

# 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** C



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

**Revision history: MAN0018881 C (English)**

Revision	Date	Description
C	2 July 2024	The catalog number for the CarrierMax™ FMR1 Control DNA Kit was updated to 952414.
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.

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

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# Product information

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**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 <sup>[1]</sup> .
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	

<sup>[1]</sup> 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](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.fisherscientific.com) or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

**Table 2 Materials for generating PCR products**

Item	Source
<b>Instruments and equipment</b>	
One of the following thermocyclers: <sup>[1]</sup> <ul style="list-style-type: none"> <li>Veriti™ Thermal Cycler</li> <li>ProFlex™ 96-well PCR System</li> <li>GeneAmp™ PCR System 9700</li> </ul>	Contact your local sales office.
Benchtop microcentrifuge	MLS
Vortex mixer	MLS
Adjustable micropipettors	MLS
<b>Plates and other consumables</b>	
PureLink™ Genomic DNA Mini Kit or equivalent <sup>[2]</sup>	<a href="#">K1820-00</a>
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	<a href="#">4306737</a>
MicroAmp™ Clear Adhesive Film, or equivalent	<a href="#">4306311</a>
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	<a href="#">AM12450</a>
Aerosol-resistant pipette tips	MLS
Other plastic consumables	<a href="https://www.thermofisher.com/plastics">thermofisher.com/plastics</a>
<b>Controls</b>	
CarrierMax™ FMR1 Control DNA Kit	952414

<sup>[1]</sup> You can use an equivalent thermal cycler. Optimize the protocols for other thermal cyclers.

<sup>[2]</sup> 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
<b>Instruments and equipment</b>	
One of the following genetic analyzers: <ul style="list-style-type: none"> <li>3500/3500xL Genetic Analyzer with: <ul style="list-style-type: none"> <li>3500 Data Collection Software (v3.3 or later; Windows™ 10 operating system)</li> </ul> </li> <li>SeqStudio™ Genetic Analyzer with: <ul style="list-style-type: none"> <li>(Recommended) SeqStudio™ Plate Manager</li> </ul> </li> </ul>	Contact your local sales office.
Biosafety cabinet	MLS
Benchtop microcentrifuge	MLS
Vortex mixer	MLS
Adjustable micropipettors	MLS
<b>General reagents</b>	
Hi-Di™ Formamide	<a href="#">4311320</a>
CarrierMax™ A5D Matrix Standard Kit	952364
<b>Consumables and reagents for the 3500/3500xL Genetic Analyzer</b>	
Septa Cathode Buffer Container (for the 3500 series Genetic analyzers)	4410715
Septa for 3500/3500xL Genetic Analyzers, 96 well	4412614
Anode Buffer Container	<a href="#">4393927</a>
Cathode Buffer Container	<a href="#">4408256</a>
Polymer, POP-7™ (96)	<a href="#">A26073</a>
Conditioning reagent	<a href="#">4393718</a>
Capillary array, 8-Capillary, 50-cm	<a href="#">4404685</a>
Capillary array, 24-Capillary, 50-cm	<a href="#">4404689</a>
Retainer & Base Set (Standard) for 3500/3500xL Genetic Analyzers, 96 well	<a href="#">4410228</a>
Retainer & Base Set (Standard) for 3500/3500xL Genetic Analyzers, 8 tube	4410231
<b>Consumables and reagents for the SeqStudio™ Genetic Analyzer</b>	
Septa for SeqStudio™ Genetic Analyzer, 96 well	<a href="#">A35641</a>
Septa for SeqStudio™ Genetic Analyzer, 8 strip	<a href="#">A35643</a>
SeqStudio™ Cartridge or SeqStudio™ Cartridge v2	A33671 or <a href="#">A41331</a>

**Table 3** Materials for capillary electrophoresis *(continued)*

Item	Source
Cathode Buffer Container	<a href="#">A33401</a>
Reservoir Septa	<a href="#">A35640</a>
<b>Plates and other consumables</b>	
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	<a href="#">4306737</a>
MicroAmp™ Optical 96-Well Reaction Plate	<a href="#">N8010560</a>
MicroAmp™ Clear Adhesive Film, or equivalent	<a href="#">4306311</a>
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	<a href="#">AM12450</a>

**Table 4** Materials to analyze data

Item	Source
GeneMapper™ Software v5.0 or later	Contact your local sales office.
CarrierMax™ Software	Complimentary download from <a href="https://thermofisher.com/carriermax-fmr1">thermofisher.com/carriermax-fmr1</a> .

## Workflow

### System workflow

**Chapter 2, Prepare for capillary electrophoresis**

**Chapter 3, Prepare samples and run the PCR**

**Chapter 4, Analyze the results**



# Prepare for capillary electrophoresis

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## 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.

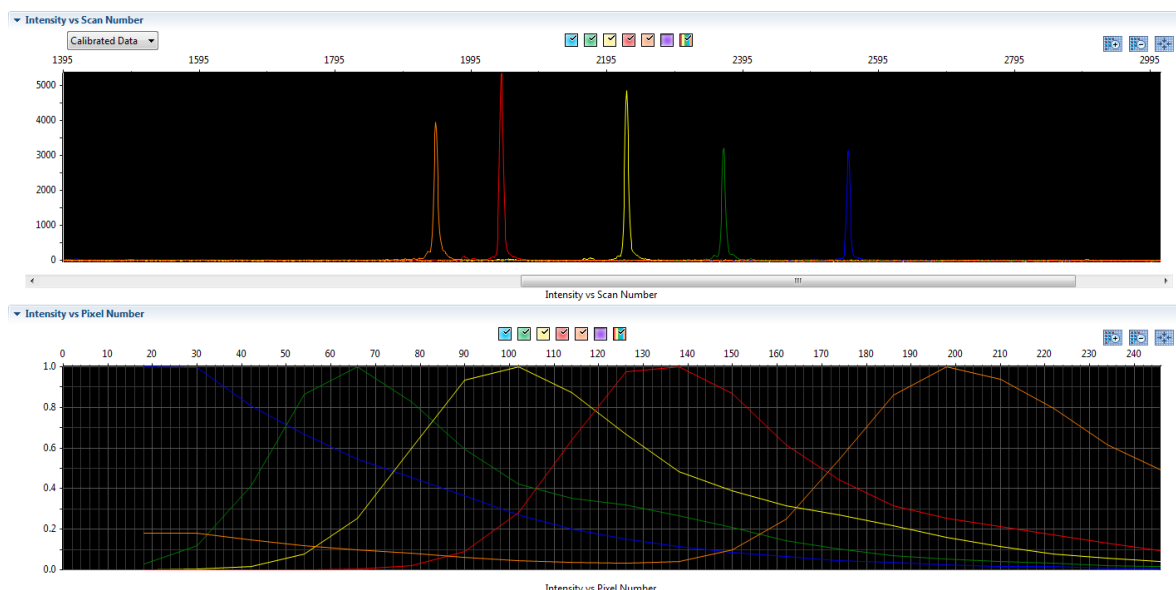
1. (First time only) Create an **A5D Matrix Standard** dye set in the 3500 Series Data Collection Software.
  - a. Go to **Library**, select **Dye Set**, then click **Create**.
  - b. Go to the **Dye Set Name**, then enter **A5D**. Choose **Matrix Standard** in **Chemistry**.
  - c. Go to **Dye Set Template**, 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

Create New Instrument Protocol

Setup an Instrument Protocol

Instrument Protocol Setup Help ?

Application Type: Fragment Capillary Length: 50 cm Polymer: POP7

Dye Set: A5D ☐ Disable Name Filter

Instrument Protocol Properties

\* Run Module: FragmentAnalysis50\_POP7xl Run Modules for 24 capillary are only available in the list.

\* Protocol Name: FMR1\_Assay ☐ Locked

Description:

Oven Temperature (°C): 60 Run Voltage (kVolts): 16.5 PreRun Voltage (kVolts): 15 Injection Voltage (kVolts): 1.2

Run Time (sec.): 2700 PreRun Time (sec.): 180 Injection Time (sec.): 23 Data Delay (sec.): 1

▶ Advanced Options

Close Save

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**.

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## 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 Baseline (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

Sizing Range:

Full

Size Calling Method:

Local Southern

Analysis Start Point:

0

Sizing Start Size:

0

Primer Peak:

Present

Analysis Stop Point:

1000000

Sizing Stop Size:

100000

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**.

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## Create the assay

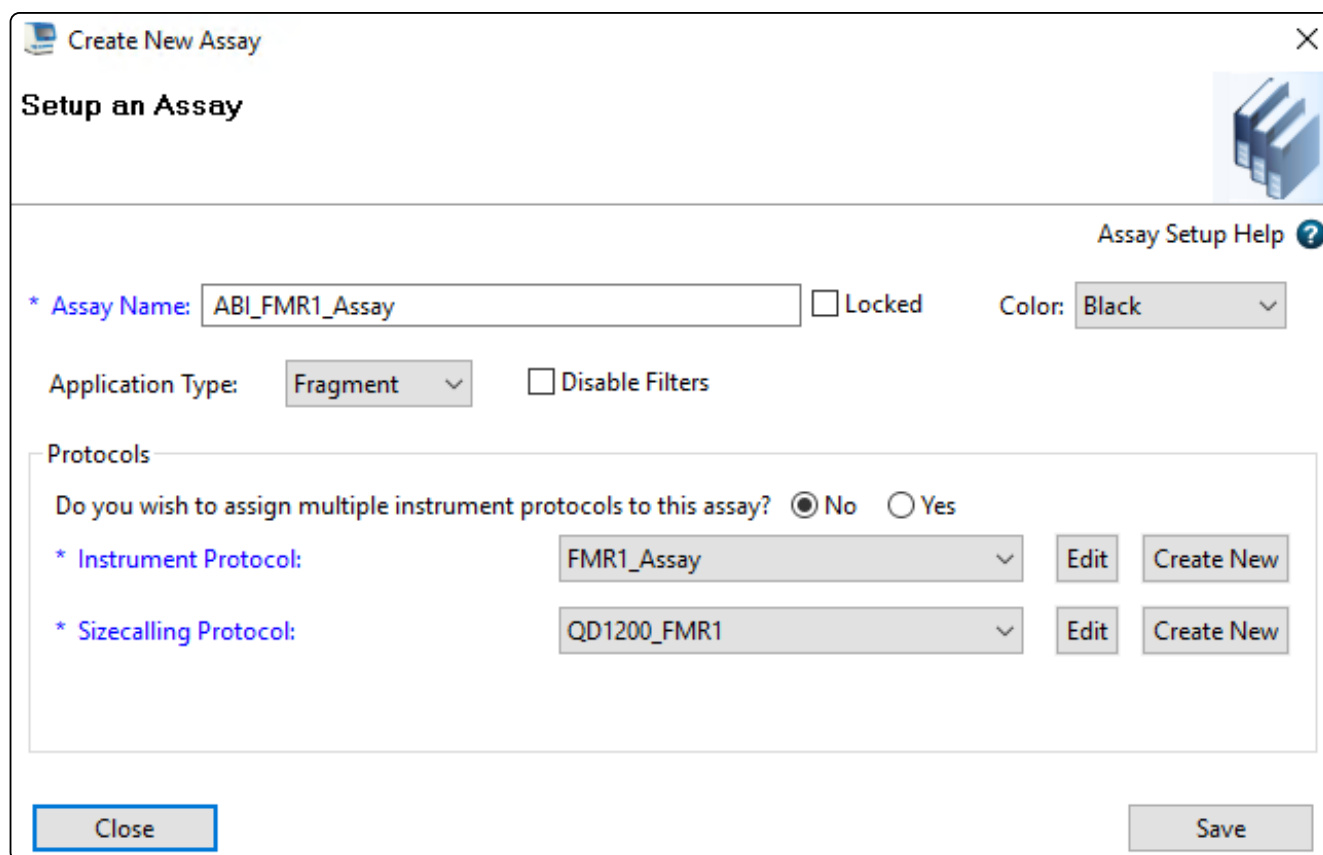
1. Navigate to the **Assays** library.

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)



**Create New Assay**

**Setup an Assay**

Assay Setup Help ?

\* Assay Name:  ☐ Locked Color:

Application Type:  ☐ Disable Filters

**Protocols**

Do you wish to assign multiple instrument protocols to this assay? ☒ No ☐ Yes

\* Instrument Protocol:

\* Sizecalling Protocol:

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**.

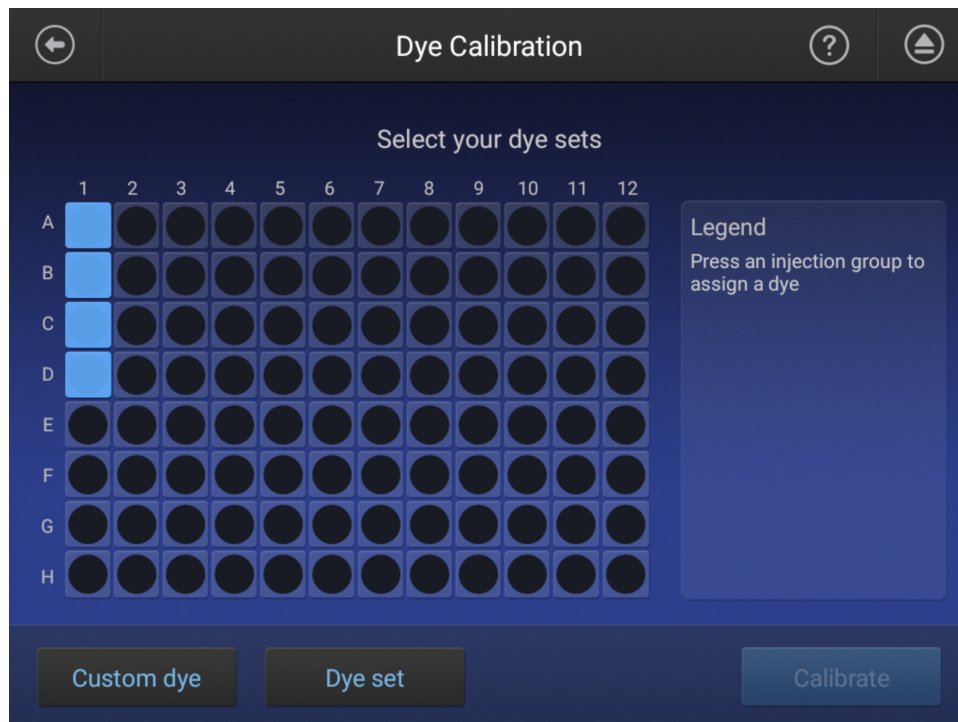
# SeqStudio™ Genetic Analyzer

## 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.

← Add Dye Set ? ↗

Select an existing dye set

Dye set	Chemistry standard
J6 (DS-36)™	Matrix standard
G5 (DS-33)™	Matrix standard
F (DS-32)™	Matrix standard
E5 (DS-02)™	Matrix standard
E_BigDye™ Terminator v1.1	Matrix standard
E_BigDye™ Terminator v1.1	Sequencing standard
D (DS-30)™	Matrix standard

Cancel

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

Dye Set Parameter ×

Matrix condition number upper limit  
6.0

Locate start point after scan  
500

Locate start point before scan  
5000

Limit scans To  
32500

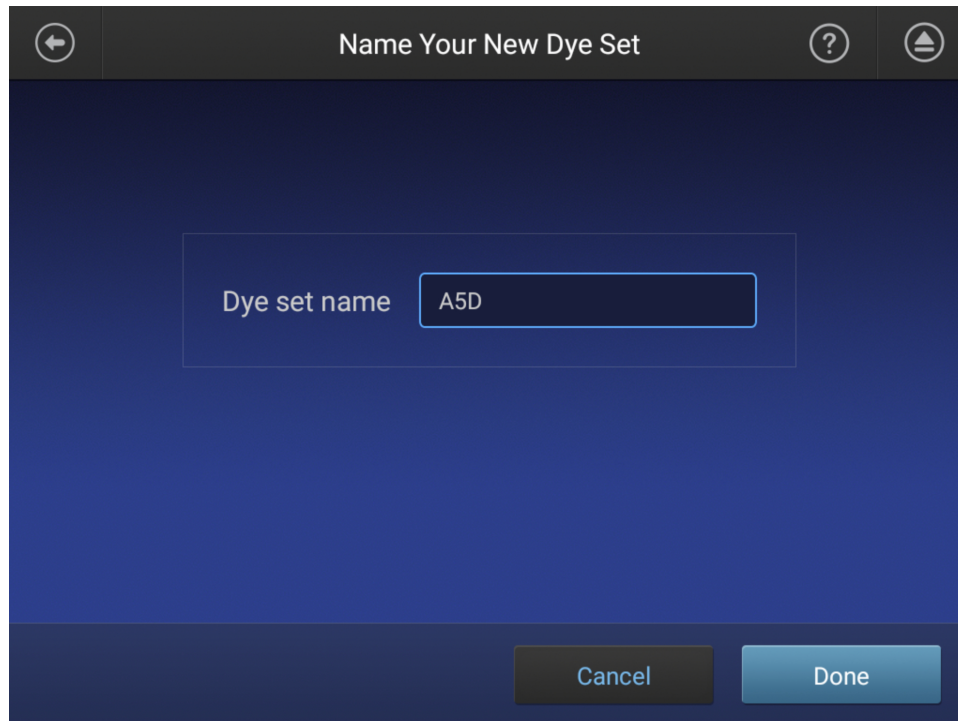
Sensitivity  
0.4

Minimum quality score

1 2 3  
4 5 6  
7 8 9  
- 0  
← × .

Next

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



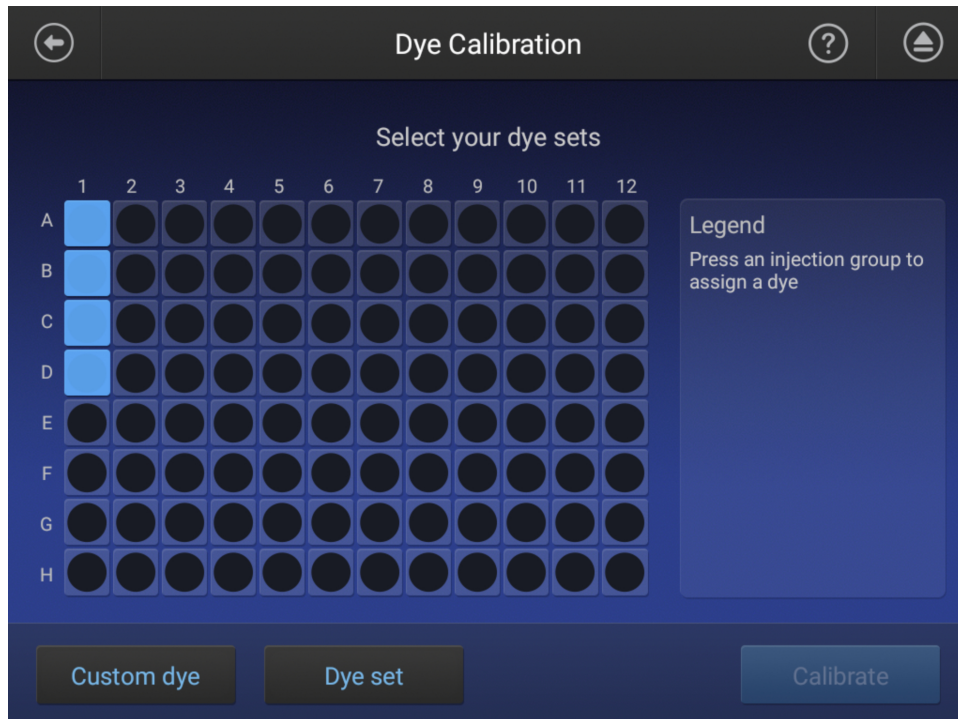
The screenshot shows a mobile application interface for naming a new dye set. The title bar at the top reads "Name Your New Dye Set". Below the title bar, there is a text input field with the label "Dye set name" and the text "A5D" entered. At the bottom of the screen, there are two buttons: "Cancel" and "Done".

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

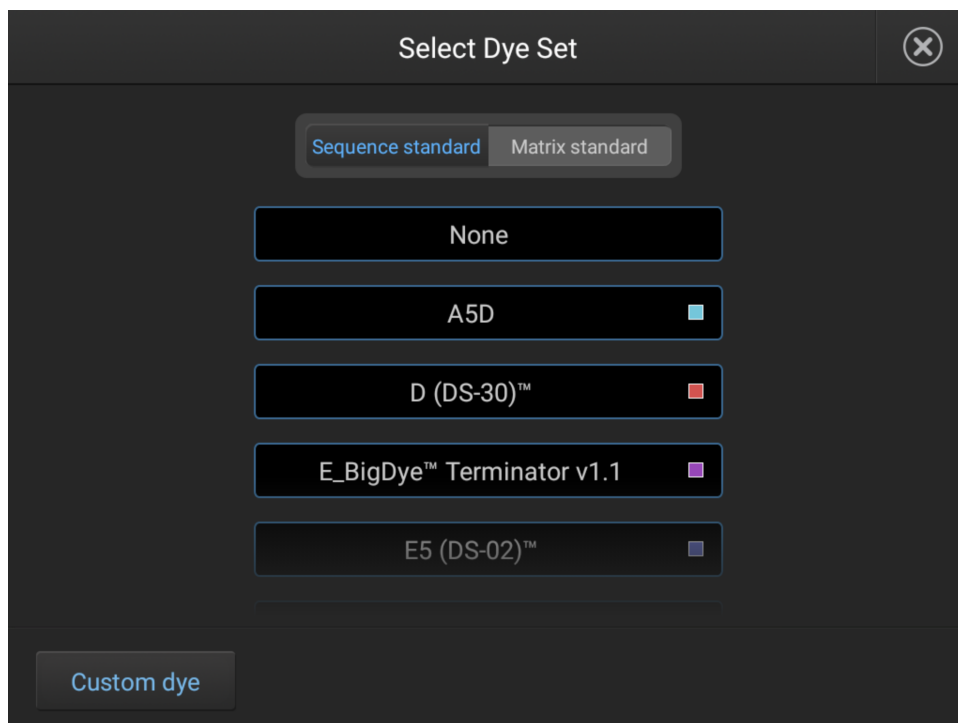
7. Return to the home screen, tap ⚙ **Settings** ▶ **Maintenance and Service** ▶ **Calibration** ▶ **Dye Calibration**.



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

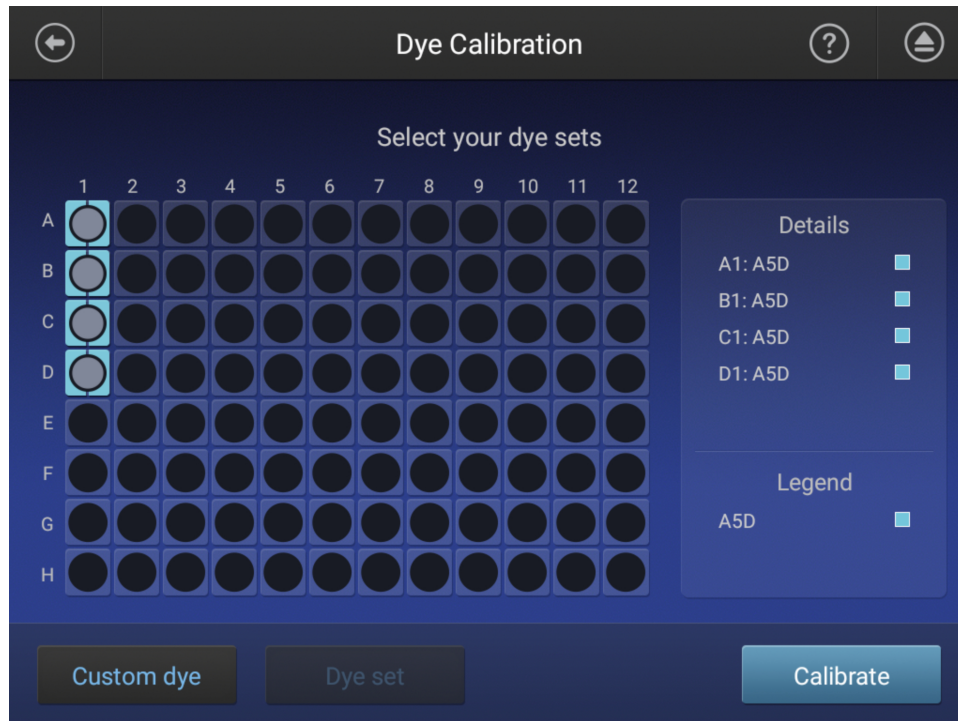


9. Tap **Matrix Standard**, then select the **A5D** matrix standard.





10. Tap **Calibrate**.



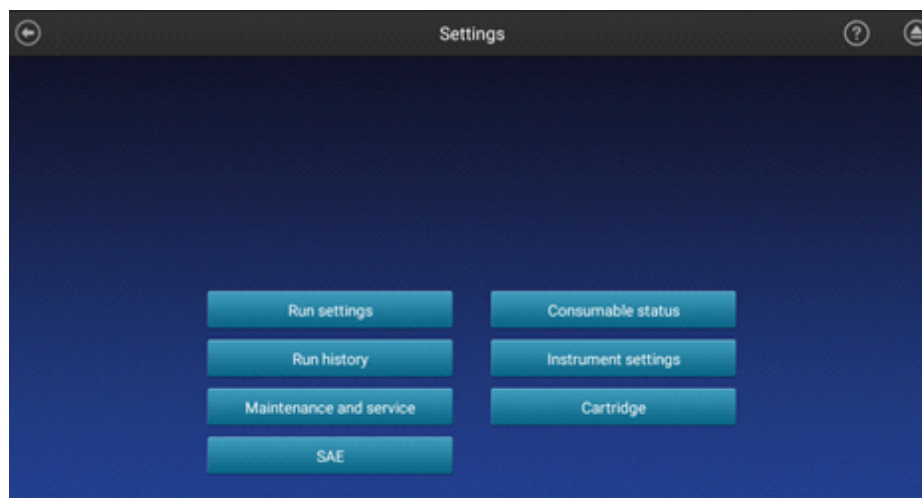
The calibration run starts.

**IMPORTANT!** If the dye calibration fails:

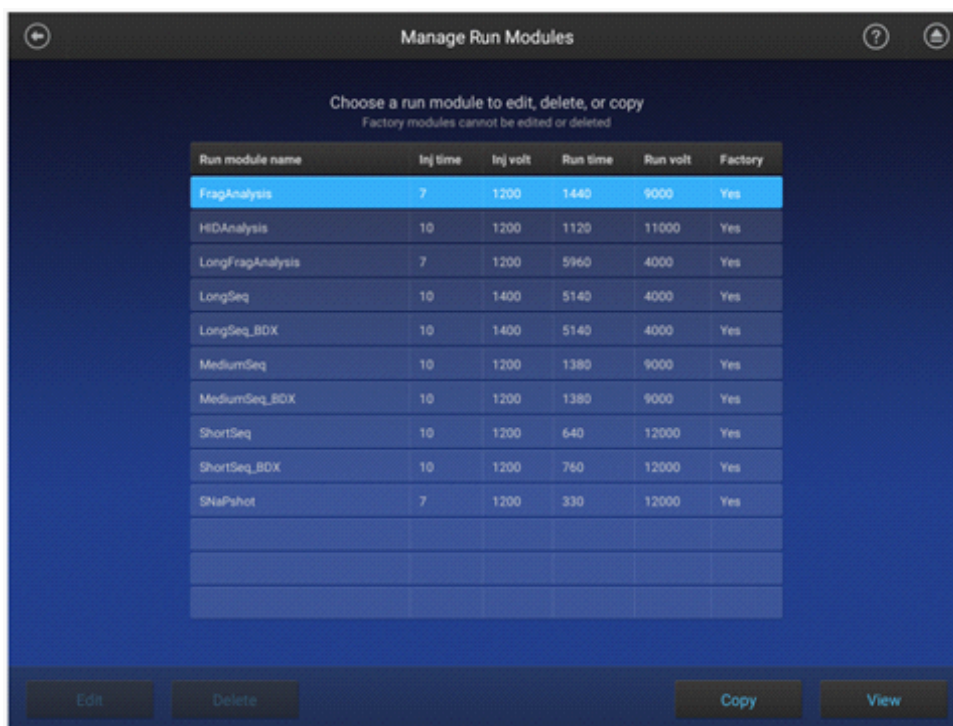
- The results of the calibration are not saved, and the calibration plate is not moved to **Run History**.
- The instrument does not allow you to rerun the plate setup for a failed calibration. Close the calibration screen, then start a new calibration.

## 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

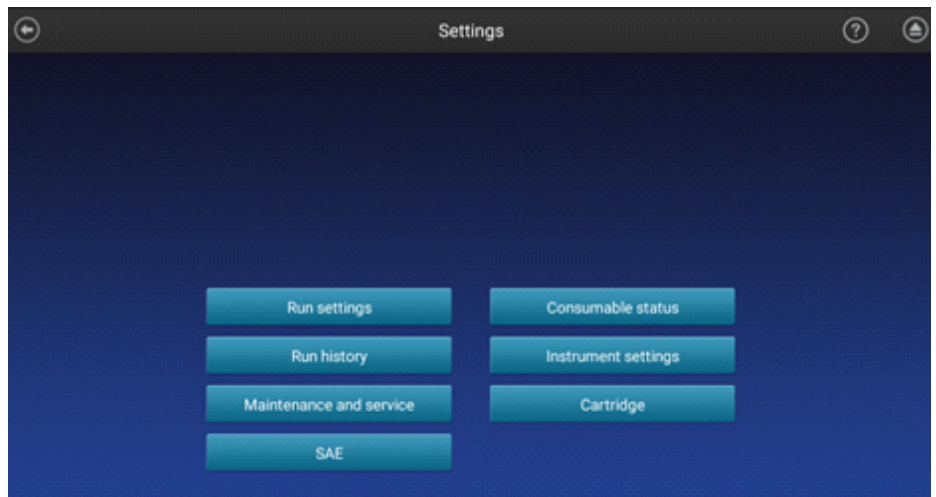
The screenshot shows the 'Edit Run Module' interface. On the left, there are four input fields: 'Injection time' with the value '9' (range [1, 600]), 'Injection voltage' with the value '1600' (range [0, 13000]), 'Run time' with the value '3300' (range [200, 14000]), and 'Run voltage' with the value '6000' (range [0, 13000]). The 'Run voltage' field is highlighted with a red border. To the right of these fields is a numeric keypad with digits 1-9, 0, and a decimal point, as well as a minus sign and a 'Next' button. A back arrow button is also present.

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 standard 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**

**Edit Size Standard**

Name: QD1200      Dye (Color): Orange

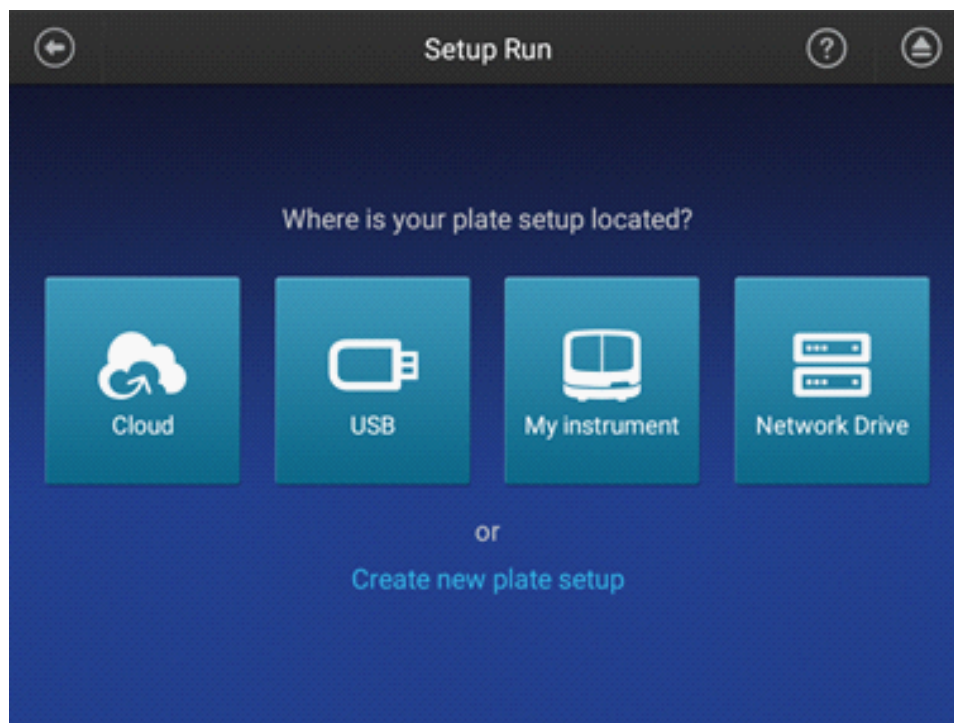
70.0	80.0	100.0	120.0	140.0	160.0
180.0	200.0	225.0	250.0	275.0	300.0
325.0	350.0	375.0	400.0	425.0	450.0
475.0	500.0	525.0	550.0	600.0	650.0
700.0	750.0	800.0	850.0	900.0	950.0
1000.0	1100.0	1200.0			

Add      Delete      Cancel      Done

4. Tap **Done**.

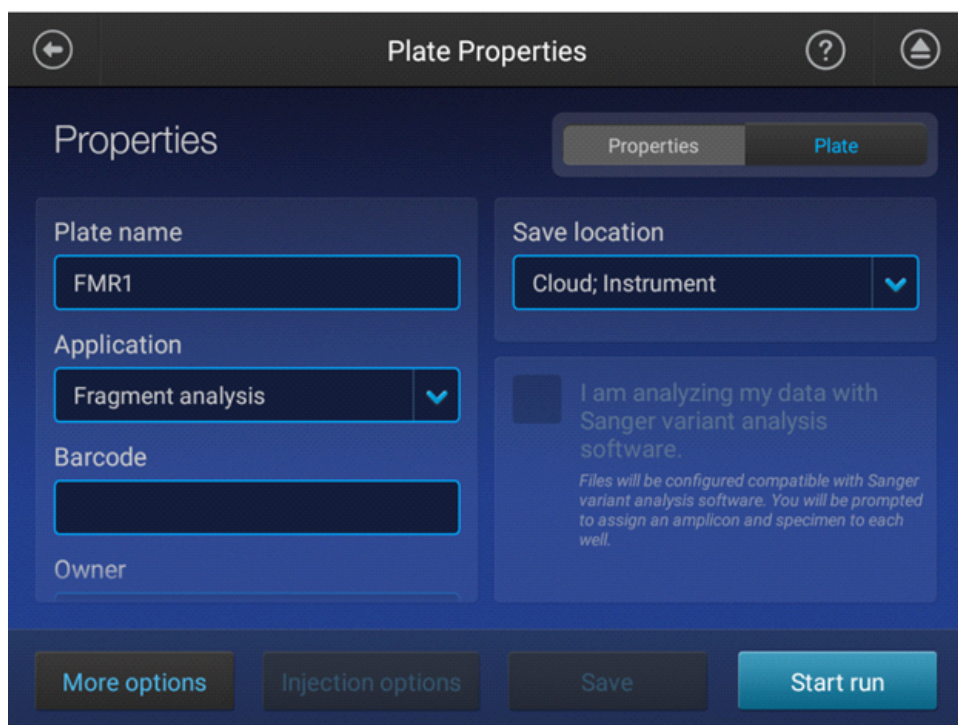
## Create a new plate setup

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





The screenshot shows the 'Plate Properties' window with the 'Properties' tab selected. The 'Plate name' field contains 'FMR1'. The 'Application' dropdown is set to 'Fragment analysis'. The 'Save location' dropdown is set to 'Cloud; Instrument'. There is a checkbox for 'I am analyzing my data with Sanger variant analysis software.' which is currently unchecked. Below this is a note: 'Files will be configured compatible with Sanger variant analysis software. You will be prompted to assign an amplicon and specimen to each well.' At the bottom, there are buttons for 'More options', 'Injection options', 'Save', and 'Start run'.

Plate Properties

Properties

Plate name: FMR1

Application: Fragment analysis

Barcode:

Owner:

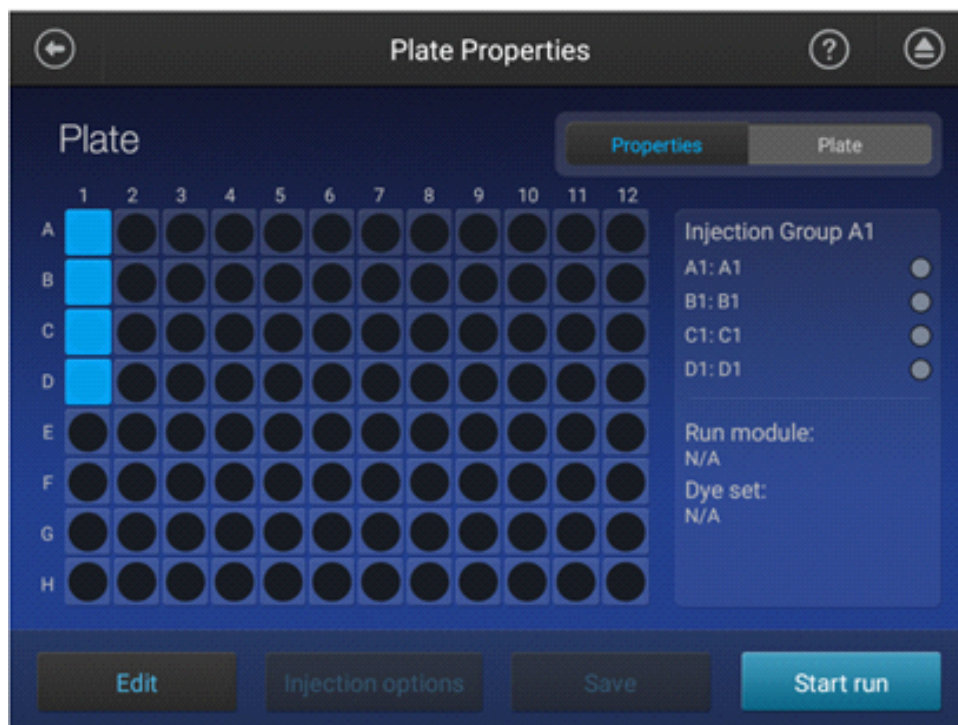
Save location: Cloud; Instrument

☐ I am analyzing my data with Sanger variant analysis software.

Files will be configured compatible with Sanger variant analysis software. You will be prompted to assign an amplicon and specimen to each well.

More options Injection options Save Start run

3. Tap the **Plate** tab.
4. Highlight the **Injection Groups**, then tap **Edit**.



The screenshot shows the 'Plate Properties' window with the 'Plate' tab selected. It displays a 12x8 grid of wells. The first column (A1-D1) is highlighted in blue. To the right of the grid, there is a section for 'Injection Group A1' with four rows: 'A1: A1', 'B1: B1', 'C1: C1', and 'D1: D1', each with a radio button. Below this, there are fields for 'Run module: N/A' and 'Dye set: N/A'. At the bottom, there are buttons for 'Edit', 'Injection options', 'Save', and 'Start run'.

Plate Properties

Plate

Injection Group A1

A1: A1

B1: B1

C1: C1

D1: D1

Run module: N/A

Dye set: N/A

Edit Injection options Save Start run

5. In the **Edit Plate** screen, make the following selections.

- **Run module—ABI\_FMR1\_Assay**
- **Size standard—QD1200**
- **Dye set—A5D**

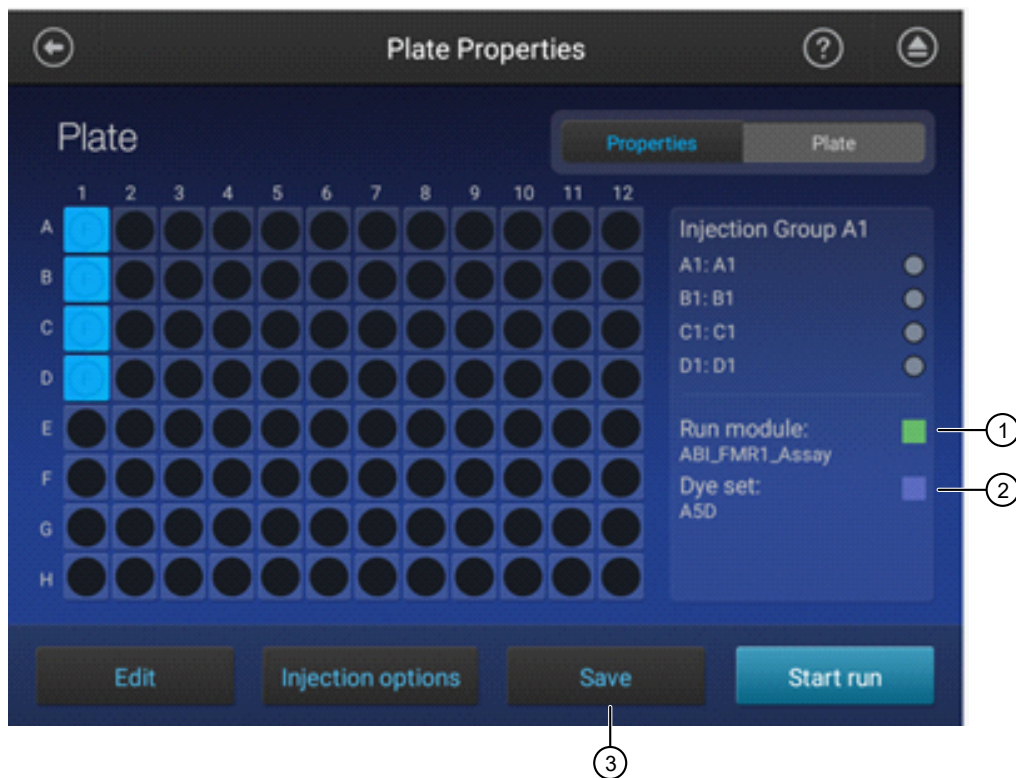
The screenshot shows the 'Edit Plate' screen with the following configuration:

Field	Value
Selected Injections	A1-D1
Sample name	A1, B1, C1, D1
Run module	ABI_FMR1_Assay
Size standard	QD1200
Dye set	A5D

Buttons at the bottom: Clear wells, Cancel, Done.

6. Tap **Done**.

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



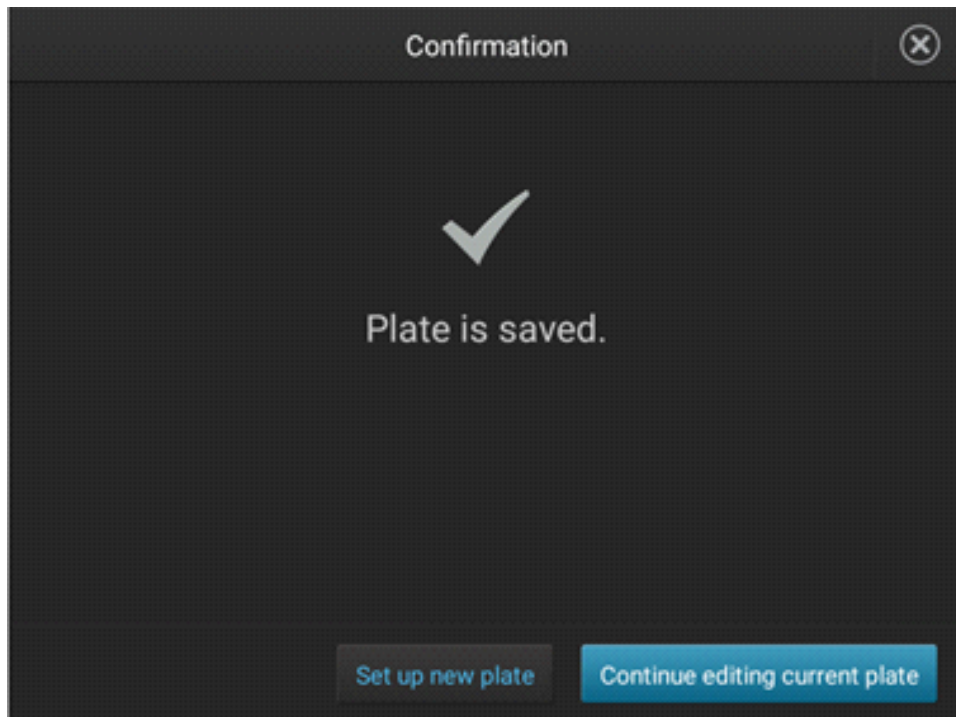
① **Run module**

② **Dye set**

③ **Save**

The plate is saved on the instrument.

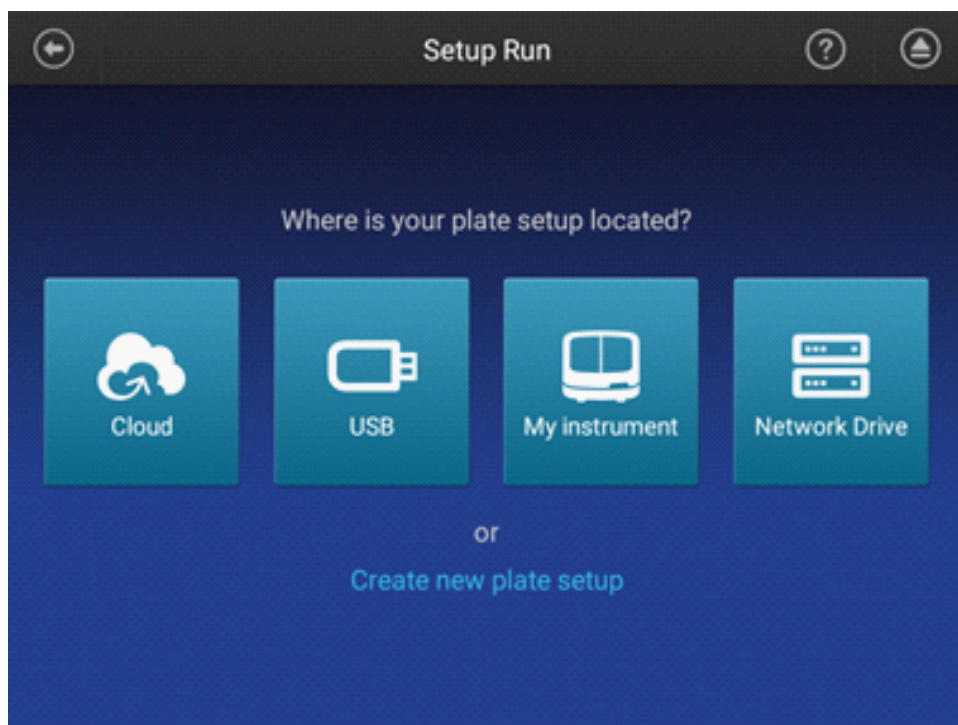




## Export the SeqStudio™ Genetic Analyzer plate setup to the Cloud

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.

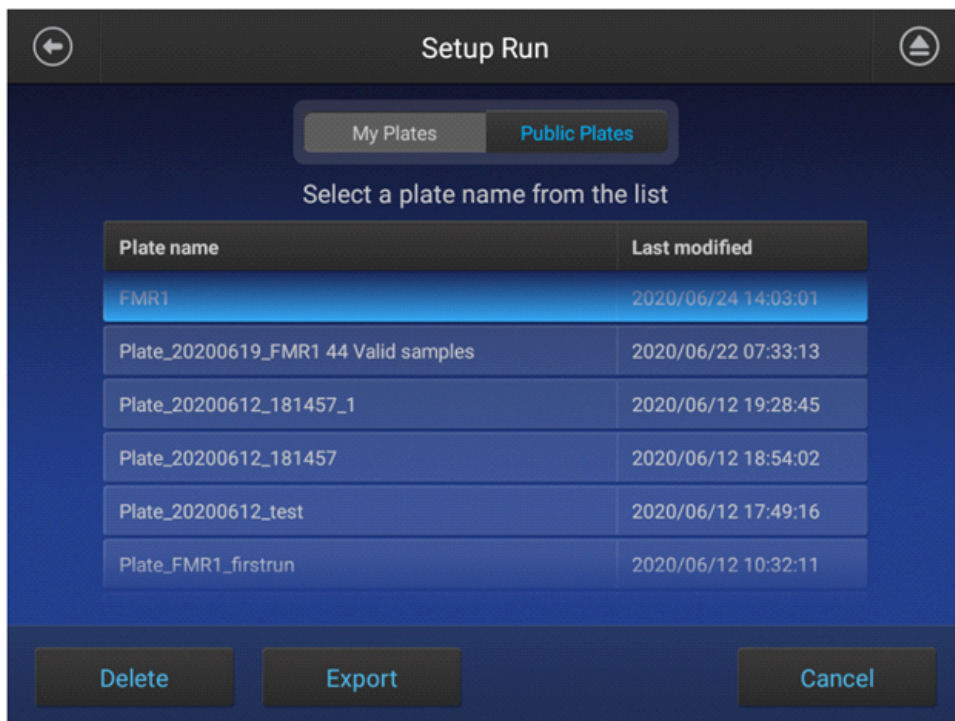
1. On the SeqStudio™ Genetic Analyzer homescreen, tap **Setup run ▶ My instrument**.



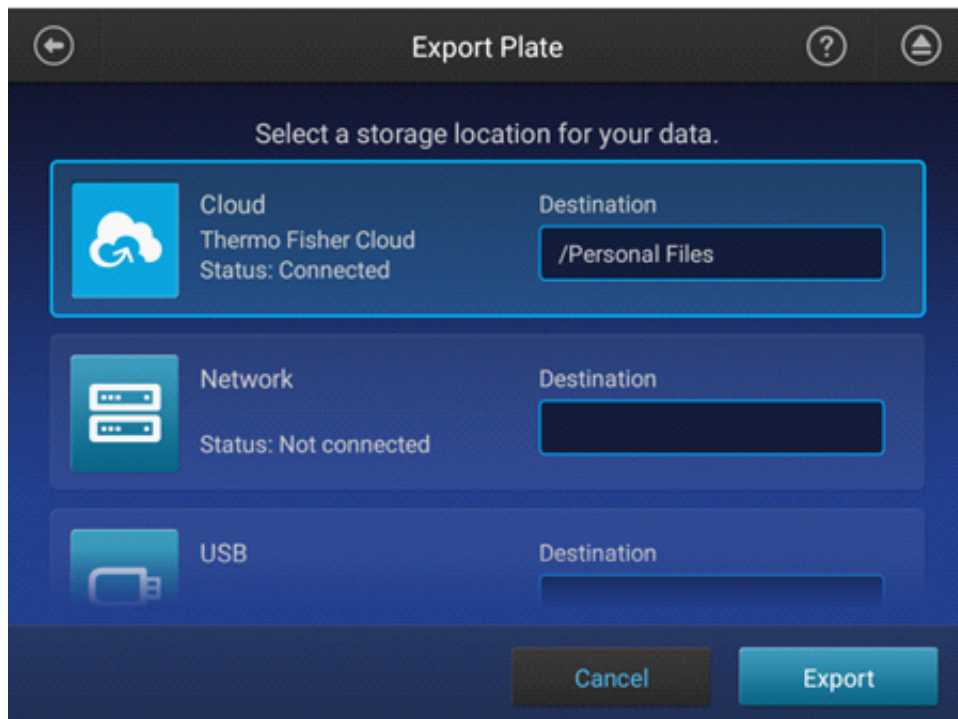
2. Tap **Manage**.



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

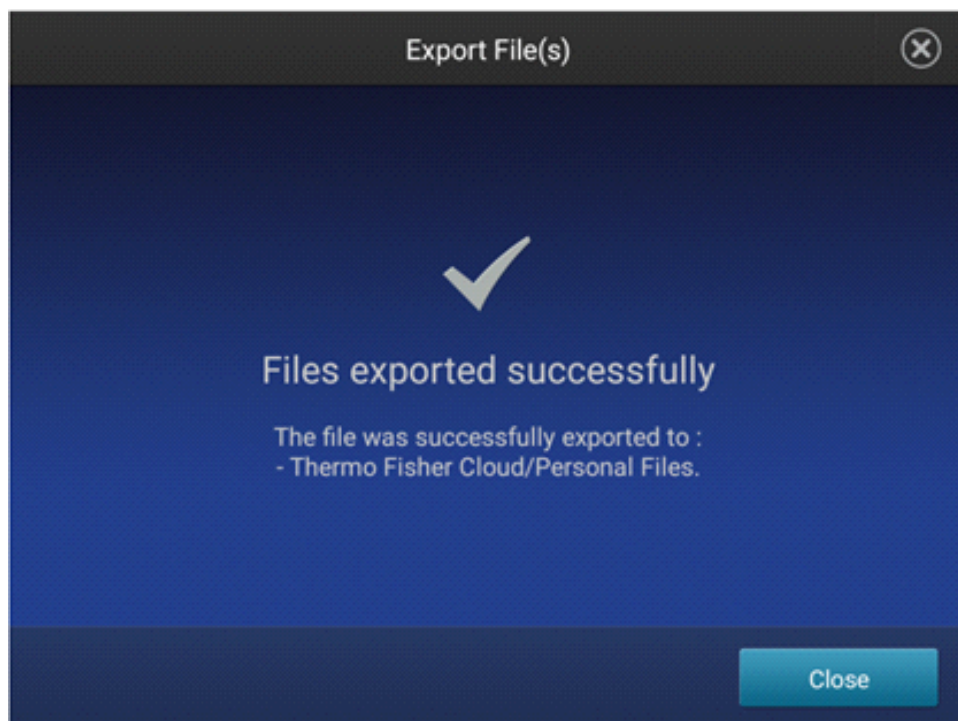


4. Select the **Cloud** storage destination, then tap **Export**.

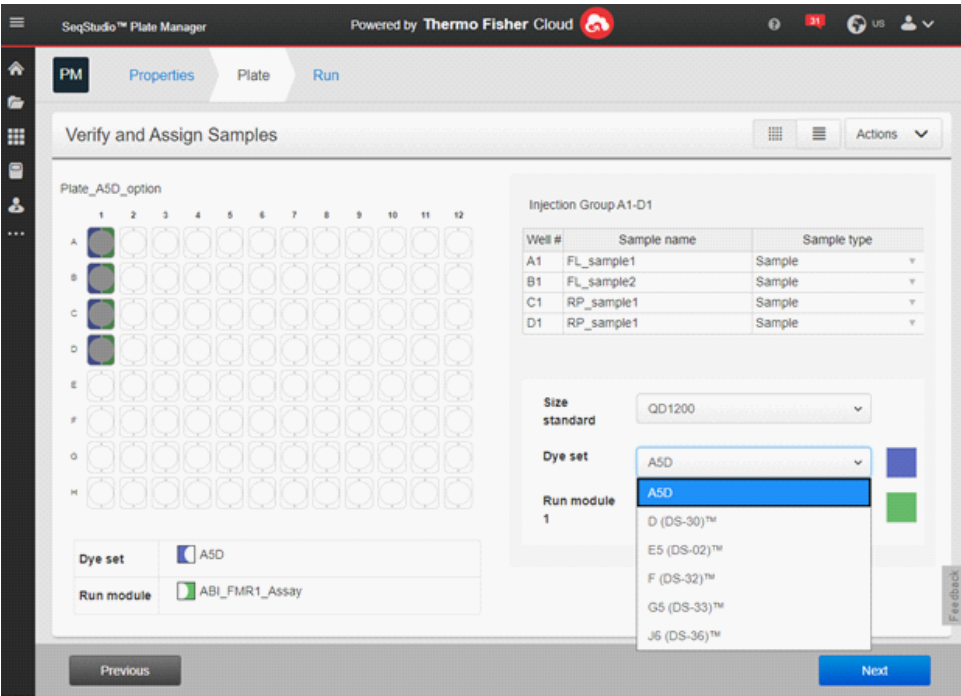


The plate setup is exported to the **Thermo Fisher Cloud** (PSM file).

5. Tap **Close**.



**Note:** The **A5D** dye set is now available 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 .....	34
■ Set up the PCR reactions .....	35
■ Run the PCR .....	36
■ 3500/3500xL Genetic Analyzer .....	37
■ SeqStudio™ Genetic Analyzer .....	39
■ Naming conventions .....	40

### 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 <sup>[1]</sup>	
	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
<b>Total volume</b>	<b>14 µL</b>	<b>14 µL</b>

<sup>[1]</sup> 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  $\mu$ L PCR product + 39  $\mu$ L of nuclease-free water.
- Dilute repeat primer (RP) PCR products 1:15. For example, 1  $\mu$ L PCR product + 14  $\mu$ L 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 $\mu$ L
Hi-Di™ Formamide	8.7 $\mu$ L
<b>Total volume</b>	<b>9 <math>\mu</math>L</b>

---

**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  $\mu$ L of the Hi-Di™ Formamide and CarrierMax™ FMR1 Reagent QD1200 Size Standard mix
  - 1  $\mu$ L of diluted PCR product (see “Run the PCR” on page 36)

---

**Note:** For blank wells, add 10  $\mu$ L 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™ Software and 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

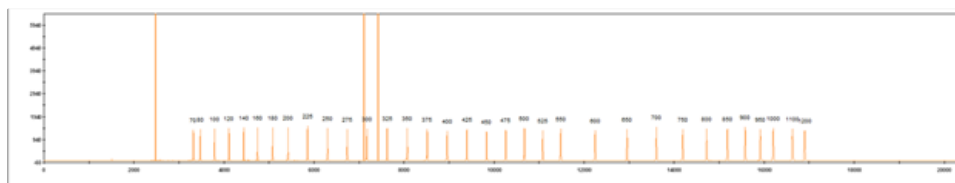
■ Data analysis workflow .....	42
■ Analyze the data with GeneMapper™ Software .....	44
■ Analyze the data with CarrierMax™ Software .....	62

## Data analysis workflow

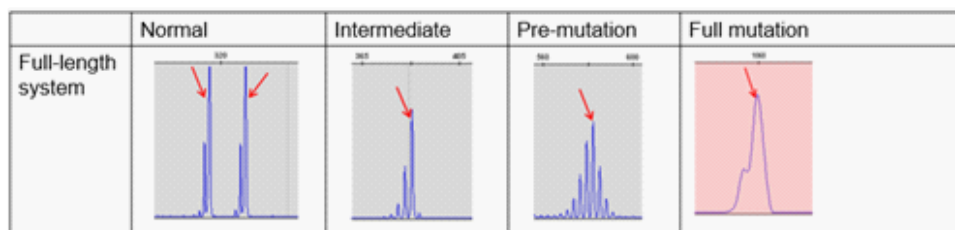
## Create a project and analyze sample files with GeneMapper™ Software

**Note:** Before first use, the latest instrument-specific settings files must be imported. See “About importing files into the GeneMapper™ Software database” on page 44.

### Determine sizing quality (SQ), then correct as required

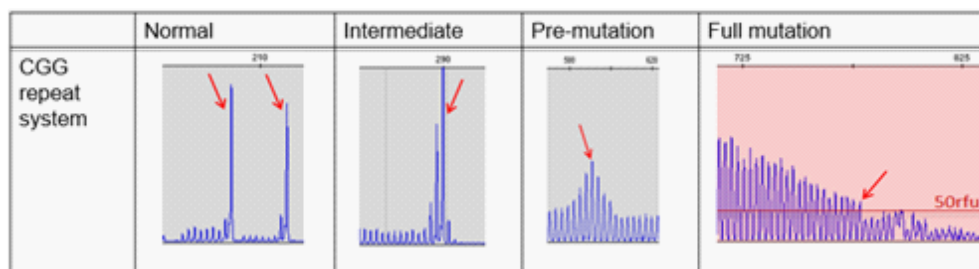


### Review the full length (FL) peak results



For samples with possible full mutation alleles, see step 4 or go to the “Results troubleshooting” on page 72.

### 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 72.

# 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.  <b>IMPORTANT!</b> Use only panel and bin TXT files that are provided by Thermo Fisher Scientific. If you use panel and bin files that you create in the GeneMapper™ Software, you may see off-ladder alleles.	“Import panel and bins” on page 45
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 47
Size Standard	XML	Defines the sizes of the fragments present in the size standard.	“Import the size standard” on page 46
Table Settings	XML	Hide or show columns, filter results, sort order.	“Import table settings and plot settings” on page 48
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.	

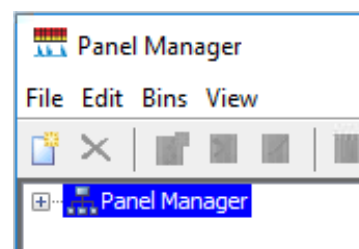


## Import panel and bins

**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.

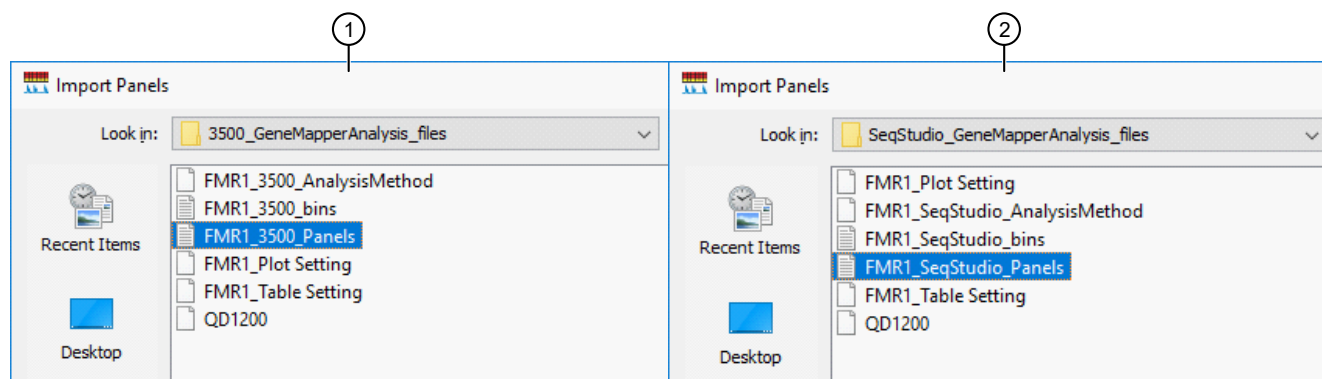
1. Start the GeneMapper™ Software, then log in with the appropriate user name and password.
2. Select **Tools ▶ Panel Manager**.
3. In the left pane, click **Panel Manager**.

**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](http://thermofisher.com/carriermax-fmr1).



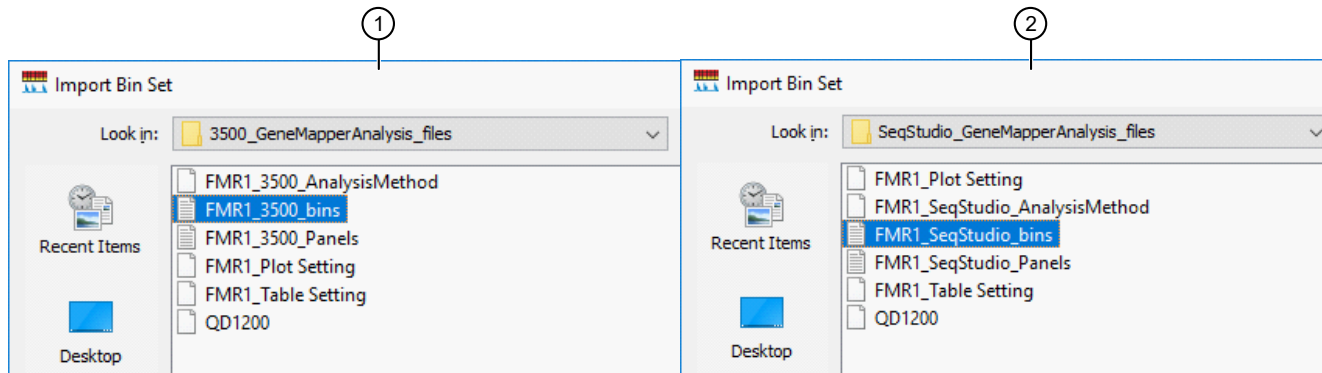
- ① 3500/3500xL Genetic Analyzer
- ② SeqStudio™ Genetic Analyzer

- 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.

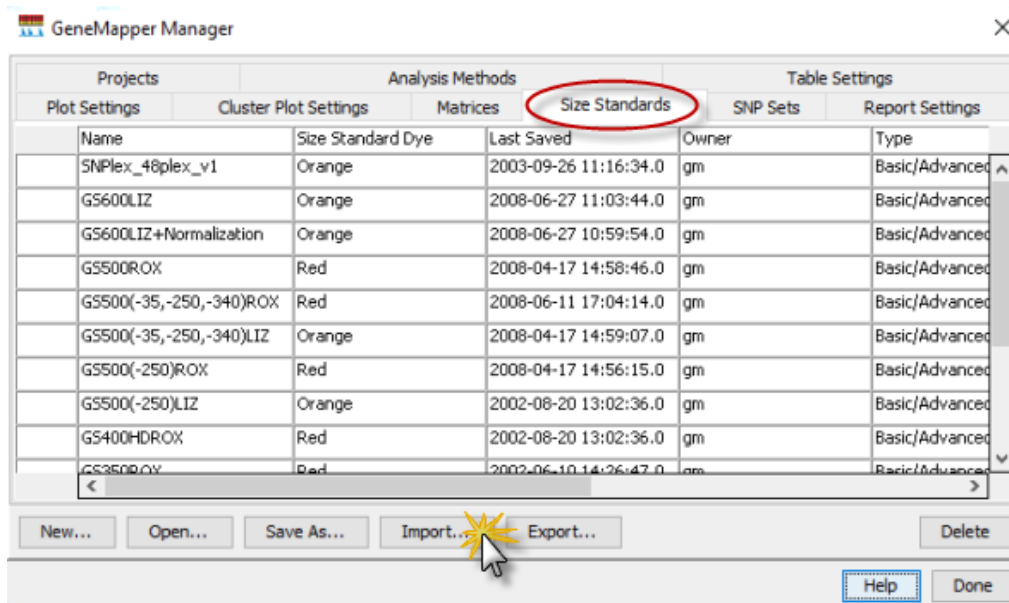
---

## Import the size standard

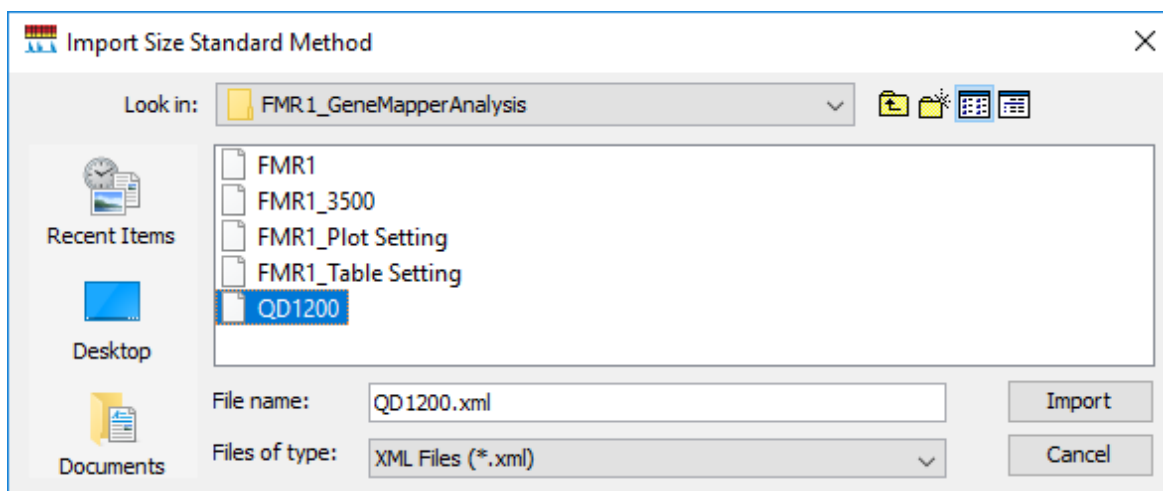
Import the latest size standard from the website into the GeneMapper™ Software database:

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.

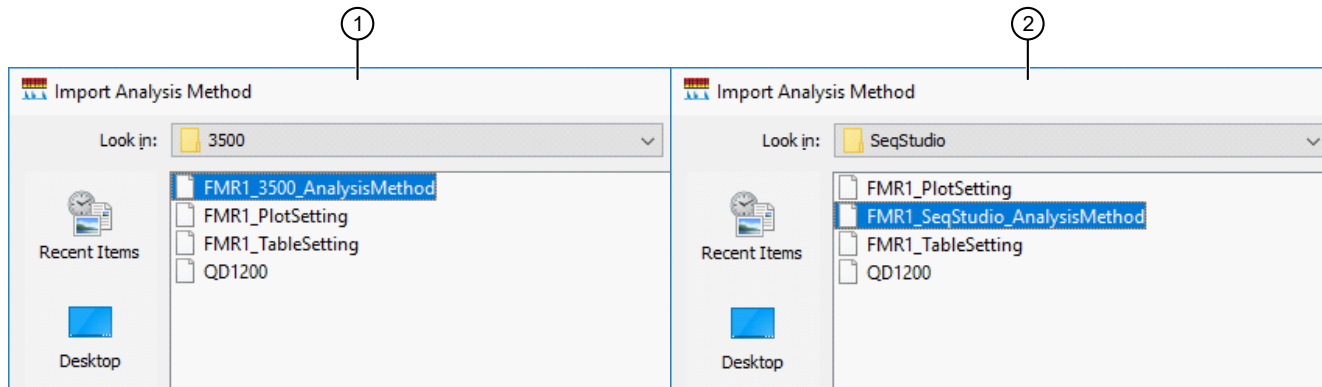


5. Click **Import**.
6. Click **Done**.

## 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

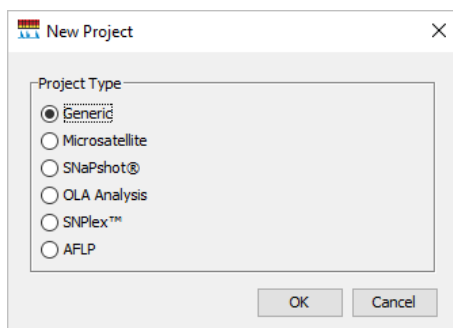
5. Click **Done**.

## Import table settings and plot settings

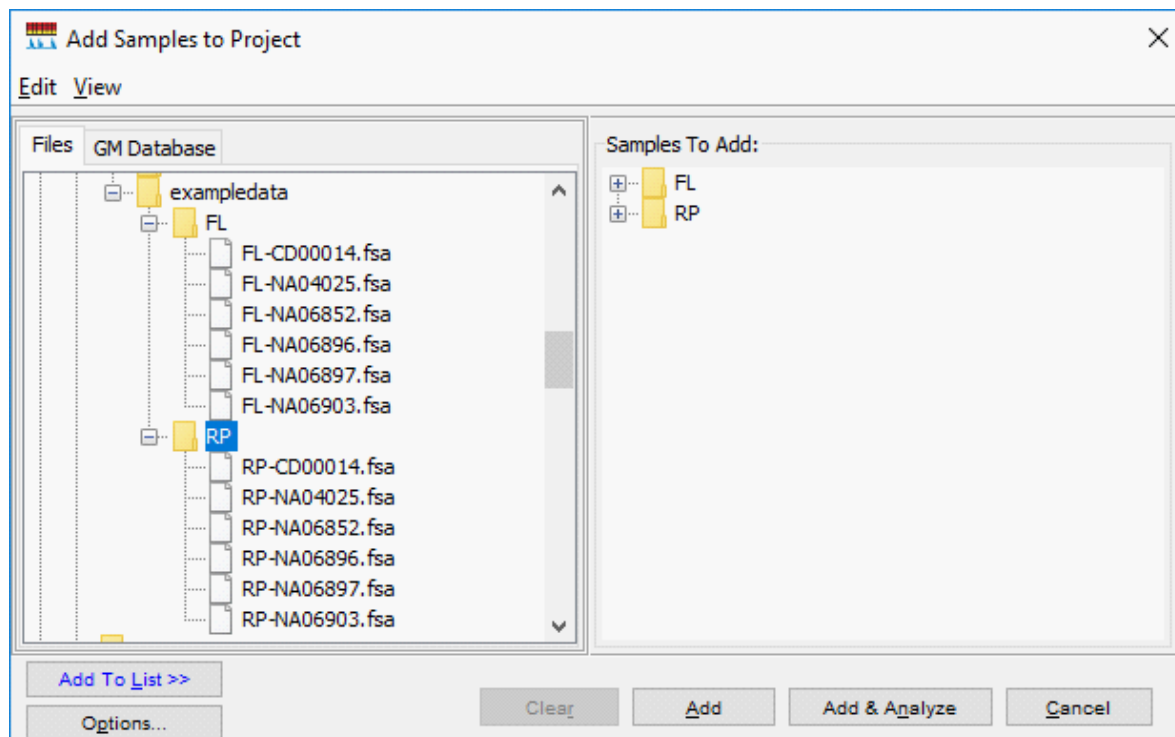
1. In the GeneMapper™ Software, select **Tools ▶ GeneMapper Manager**.
2. In the **Table Settings** tab, click **Import**.
3. Navigate to, then select, the appropriate table setting file.
4. Click **Import**.
5. In the **Plot Settings** tab, click **Import**.
6. Navigate to, then select, the appropriate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

## Create a project and analyze sample files with GeneMapper™ Software

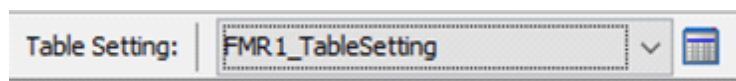
1. If a project is currently open, select **File ▶ New Project**, then select **Generic**.



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.

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

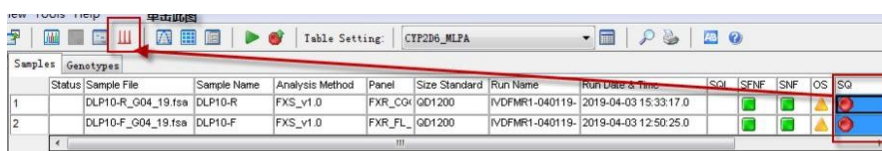
- c. If this is the first time the analysis method is used: Double-click the analysis method in the first row, then click the **Allele** tab. Select the bin set you imported.

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.

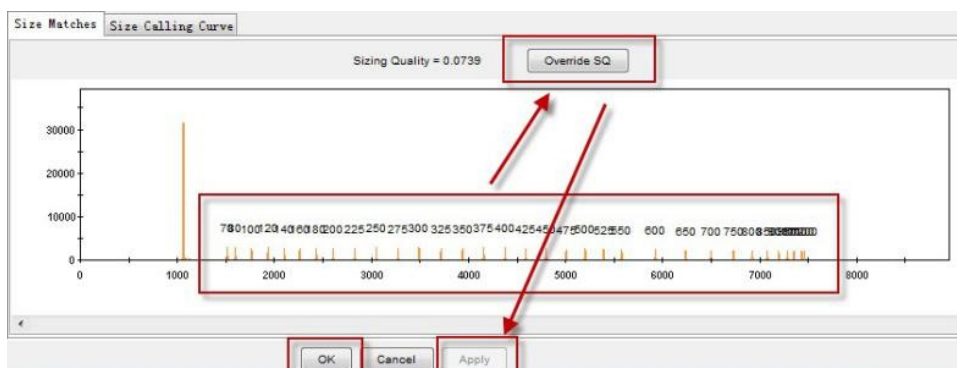
5. Click  (**Analyze**), enter a name for the project (in the **Save Project** dialog box), then click **OK** to start analysis.

- 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.




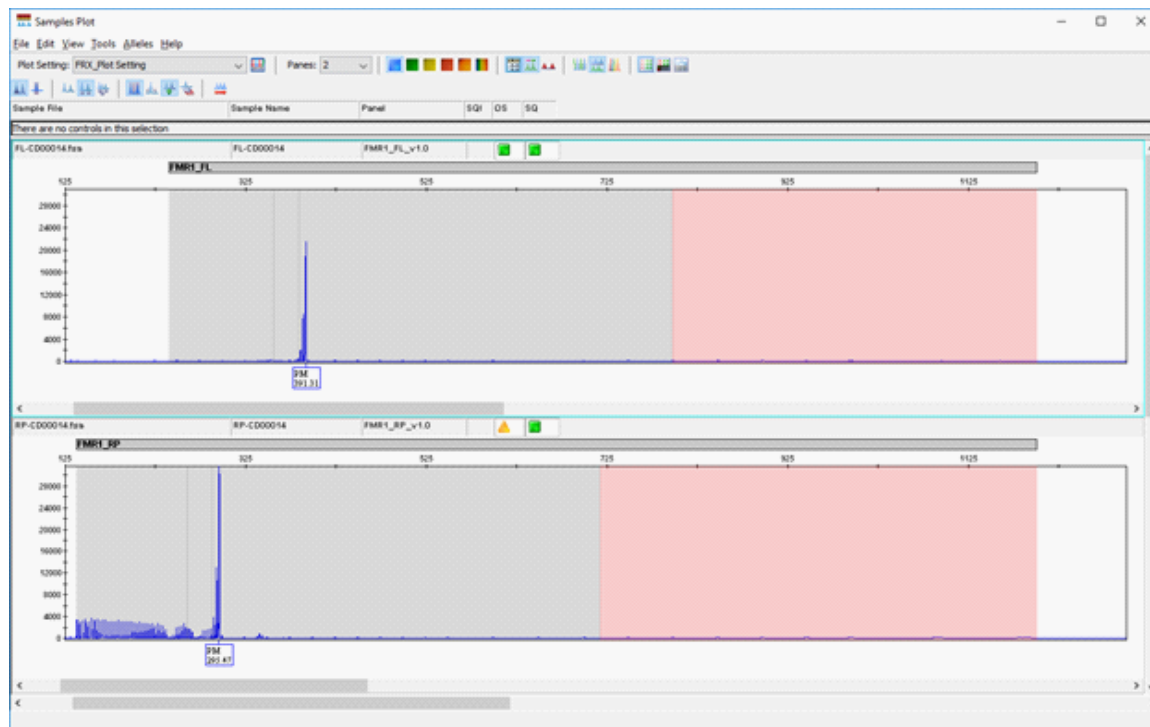
Sample	Status	Sample File	Sample Name	Analysis Method	Panel	Size Standard	Run Name	Run Date & Time	SQ	SFNF	SNF	OS	SQ
1		DLP10-R_004_19.fsa	DLP10-R	FXS_v1.0	FXR_CGX	GD1200	IVDFMR1-040119-	2019-04-03 15:33:17.0					
2		DLP10-F_004_19.fsa	DLP10-F	FXS_v1.0	FXR_FL	GD1200	IVDFMR1-040119-	2019-04-03 12:50:25.0					

**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**.

GeneMapper® Software Online Help

Hide Back Forward Print

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GeneMapper® Software Online Help

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**Applied Biosystems**

### Samples/Genotypes Plot

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

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

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

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

**Bins**

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

**Labels**

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

**Genotype Calls**

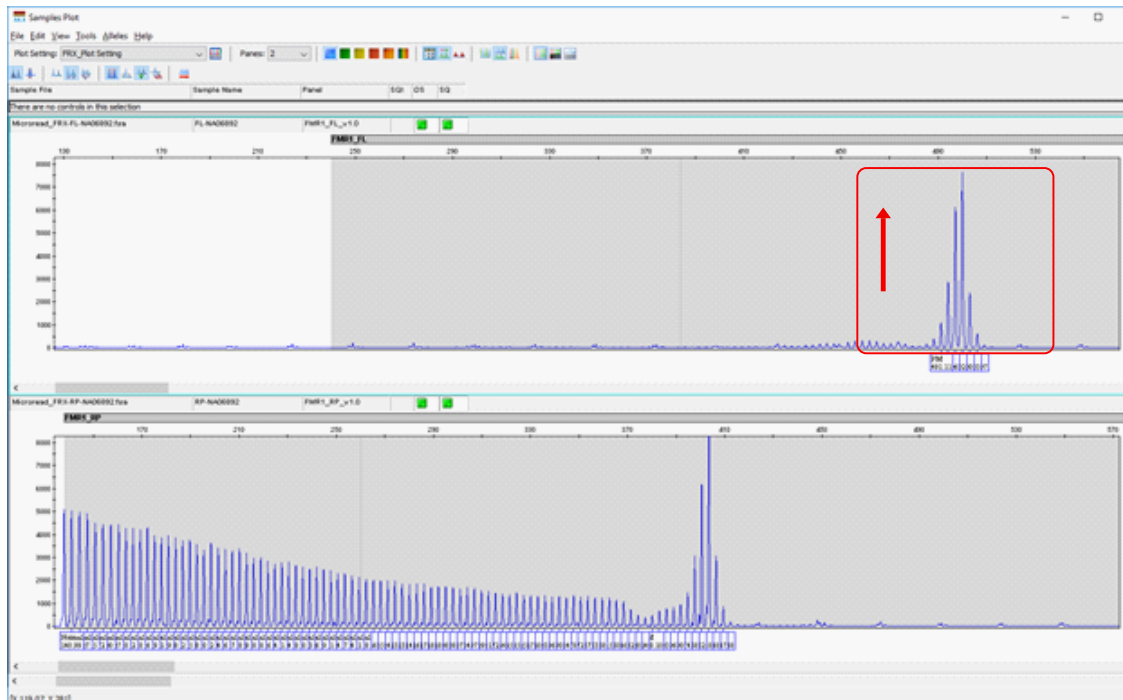
- Modify alleles
- Rename peaks



2. Select the plot setting that determines the headers that are displayed in sample and genotype plot:  
Click the arrow next to the **Plot Setting** list at the top of the screen, then select the setting.
3. To zoom on the plot, select **View ▶ Zoom**, or:
  - To zoom on the x- axis, place the cursor on the x-axis *at the top of the plot*, then click-drag to the right.



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



- To unzoom double-click the x-axis.

- 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 68.

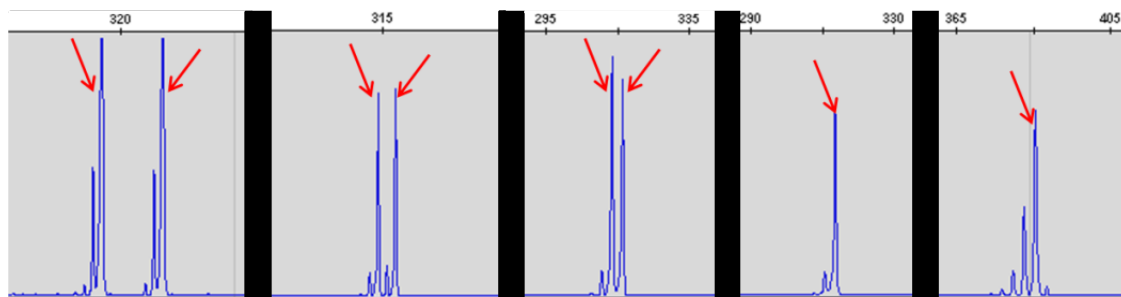
## Determine sizing quality (SQ), then correct as required

- In the sample table, select a sample with a SQ label, then click (Size Match Editor).
- In the Size Match Editor, select **Add**, **Delete**, or **Change** for individual peaks as needed.
- 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 53.
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 68.
  - a. Select full length (FL) peaks for normal alleles between 240.0-355.5 bp and intermediate alleles between 355.6-383.9 bp.

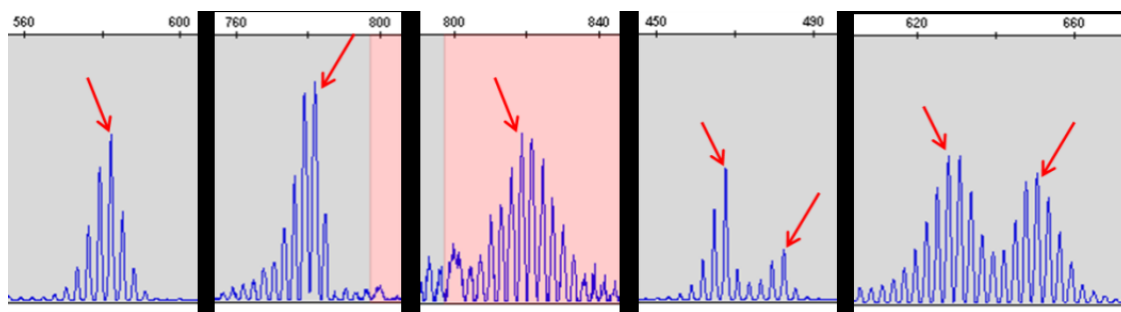
Select the highest signal peak as the full length gene-specific target peak, generally the right-most peak as there may be multiple peaks in this range.



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.
 

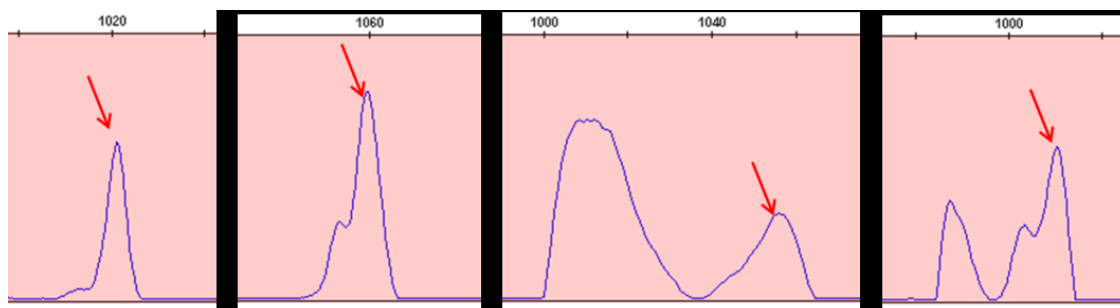
Select the highest signal peaks, generally the center peak for multi-peak alleles or peaks in the same size range. Premutation or full mutation alleles can display a single peak population less than 8 peaks from center to end, or a complex distributions of peaks.



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

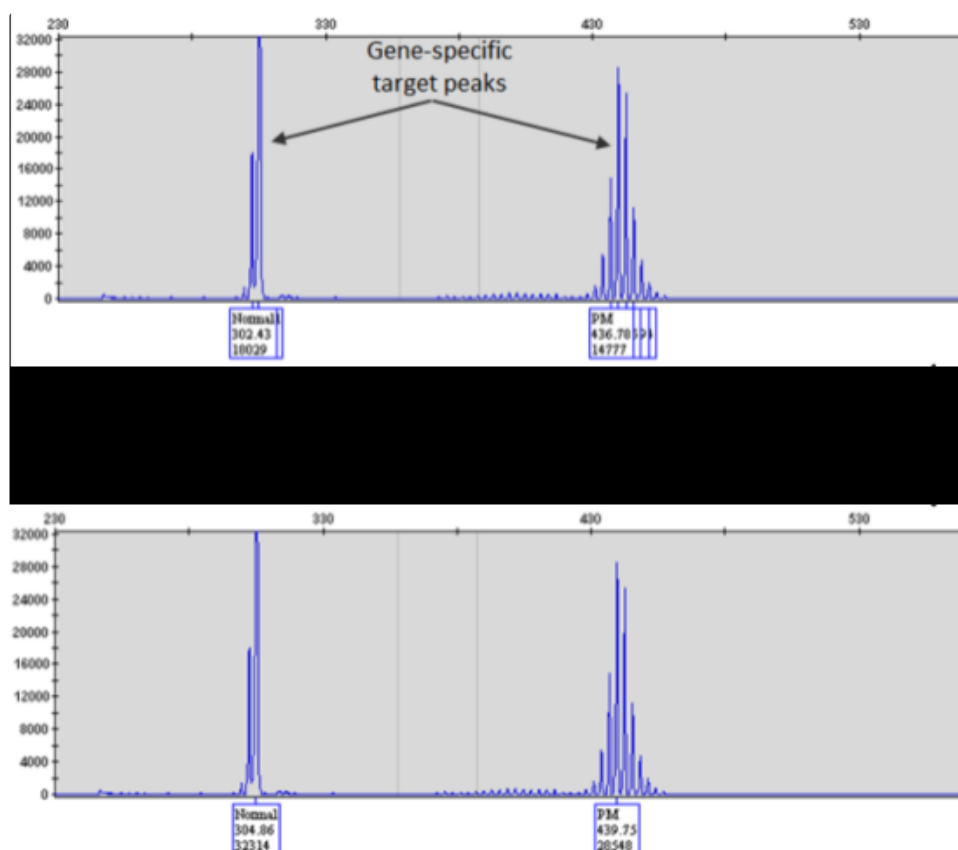
- c. Select full length (FL) peaks for full mutation alleles >798 bp.

**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.

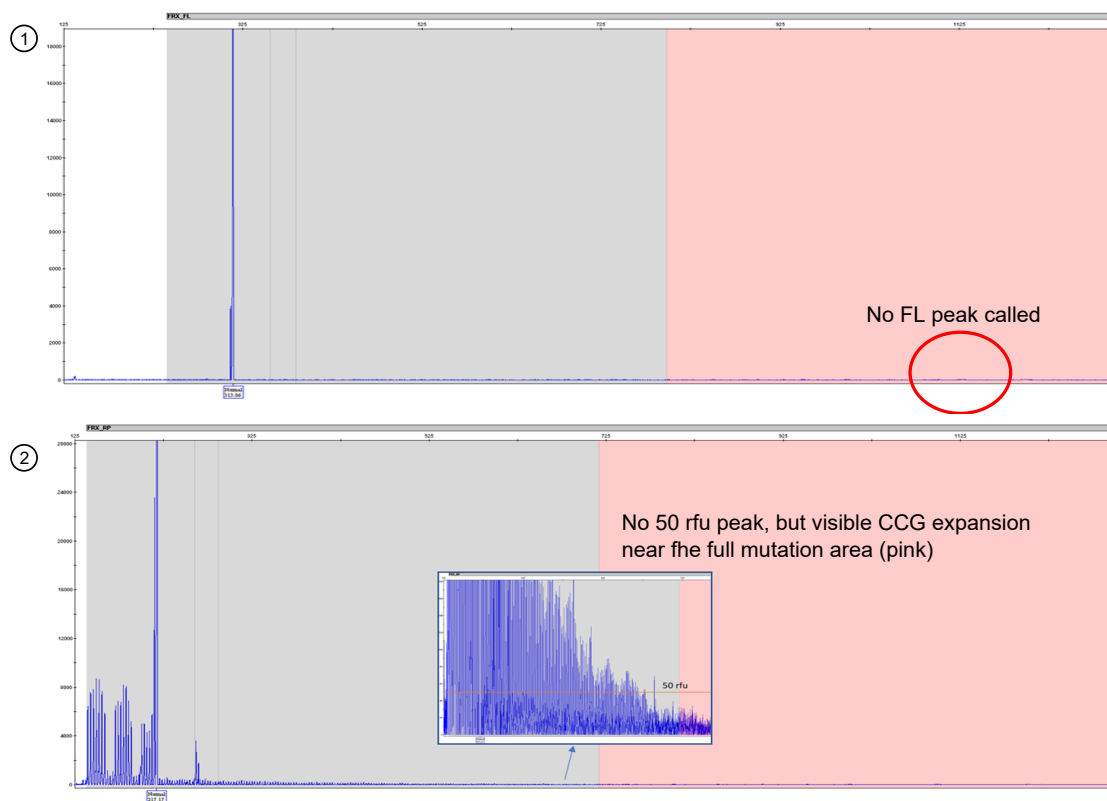


Example electropherogram of Full Length (FL) PCR product with default analysis settings (top) and only gene-specific target peaks selected from the electropherogram (bottom).

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.

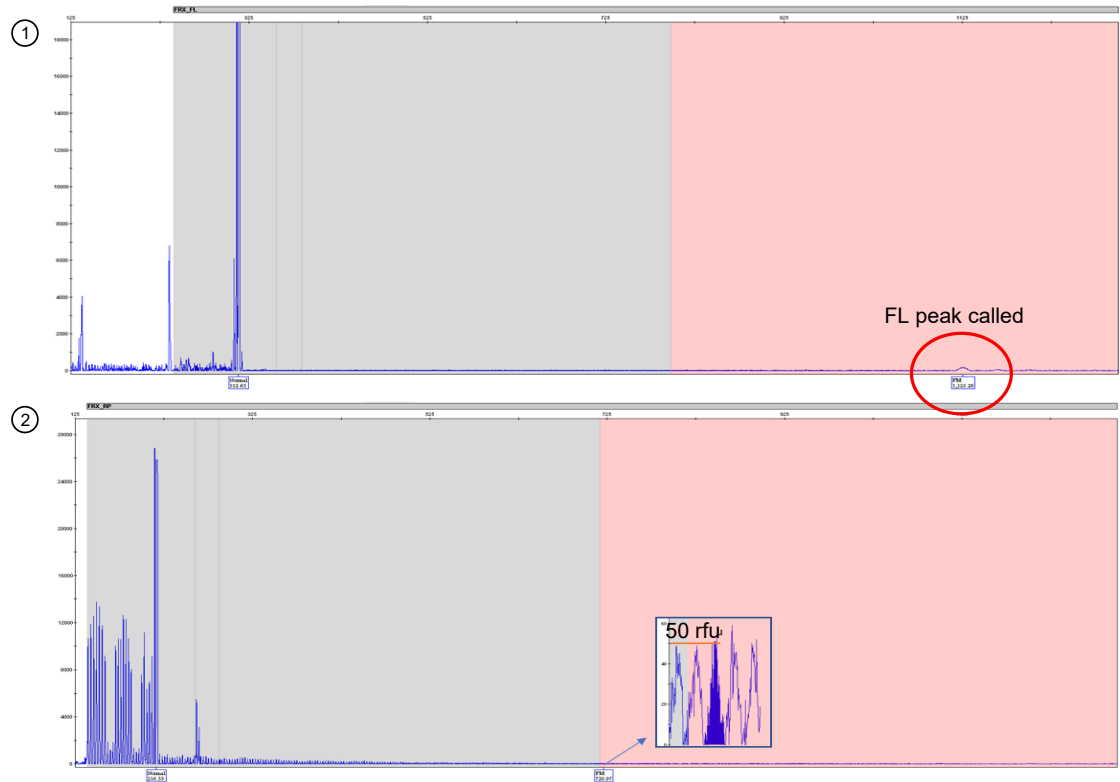
- b. If there is CGG expansion, re-run FL and RP reactions with undiluted sample, then check the FL results again.

**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

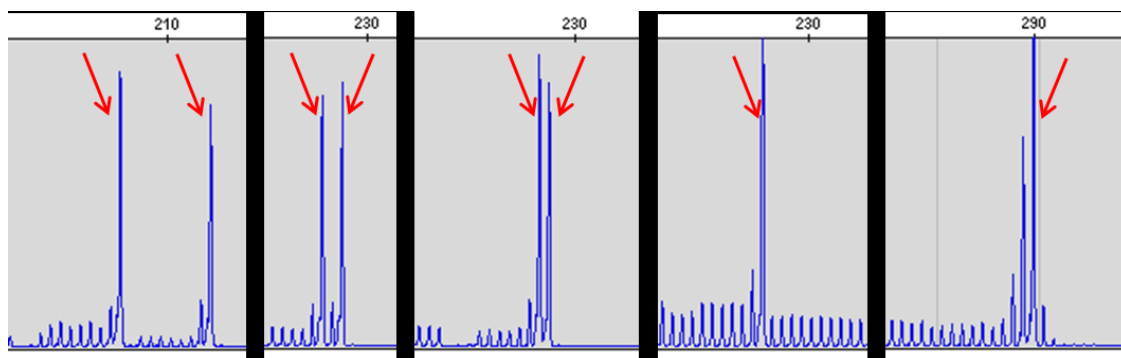
- ① Electropherogram of FL reactions (undiluted)
- ② Electropherogram of RP reactions (undiluted)

## Review the repeat primers (RP) peak results

1. Perform a quality control check by following the guidelines in “Results guidelines” on page 53.
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 68.

- 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.

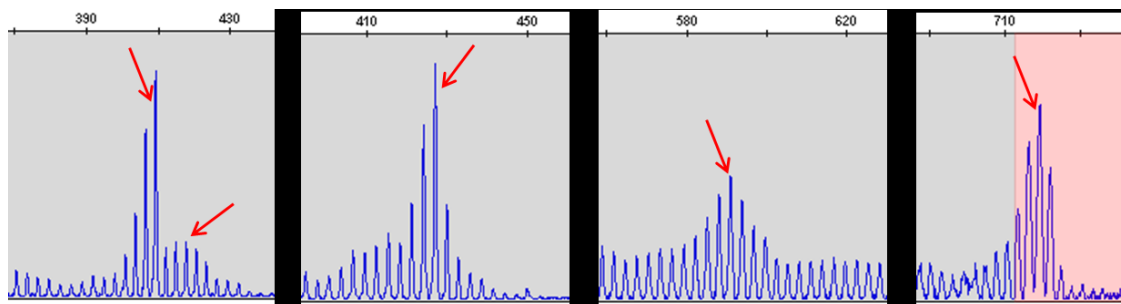
Select the highest signal peak, generally the right-most peak as there may be multiple peaks in this range. "Step signal" peaks appear. These are peaks < 2 peaks from the highest peak to the right end, or the left continuous peaks more than one times higher than the right.



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.

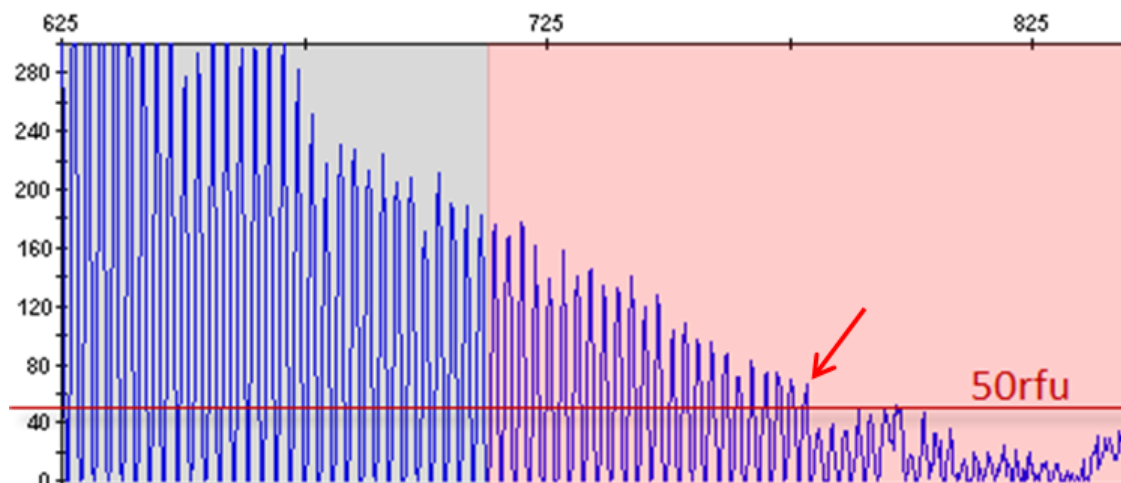


Example of RP peaks for premutation alleles between 287.1-716.9 bp.



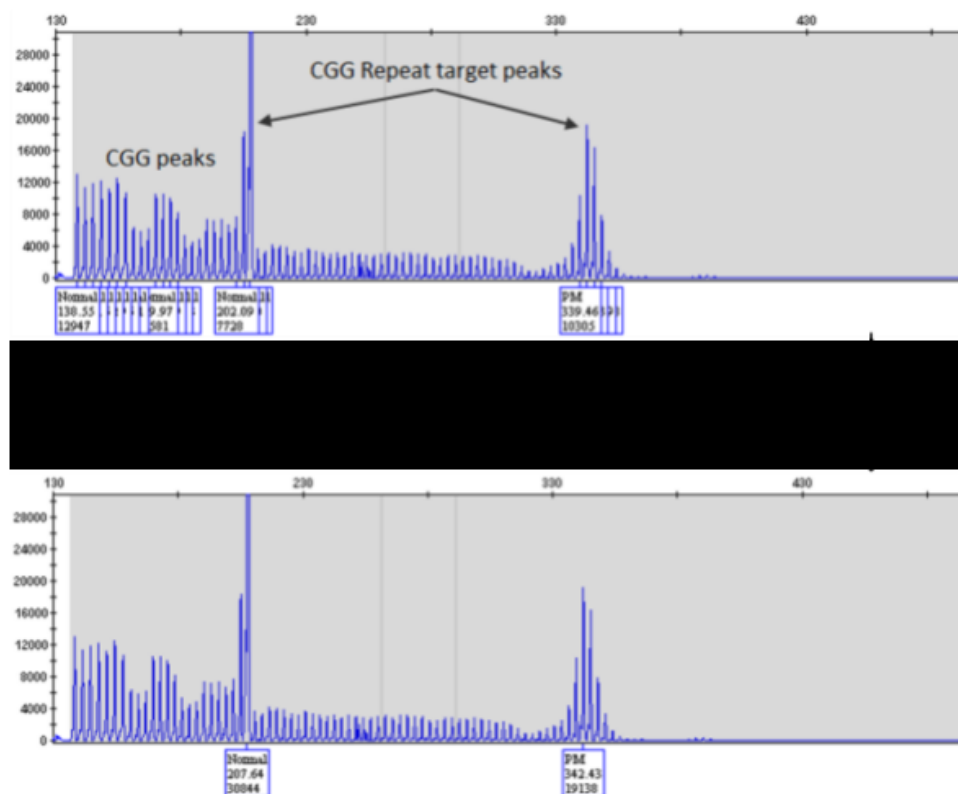
- c. Select full length (RP) peaks for full mutation alleles >717 bp.

**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 72.

## Export the GeneMapper™ Software genotypes table

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

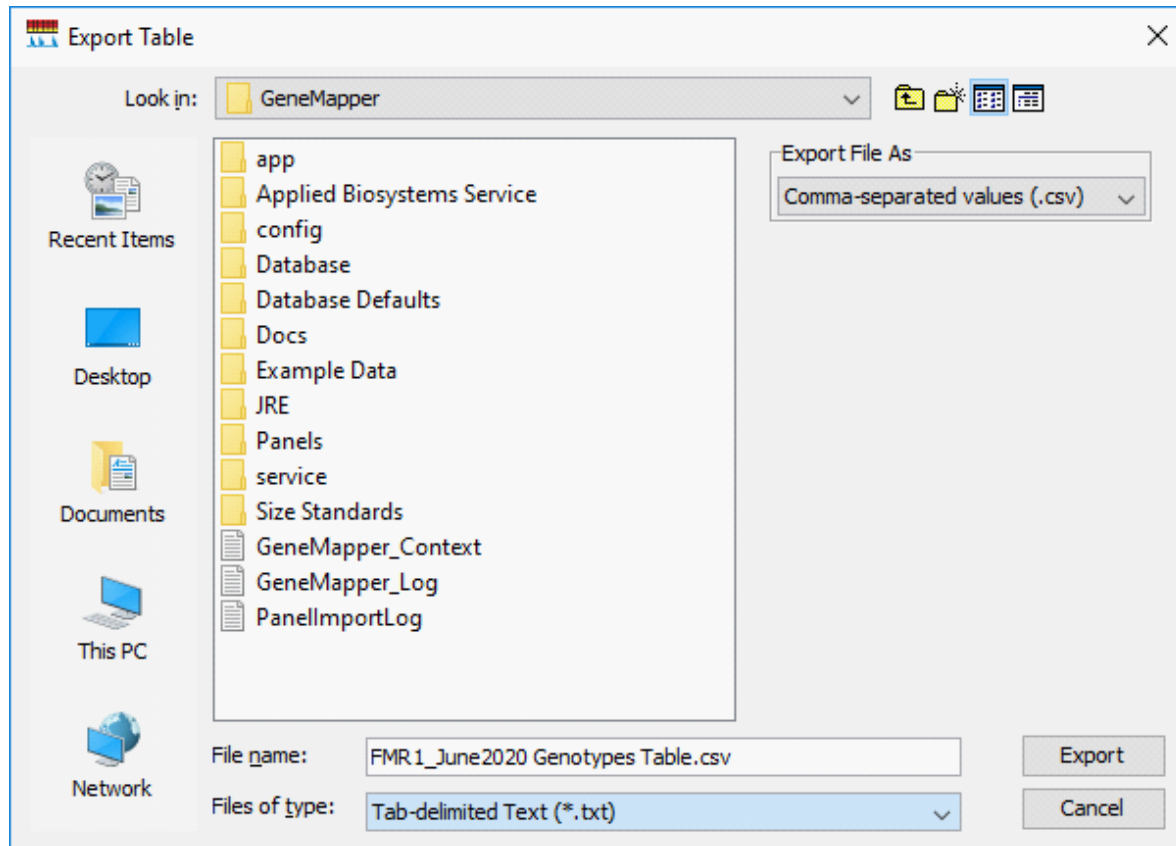
	Sam	Sample Name	Panel	Marker	Dye	Allele 1	Allele 2	Size 1	Size 2	Height
20	Mic	FL-NA20240	FRX_FL_v1.0	FRX_FL	B	Normal	PM	315.55	467.42	273
21	Mic	FL-NA20241	FRX_FL_v1.0	FRX_FL	B	Normal	PM	312.3	576.31	320
22	Mic	FL-NA20242	FRX_FL_v1.0	FRX_FL	B	Normal	PM	315.15	443.96	296
23	Mic	FL-NA20244	FRX_FL_v1.0	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 separate 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](https://thermofisher.com/carriermax-fmr1).
2. Download the **CMAXSetup.exe** file.

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

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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 62. Open the file.

[illegible]

**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 72.
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 72.
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 72.





# Troubleshooting

■ PCR and CE troubleshooting .....	66
■ Peak troubleshooting for the CarrierMax™ FMR1 Reagent Kit .....	68
■ GeneMapper™ Software troubleshooting .....	71
■ Results troubleshooting .....	72

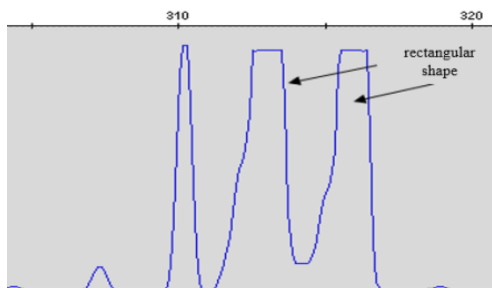
## PCR and CE troubleshooting

Observation	Possible cause	Recommended action
Faint or no signal from the controls and test samples at all loci	The <b>PCR Reaction Mix</b> 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.

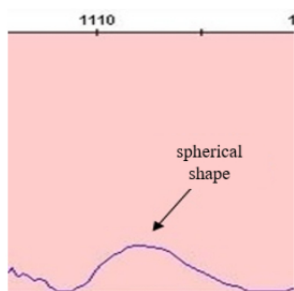
Observation	Possible cause	Recommended action
Poor peak height balance	Too much DNA. Excess DNA causes low-size peaks to appear much higher.	Use the recommended amount of DNA.
	The test sample contains a high concentration of PCR inhibitor (for example, heme compounds or certain dyes)	Quantitate the 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

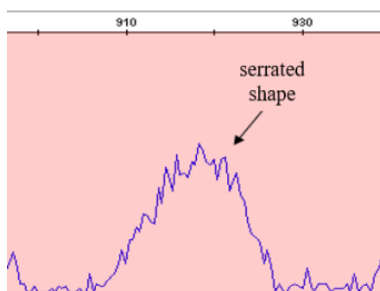
### Abnormal peak shape examples



Rectangular shaped peaks

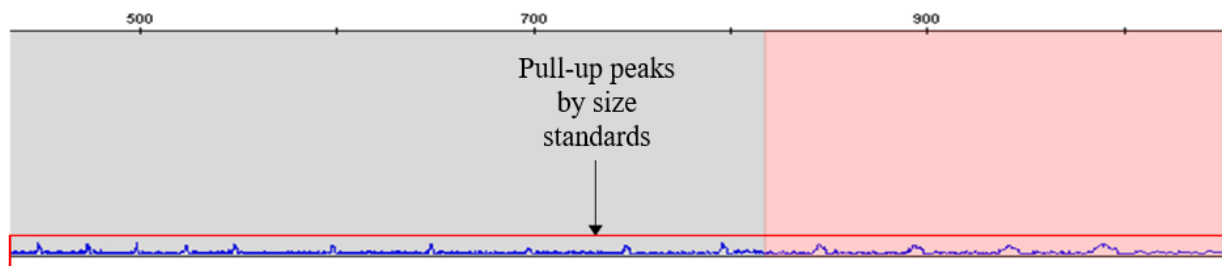


Spherical shaped peaks



Serrated shaped peaks

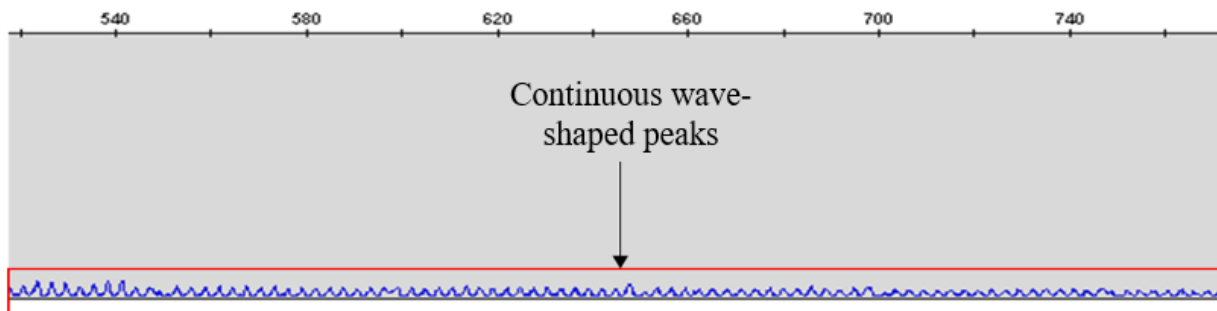
### Pull up peaks example



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

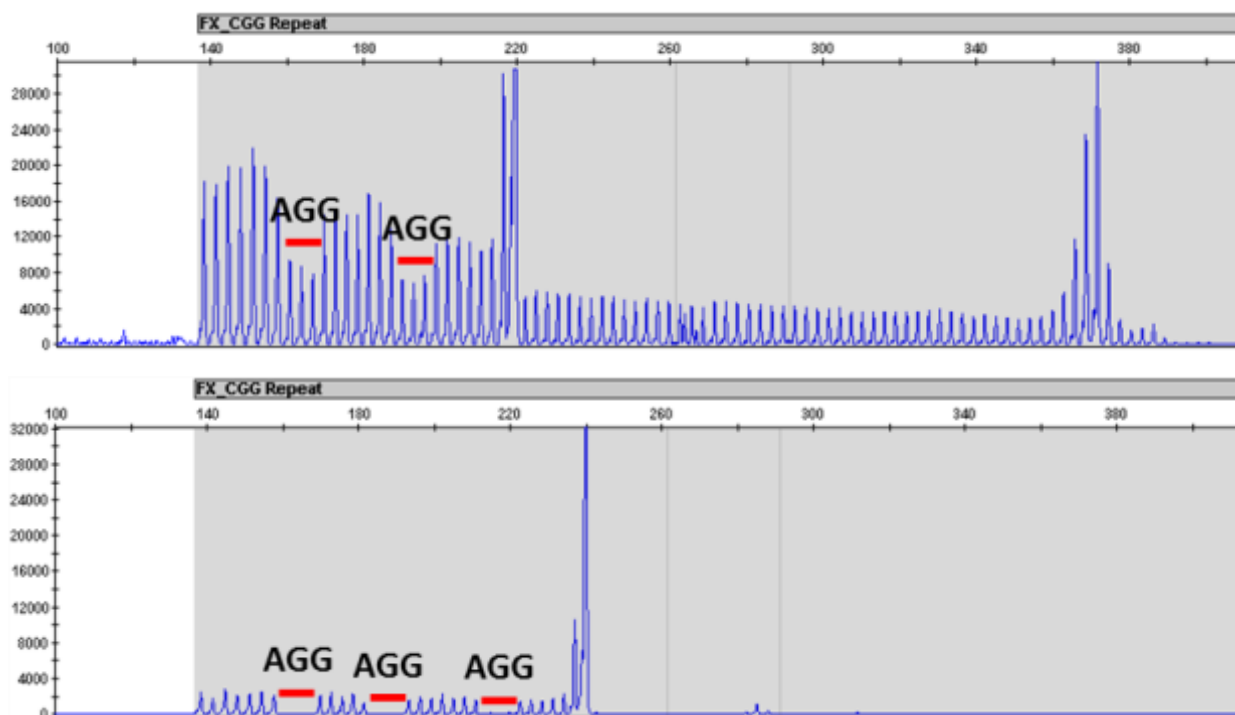
## Continuous waves example

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.

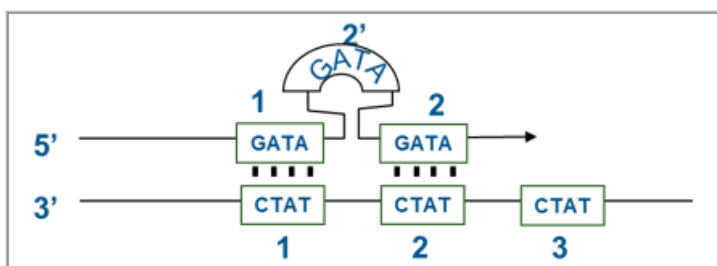


Figure 4 Plus stutter is caused by slippage in the top DNA strand. Example of tetranucleotide stutter (one unit = 4 bp).

### 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


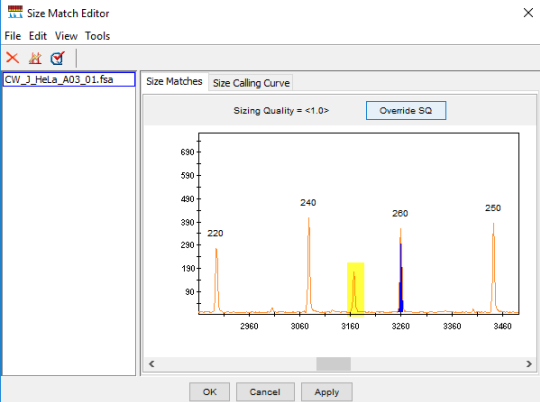
Pull-up peaks are observed if simultaneous fluorescence occurs in multiple dye color channels. Pull-up is caused by the overlapping emission spectrum of fluorescent dyes and usually occurs when the fluorescence signals approach the dynamic range of the instrument.

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

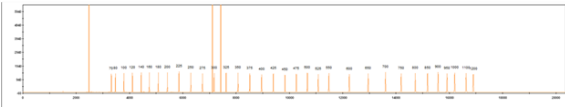
# GeneMapper™ Software troubleshooting

## 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 53.

Observation	Possible cause	Recommended action
When you click  ( <b>Analyze</b> ), 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 <b>View ▶ Sample Info</b> . Review the information in the <b>Error Message</b> section of the screen.
		Correct the issue indicated in the <b>Error Message</b> section of the screen.
Cannot select peak in <b>Size Match Editor</b> 	The peak signal is below the <b>Peak Amplitude Threshold</b> 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.
<b>No Sizing Data error</b> 	Offscale signal of sample peaks (orange pull-up peaks).	Further dilute the FMR1 sample prior to capillary electrophoresis or go to the <b>Size Match Editor</b> , 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.





# 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](https://www.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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020  
[www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf](http://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf)
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)  
[www.who.int/publications/i/item/9789240011311](http://www.who.int/publications/i/item/9789240011311)



# Documentation and support

## Related documentation

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

## Customer and technical support

Visit [thermofisher.com/support](http://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)

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

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## 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](http://www.thermofisher.com/us/en/home/global/terms-and-conditions.html). If you have questions, contact Life Technologies at [www.thermofisher.com/support](http://www.thermofisher.com/support).

