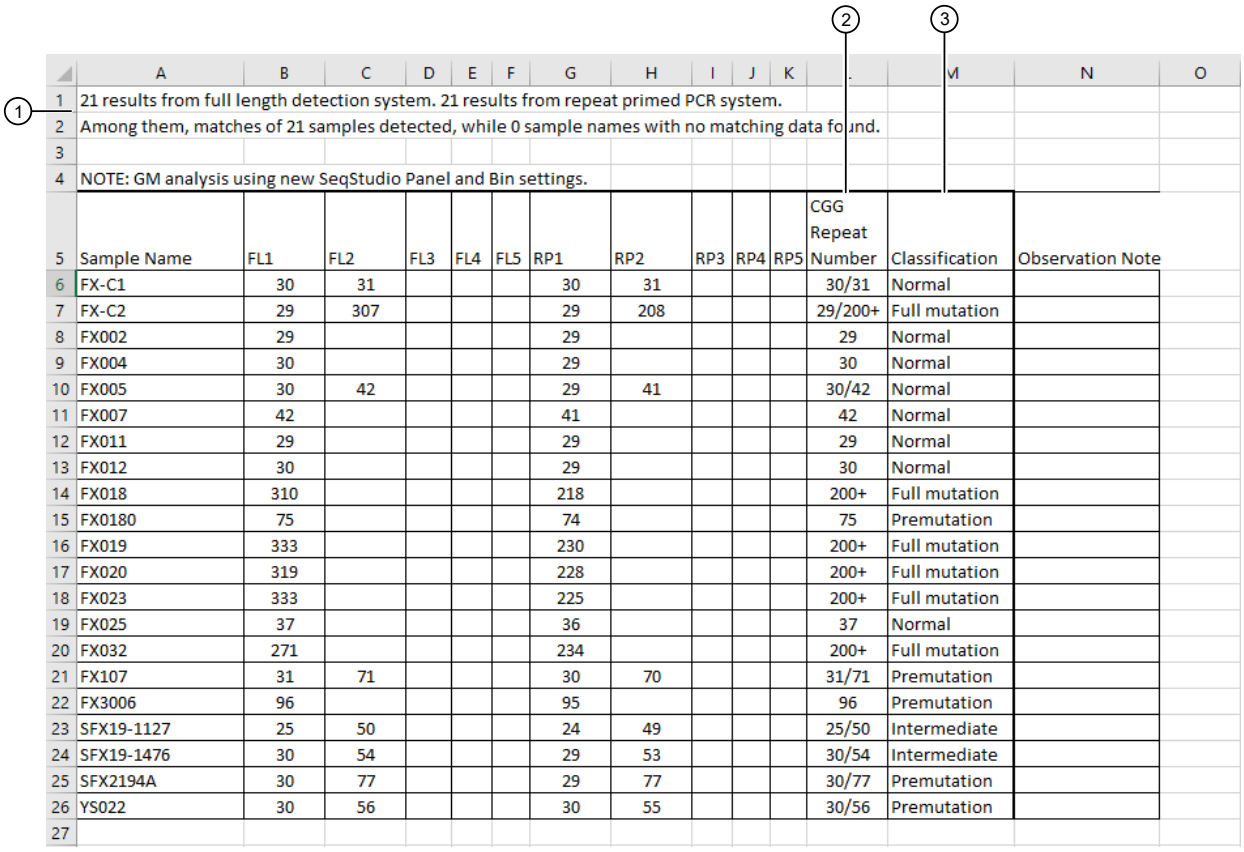
① Assay tab
② **Defaults** button. Use to revert to default instrument parameters.
③ Ellipses. Use to select the **RP Data File**.
④ Ellipses. Use to select the **FL Data File**.
⑤ Ellipses. Use to select the **Output File**.
⑥ Instrument dropdown list
⑦ **Analyze** button

Note: The default instrument parameters change depending upon the instrument selected.

A popup window appears when the analysis is complete. Click **OK** and navigate to the output file to view the results.

View the results

Navigate to the **Output File** selected in "Import sample files" on page 61. Open the file.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	21 results from full length detection system. 21 results from repeat primed PCR system.														
2	Among them, matches of 21 samples detected, while 0 sample names with no matching data found.														
3															
4	NOTE: GM analysis using new SeqStudio Panel and Bin settings.														
5	Sample Name	FL1	FL2	FL3	FL4	FL5	RP1	RP2	RP3	RP4	RP5	CGG Repeat Number	Classification	Observation Note	
6	FX-C1	30	31				30	31				30/31	Normal		
7	FX-C2	29	307				29	208				29/200+	Full mutation		
8	FX002	29					29					29	Normal		
9	FX004	30					29					30	Normal		
10	FX005	30	42				29	41				30/42	Normal		
11	FX007	42					41					42	Normal		
12	FX011	29					29					29	Normal		
13	FX012	30					29					30	Normal		
14	FX018	310					218					200+	Full mutation		
15	FX0180	75					74					75	Premutation		
16	FX019	333					230					200+	Full mutation		
17	FX020	319					228					200+	Full mutation		
18	FX023	333					225					200+	Full mutation		
19	FX025	37					36					37	Normal		
20	FX032	271					234					200+	Full mutation		
21	FX107	31	71				30	70				31/71	Premutation		
22	FX3006	96					95					96	Premutation		
23	SFX19-1127	25	50				24	49				25/50	Intermediate		
24	SFX19-1476	30	54				29	53				30/54	Intermediate		
25	SFX2194A	30	77				29	77				30/77	Premutation		
26	YS022	30	56				30	55				30/56	Premutation		
27															

Figure 3 Results (XLS output)

① Rows 1–2 display the number of matched files imported.

Note: If the numbers of files imported doesn't match the number of files matched, there was a sample naming discrepancy.

② **CGG Repeat Number**—number of CGG repeats from each peak (FL primers).

③ **Classification**—assay result.

Classification	Description
Full mutation	**>200** CGG repeats.
Normal	**<45** CGG repeats.
Gray zone	**45-54** CGG repeats.
Premutation	**55-200** CGG repeats.
No Call. 3+ peaks detected. Please refer to user guide.	This indicates sample contamination or mosaicism. See the "Results troubleshooting" on page 71.
No Call. Different Classifications detected.	The maximum FL data and maximum RP data do not match. Re-run the PCR with undiluted PCR product. If FL or RP data are still inconsistent, please re-run the PCR with more input DNA. If still inconsistent, use the FL peak data, or see the "Results troubleshooting" on page 71.

(continued)

Classification	Description
No Call. Reamplify and reanalyze. Please refer to user guide.	No FL data was found and the RP data is > 200. This can indicate a failure of the FL PCR. Repeat the assay. See the "Results troubleshooting" on page 71.

A

Troubleshooting

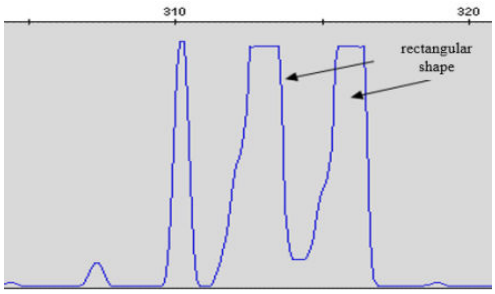
PCR and CE troubleshooting

Observation	Possible cause	Recommended action
Faint or no signal from the controls and test samples at all loci	The **PCR Reaction Mix** reagents were not vortexed thoroughly before aliquoting	All mixes should be thawed at room temperature, then vortexed thoroughly before aliquoting.
	Incorrect volume of reagents added.	Repeat amplification ensuring that all components have been added correctly.
	Insufficient sample quantity or quality	Ensure that both sample quantity and quality are at recommended levels.
	Incorrect thermal cycler parameters	Use the recommended thermal cycler parameters.
	Insufficient PCR product injected（and the peaks of size standards are also low or absent)	Check the sample volume to ensure capillaries are going into the sample. Check for bubbles at the bottom of the sample, if present briefly centrifuge samples to remove the bubbles in the well.
	Degraded Hi-Di™ Formamide	Use the recommended storage conditions for Hi-Di™ Formamide . Do not thaw and refreeze multiple times.
Signal obtained for the positive control, but not the test samples	Quantity of the test DNA sample is too low.	Quantitate DNA, then add 40 ng of DNA. Repeat the test to ensure that each target peak is above 50 rfu.
	The test sample contains a high concentration of PCR inhibitor (for example, heme compounds or certain dyes)	Quantitate the DNA, then add the minimum necessary volume. Repeat the test.
		Purify the sample, then repeat the test.
	The sample DNA is degraded.	Evaluate the sample DNA quality by running an agarose gel. If the DNA is degraded, reamplify with an increased amount of DNA.

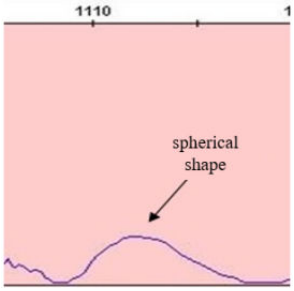
Observation	Possible cause	Recommended action
Poor peak height balance	Too much DNA. Excess DNA causes low-size peaks to appear much higher.	Use the recommended amount of DNA.
	The test sample contains a high concentration of PCR inhibitor (for example, heme compounds or certain dyes)	Quantitate the DNA, then add the minimum necessary volume. Repeat the assay.
		Purify the sample. Repeat the assay.
	The sample DNA is degraded.	Re-extract the sample DNA.
	The PCR Reaction Mix reagents were not vortexed thoroughly before aliquoting.	All mixes should be thawed at room temperature, then vortexed thoroughly before aliquoting.
	The PCR reaction mix was not vortexed thoroughly before aliquoting.	The PCR reaction mix should be vortexed for 10 seconds before aliquoting it into the reaction plate.

Peak troubleshooting for the CarrierMax™ FMR1 Reagent Kit

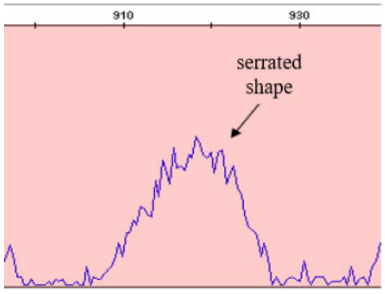
Abnormal peak shape examples



Rectangular shaped peaks

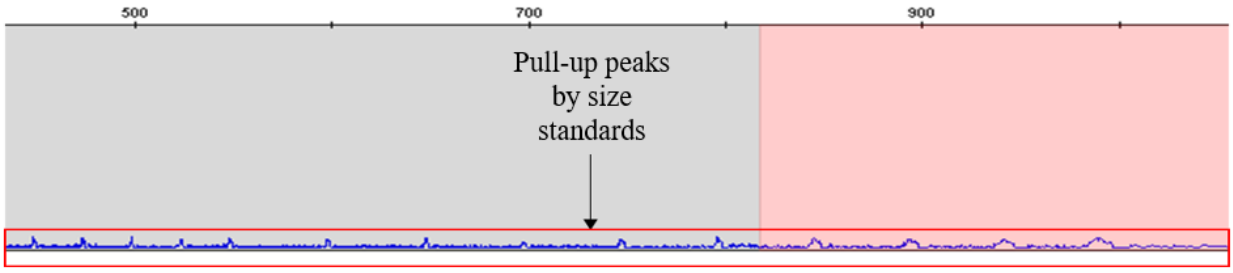


Spherical shaped peaks



Serrated shaped peaks

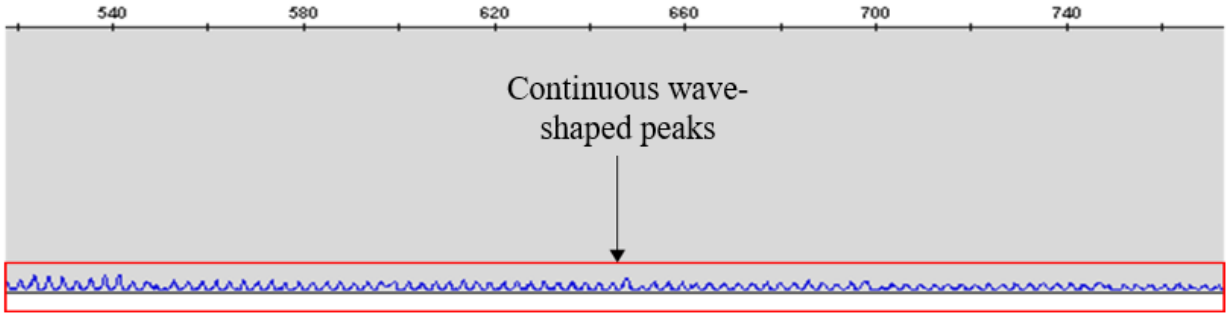
Pull up peaks example



Small peaks, corresponding to the size standards, can occur. These peaks are referred to as pull up peaks.

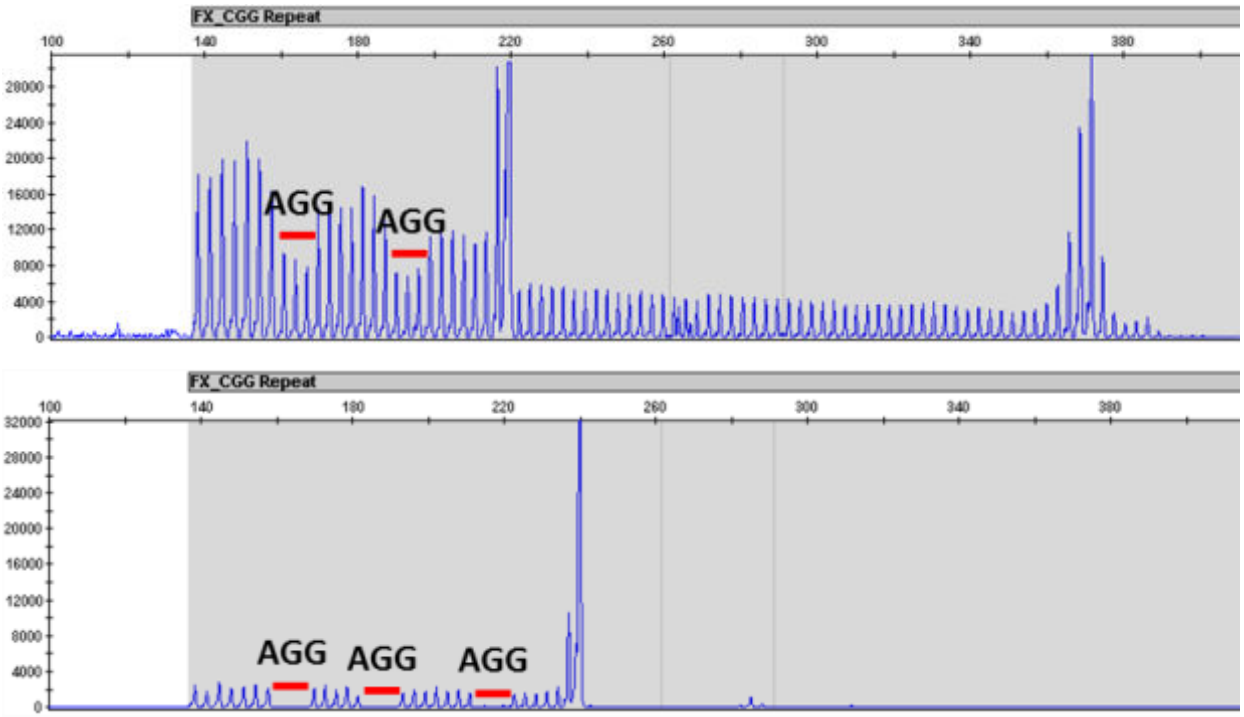
Continuous waves example

Invalid peaks generated in the FL amplification system. The wave-shaped peaks are 3 bp apart and lack an obvious main peak.



AGG insertion example

In the RP amplification system, AGG insertion can cause a decrease in peak heights.



Causes of extra peaks

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

Stutter peak definition

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

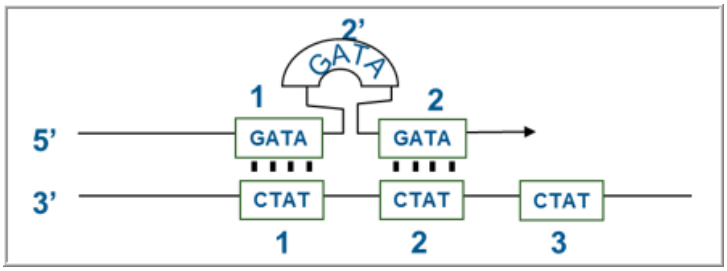


Figure 4 Plus stutter is caused by slippage in the top DNA strand. Example of tetranucleotide stutter (one unit = 4 bp).

Artifact peak definition

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

Pull-up peak definition

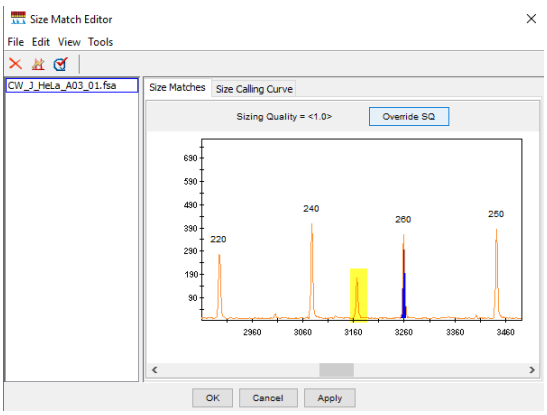
Pull-up peaks are observed if simultaneous fluorescence occurs in multiple dye color channels. Pull-up is caused by the overlapping emission spectrum of fluorescent dyes and usually occurs when the fluorescence signals approach the dynamic range of the instrument.

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

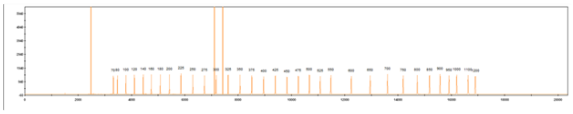
GeneMapper™ Software troubleshooting

Troubleshoot a failing SQ in GeneMapper™ Software

1. In the sample table, select a sample with a **SQ** label, then click **(Size Match Editor)**.
2. Follow the steps to **Add**, **Delete**, or **Change** as described in "Determine sizing quality (SQ), then correct as required" on page 52.

Observation	Possible cause	Recommended action
When you click **(Analyze)**, error message is displayed "There are samples that do not meet analysis requirements. Please see Error Message in the info view of each sample."	Multiple possible causes.	Select a sample, then select **View ▸ Sample Info**. Review the information in the **Error Message** section of the screen. Correct the issue indicated in the **Error Message** section of the screen.
Cannot select peak in **Size Match Editor** 	The peak signal is below the **Peak Amplitude Threshold** in the analysis method.	Dilute the PCR product further. Increase the amount of size standard.

Results troubleshooting

Observation	Possible cause	Recommended action
Two or more FL peaks in male samples	The PCR products were not denatured before adding Hi-Di™ Formamide.	Denature the PCR products. Repeat the assay.
	The male sample may be a chimera.	Re-amplify the sample to confirm the results. If similar, karyotyping is needed for confirmation.
Loss of FL peaks (RP peaks are >200)	The FL peak size is too long to find in the result map.	Re-amplify from stock genomic DNA to confirm the results. If the results are unchanged, then use the RP results.
No Sizing Data error 	Offscale signal of sample peaks (orange pull-up peaks).	Further dilute the FMR1 sample prior to capillary electrophoresis or go to the **Size Match Editor**, then edit each size peak manually. Save, then re-analyze the sample.
	Peaks below the signal threshold.	Re-run the sample and increase the amount of size standard so that the signal is above the minimum peak amplitude threshold.
	The larger size standards, 1100 or 1200, may be missing due to slower sample migration. Slower sample migration can be related to expired or old consumables.	Replace expired consumables or consumables that have been on the system for more than recommended amount of time. Re-run the samples with fresh reagents.
2+ target peaks in male samples or 3+ target peaks in female samples	The wrong sample was run.	Check sample records, or perform an identification test to confirm the sample information.
	There was problem in the experiment (for example, contamination, mixed samples, or an error in sample input.	Repeat the extraction, amplification, or detection as needed.
	There was problem in the sample (for example, mosaicism or X chromosome aneuploidy)	Perform additional tests to confirm the cause.

Observation	Possible cause	Recommended action
Conflicting classifications between FL and RP systems	Sample has full mutation allele with insufficient PCR product.	First, please run the undiluted PCR product, and if FL or RP data are still inconsistent, rerun the PCR with more input DNA.

B

Safety

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

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

Chemical safety

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

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

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:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/publications/i/item/9789240011311

C Documentation and support

Related documentation

Document	Publication number
CarrierMax™ A5D Matrix Standard Kit Product Information Sheet	MAN0018893
3500/3500xL Genetic Analyzer with 3500 Series Data Collection Software v3.3 User Guide	100079380
SeqStudio™ Genetic Analyzer Instrument and Software User Guide	MAN0018646
GeneMapper™ Software 5 Installation and Administration User Guide	4476603
GeneMapper™ Software v4.1 Quick Reference Guide	4403615

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- 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

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