

TaqMan™ SARS-CoV-2 Mutation Panel

USER GUIDE

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Revision	Date	Description
C.0	11 August 2021	<ul style="list-style-type: none">Added section for “P681H and P681R mutation assay analysis”.
B.0	4 May 2021	<ul style="list-style-type: none">Updated the product description to specify that some of the assays are stocked in advance.Updated ordering information.Updated the supporting documentation provided with the shipment to include Certificates of Conformance.Added instructions to set up a run on the 7500 Fast Dx Real-Time PCR instrument.Added instructions to analyze the data with QuantStudio™ Design and Analysis Software v2 if the run was performed on a 7500 series of real-time PCR instrument, including the 7500 Fast Dx Real-Time PCR instrument.Updated the order of probe and primer binding in the assay description.
A.0	5 March 2021	New document for the TaqMan™ SARS-CoV-2 Mutation Panel.

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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.

About this guide

This document provides guidance for the detection of emerging SARS-CoV-2 mutants in respiratory tract samples that have previously had SARS-CoV-2 nucleic acid detected. The Applied Biosystems™ TaqMan™ SARS-CoV-2 Mutation Panel assays contain probes to detect reference and mutation sequences. The assays in the TaqMan™ SARS-CoV-2 Mutation Panel are combined with TaqPath™ 1-Step RT-qPCR Master Mix, CG and samples that contain SARS-CoV-2 nucleic acid.

This procedure is optimal for a minimum of 6–8 samples.

Real-time RT-PCR is performed with a QuantStudio™ 5 Real-Time PCR Instrument or an equivalent Applied Biosystems™ real-time PCR instrument.

Data are analyzed with the QuantStudio™ Design and Analysis Software v2.5 or later and the Genotyping Analysis Module.

The TaqMan™ SARS-CoV-2 Mutation Panel is a research use only tool for surveillance.

IMPORTANT! The procedures in this document have not been optimized for detection of SARS-CoV-2 mutations. It is the responsibility of laboratories to design and validate their own experimental design and analysis parameters.

Product description

The Applied Biosystems™ TaqMan™ SARS-CoV-2 Mutation Panel use TaqMan™ 5' nuclease chemistry for amplifying and detecting specific variants in purified genomic RNA samples.

Each TaqMan™ SARS-CoV-2 Mutation Panel assay contains the following items:

- Sequence-specific forward and reverse primers to amplify the target sequence region.
The reverse primer also primes reverse transcription of the SARS-CoV-2 genomic RNA sequences.
- Two TaqMan™ probes with a minor groove binder (MGB), a non-fluorescent quencher (NFQ), and the following 5' reporter dyes:
 - One VIC™ dye-labeled probe to detect the reference sequence
 - One FAM™ dye-labeled probe to detect the mutation sequence

Go to <https://www.thermofisher.com/mutationpanel> for a list of available assays. The following information is provided for each assay:

- Mutation
- Targeted gene
- Assay ID

Some of the assays are manufactured and stocked in advance. The assays that are stocked in advance are listed at <https://www.thermofisher.com/mutationpanel>.

Context sequences

Reporter dye information for the TaqMan™ SARS-CoV-2 Mutation Panel is represented in the assay context sequence, which is the nucleotide sequence surrounding the mutation site in the SARS-CoV-2 reference genome (hCoV-19/Wuhan/WIV04/2019; GISAID EPI_ISL_402124). The reference and mutant nucleotide alleles are included in brackets, where the order of the alleles corresponds to the association with probe reporter dyes, where [VIC™ dye / FAM™ dye].

An example is shown in the following table. The context sequence for the N501Y mutation is:

CCAACCCACT [A/T] ATGGTGTG.

It is recommended to use the mutation target name as the SNP Assay name in the software.

Mutation	Assay ID	VIC™ dye probe (reference allele)	FAM™ dye probe (mutant allele)
N501Y	ANPRYZA	A	T (U in the RNA genome)

Storage and stability

Store the TaqMan™ SARS-CoV-2 Mutation Panel assays at –25°C to –15°C in the dark.

IMPORTANT! Protect the assays from direct exposure to light. Excessive exposure to light can affect the fluorescent probes.

Limit freeze-thaw cycles. If you expect to freeze-thaw multiple times, consider creating aliquots of the assay to minimize freeze-thaw cycles.

The assays are stable for up to 5 years after the manufacturing date. The manufacturing date is printed on each assay tube.

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.

IMPORTANT! The customer is responsible for performing all of the necessary validations to run this assay.

Item	Source
Real-time PCR instrument	
An Applied Biosystems™ real-time PCR instrument compatible with VIC™ dye and FAM™ dye. The assay has been tested with the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument.	Contact your local sales office
Software	
QuantStudio™ Design and Analysis Software v2.5 or later with the Genotyping Analysis Module	thermofisher.com/us/en/home/global/forms/life-science/quantstudio-6-7-pro-software
Equipment	
Centrifuge with plate adapter	MLS
Microcentrifuge	MLS
Laboratory mixer, vortex or equivalent	MLS
Single and multichannel adjustable pipettors (1.00 µL to 1,000.0 µL)	MLS
Tubes, plates, and other consumables	
Real-time PCR plate	thermofisher.com/plastics

(continued)

Item	Source
MicroAmp™ Optical Adhesive Film	4311971, 4360954
MicroAmp™ Adhesive Film Applicator	4333183
Nonstick, RNase-free microcentrifuge tubes	thermofisher.com/plastics
Sterile aerosol barrier (filtered) pipette tips	thermofisher.com/pipettetips
Nuclease-free Water (not DEPC-Treated)	MLS

Reagents

Reagents	Cat. No.
TaqPath™ 1-Step RT-qPCR Master Mix, CG	A15299 (5 × 1 mL) A15300 (1 × 10 mL)
Optional controls	
AcroMetrix™ Coronavirus 2019 (COVID-19) RNA Control (RUO)	954519
GeneArt™ DNA string or plasmid controls	https://www.thermofisher.com/geneart
MEGAscript™ T7 Transcription Kit (used with GeneArt™ strings to generate RNA)	AMB13345
Synthetic viral controls	Vendor of choice

Workflow

TaqMan™ SARS-CoV-2 Mutation Panel workflow

Order the assay for the mutations of interest

See Chapter 2, “Ordering information”.

Start with isolated RNA from respiratory tract samples with SARS-CoV-2 nucleic acid detected

Prepare RT-PCR reactions (page 12)

Perform PCR amplification

Use one of the following options:

- “Set up and run the real-time PCR instrument” on page 13
- “Set up and run the 7500 Fast Dx Real-Time PCR instrument” on page 15

Analyze data

Use one of the following options:

- “Analyze the data (for experiments set up with QuantStudio™ Design and Analysis Software v2.5)” on page 17
- “Analyze the data (for experiments not set up with QuantStudio™ Design and Analysis Software v2.5)” on page 19

(Optional) Export the data (page 26)



Ordering information

Order the assays

Order assays at <https://www.thermofisher.com/mutationpanel>.

The assays that are available are listed on the website. The following information is provided for each assay:

- Mutation
- Targeted gene
- Assay ID

Click on the **Assay ID** for a specific assay, then select the size.

Shipment contents

The shipment includes the following components:

- Dispatch Note (Packing List)—Contains your order information, a list of the assays that were ordered, and the quantity of the assays shipped.
- Assay tubes—Each assay tube is identified with a label and a 2-D barcode.
- Data Sheet—Provides a summary of the assays in your shipment, the assay details, and the storage conditions.

Go to [thermofisher.com/taqmanfiles](https://www.thermofisher.com/taqmanfiles) to download the following supporting documents:

- Assay Information File (AIF)
- Certificates of Conformance
- Safety Data Sheets

For detailed information about the shipment and the AIF, see *Understanding Your Shipment* (Pub. No. MAN0017153), available at https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0017153_UnderstandYourShipment_RG.pdf.



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Guidelines for Positive Controls

Possible sources of positive controls that contain the SARS-CoV-2 assay target mutation include the following items:

- A sample in your lab that has been characterized by sequence analysis
- A commercial RNA control
- A commercial DNA control, for example GeneArt™ strings or plasmids.
Supply the mutation of interest and 100 nucleotides of flanking sequence to each side of the mutation.

Use approximately 1,000 copies of a control per 20 µL reaction. Adjust this amount as needed.

Prepare RT-PCR reactions

The recommended sample input is based on results from the sample run with an assay to detect nucleic acid from SARS-CoV-2. The average C_q (C_t) value of the ORF1ab, S gene, and N gene assay results should have a C_q cutoff of ≤ 30 C_q . This is approximate and can be adjusted as needed to conserve sample and/or achieve sufficient sample amplification.

IMPORTANT! Prepare the RT-PCR reactions according to the block type on your instrument. For example, set up the reaction for 96-well 0.1-mL plates if you are using the 7500 Fast Dx Real-Time PCR instrument.

1. Calculate the number of reactions to be performed for each assay, including controls.
It is recommended to include at least 6 test samples for each assay, one Positive Control for each assay (optional), and at least one No Template Control (NTC) for each assay.
2. Prepare a Reaction Mix in an appropriately-size tube, according to one of the following tables.

Table 1 96-well 0.2-mL plates

Component	Volume per assay ^[1]	Volume per 96-well plate ^[2]
TaqPath™ 1-Step RT-qPCR Master Mix, CG (4X)	5 μ L	528 μ L
TaqMan™ SARS-CoV-2 Mutation Panel Assay (40X)	0.5 μ L	52.8 μ L
Nuclease-free water	9.5 μ L	1,003.2 μ L
Total Reaction Mix volume	15 μL	1,584 μL

^[1] Add 10% overage.

^[2] Includes 10% overage.

Table 2 96-well 0.1-mL plates and 384-well plates

Component	Volume per assay ^[1]	Volume per 96-well plate ^[2]	Volume per 384-well plate ^[2]
TaqPath™ 1-Step RT-qPCR Master Mix, CG (4X)	2.5 μ L	264 μ L	1,056 μ L
TaqMan™ SARS-CoV-2 Mutation Panel Assay (40X)	0.25 μ L	26.4 μ L	105.6 μ L
Nuclease-free water	4.75 μ L	501.6 μ L	2,006.4 μ L
Total Reaction Mix volume	7.5 μL	792 μL	3,168 μL

^[1] Add 10% overage.

^[2] Includes 10% overage.

3. Pipet the Reaction Mix into each well of a reaction plate.
 - 15 μ L of Reaction Mix into each well of a 96-well 0.2-mL plate
 - 7.5 μ L of Reaction Mix into each well of a 96-well 0.1-mL plate or a 384-well plate
4. Add RNA sample or nuclease-free water (No Template Control) in the appropriate well of the reaction plate.
 - 5 μ L of RNA or nuclease-free water into each well of a 96-well 0.2-mL plate
 - 2.5 μ L of RNA or nuclease-free water into each well of a 96-well 0.1-mL plate or a 384-well plate

Note: The RNA sample input volume can be adjusted as needed. Adjust the volume of nuclease-free water accordingly.

5. Seal the plate thoroughly with MicroAmp™ Optical Adhesive Film.
6. Vortex the plate for 10–30 seconds to ensure proper mixing.
7. Centrifuge the plate for 1–2 minutes at $\geq 650 \times g$ (≥ 650 RCF) to remove bubbles and collect the liquid at the bottom of the wells.

Perform PCR amplification

Set up and run the real-time PCR instrument

For detailed instructions, see the user documentation for your real-time PCR instrument.

Your instrument software can be used to set up and run the experiment using the settings outlined in this section. Alternatively, QuantStudio™ Design and Analysis Software v2.5 or later can be used to set-up and run experiments for all QuantStudio™ instruments, except for the QuantStudio™ 12K Flex Real-Time PCR Instrument.

This section provides an example of instrument set-up and run instructions for the QuantStudio™ 5 Real-Time PCR Instrument with a 96-well 0.2-mL block and the QuantStudio™ Design and Analysis Software v2.5. Use the appropriate settings for your own instrument, block, and data collection software.

To set up a run with the 7500 Fast Dx Real-Time PCR instrument and SDS Software v1.4.1, see “Set up and run the 7500 Fast Dx Real-Time PCR instrument” on page 15.

For instructions to obtain the Assay Information File (AIF), see “Shipment contents” on page 10.

1. In the home screen, click **Set Up Plate**.
The **Plate Gallery** screen and the **System Templates** tab is displayed.
2. In the left pane, apply the appropriate filters for the **Instrument**, **Block**, and **Run Mode**.
3. In the left pane, select the following filters:
 - **Run Mode: Standard**
 - **Analysis: Genotyping**

Note: The run mode depends on the chemistry of the master mix. It does not depend on the reaction plate.

4. Select **Genotyping_Pre_PCR_Post**.
The **Run Method** tab is displayed.
5. In the **Reaction Volume** field, enter the appropriate reaction volume.
 - **20 μ L** for a 96-well 0.2-mL plate
 - **10 μ L** for a 96-well 0.1-mL plate or a 384-well plate
6. Modify the thermal protocol.

Step	Temperature	Time	Number of cycles
Pre-read	60°C	30 seconds	1
Reverse transcription ^[1]	50°C	10 minutes	1
Fast DNA polymerase activation ^[2]	95°C	2 minutes	1
Denaturation	95°C	3 seconds	45
Anneal / extend	60°C	30 seconds	
Post-read	60°C	30 seconds	1

^[1] The optimal temperature for reverse transcription is 48°C to 55°C.

^[2] Required for reverse transcriptase inactivation, initial denaturation, and activation of DNA polymerase.


7. In the **Plate Setup** tab, set the **Passive Reference** to **ROX**.
8. (Optional) Click **...** **► Import AIF** to import an assay information file (AIF).

Note: Use the **...** menu above the grid view to import an AIF.

The assays, reporter and quenchers, and the nucleotide alleles from the assay context sequences will be imported.

9. In the **Samples** table, click **+** (**Add**) to add samples.
10. In the **Samples** table, add a name to the **Name** field, then select an option from the **Type** dropdown list.
11. In the **SNP Assays** table, click **+** (**Add**) to add assays.
12. In the **SNP Assay** table, add a name to the **SNP Assay** field, then define the following parameters.
 - **Allele 1 Reporter: VIC**
 - **Allele 1 Quencher: NFQ-MGB**
 - **Allele 2 Reporter: FAM**
 - **Allele 2 Quencher: NFQ-MGB**

Note: The mutant allele is detected by FAM™ dye. The reference allele is detected by VIC™ dye.

13. (Optional) To edit the default **Allele 1** and **Allele 2** names displayed in the data plots and results, click  (**Edit**) in the **SNP Assays**. Edit the **Name or Base(s)** fields to contain the nucleotide base detected by the probes, found in the context sequence, or other name of choice.
If the AIF was imported, the base names for the alleles are imported into the **Name or Base(s)** fields.
14. To assign samples and SNP assays to a well, select the well, select the assay from the dropdown list in the well, then select the checkbox for the appropriate sample in the **Samples** table.
The **Task** in the **SNP Assays** table is automatically assigned.
15. In the **Run Summary** tab, review the method and the plate setup.
16. Select an instrument from the list, then start the run.

Set up and run the 7500 Fast Dx Real-Time PCR instrument

For detailed instructions, see the user documentation for the instrument.

SDS Software v1.4.1 is used to set up and run the experiment with a 7500 Fast Dx Real-Time PCR instrument. Set up the run as a standard curve assay because real-time data are required for analysis.

The data are analyzed with the QuantStudio™ Design and Analysis Software v2.5 after the experiment.

1. In the home screen, click **File ▶ New**.
2. In the **New Document Wizard** dialog box, select the following options:
 - **Assay: Standard Curve (Absolute Quantification)**
 - **Container: 96-Well Clear**
 - **Template: Blank Document**
 - **Run Mode: Fast 7500**
3. (Optional) Edit the operator name.
The operator name is populated by default, based on the account used to log into the computer.
4. (Optional) In the **Comments**, enter comments.
5. In the **Plate Name** field, enter a unique experiment name.
The name in the **Plate Name** is the name of the data file generated after the experiment is complete.
6. Click **Next**.
7. In the **Select Detectors** pane, click **New Detector**.
8. Enter the following information:
 - **Detector Name** for the mutation name, for example, **K417N**
 - (Optional) **Description**

- **Reporter dye**
 - **FAM** for the mutant allele
 - **VIC** for the reference allele
- **Quencher dye:** *None*
- **Color:** Select a different color for each allele type, reference allele or mutant allele
- *(Optional) Notes*

Note: If the detectors have been added for previous experiments, this step does not need to be completed. The detectors are saved from previous experiments.

9. Click **OK** to save the detector information entered in step 8.
The **Detector Manager** page is displayed.
10. Select the detectors for both alleles, then click **Add** to move them to the **Detectors in Document** pane.
The detectors that are defined in the SDS Software will be replaced in the QuantStudio™ Design and Analysis Software v2.5.
To view the curves in the instrument software, ensure that two detectors are defined for each assay, one for the reference allele and one for the mutant allele.
11. Click **Next**.
12. In the **Set Up Sample Plate** window, assign the detectors to the wells of the plate, then click **Finish**.
13. In the **Instrument** tab, set up the thermal protocol.

Step	Temperature	Time	Number of cycles
Reverse transcription ^[1]	50°C	10 minutes	1
Fast DNA polymerase activation ^[2]	95°C	2 minutes	1
Denaturation	95°C	3 seconds	45
Anneal / extend	60°C	30 seconds	

^[1] The optimal temperature for reverse transcription is 48°C to 55°C.

^[2] Required for reverse transcriptase inactivation, initial denaturation, and activation of DNA polymerase.

14. In the **Settings** pane, set the following options:
 - **Sample Volume (µL): 10**
 - **Run Mode: Standard 7500**
 - **Data Collection: Stage 3, Step 2 (60.0 @ 0:30)**

15. Save the file.

Option	Action
Save the file as a template for future use	Click Save As . In the next window, under Save as type , select SDS Templates (*.sdt) , then click Save .
Save the file for a single experiment	Click Save .

16. Reopen the file to connect the computer to the instrument, load the plate, then start the run on the real-time PCR instrument.

Analyze data

Analyze the data (for experiments set up with QuantStudio™ Design and Analysis Software v2.5)

A data file is generated in EDS or SDS format, depending on the real-time PCR instrument.

This section provides guidelines for data analysis using the QuantStudio™ Design and Analysis Software v2.5. This software supports viewing and analyzing data from all QuantStudio™ instruments (except for TaqMan™ OpenArray™ Plate files) and from the StepOnePlus™ Instrument, the ViiA™ 7 instrument, the 7500/7500 Fast Real-Time PCR System, and the 7900HT Real-Time PCR Instrument.

The procedure in this section is for an experiment set up with QuantStudio™ Design and Analysis Software v2.5. If an experiment was set up with another software, see “Analyze the data (for experiments not set up with QuantStudio™ Design and Analysis Software v2.5)” on page 19.

After the experiment has been run, use the analysis software associated with your real-time PCR instrument to process and analyze your data.

1. Open a data file.

Note: QuantStudio™ Design and Analysis Software v2 requires data files created on the compatible instruments to be saved as a new data file. Click **Actions** ▶ **Save As**, then save the data file with a new name.

2. Click **Actions** ▶ **Analysis Modules**, then select **Genotyping**.
If the **Genotyping** tab is not available, click **Analyze** ▶ **Analysis Modules**.
3. In the **Analysis Modules** dialog box, select **Genotyping**, then click **OK**.
4. Navigate to the **Genotyping** tab.
5. In the **Actions** dropdown list, click **Genotyping Analysis Setting**.
6. In the **Genotyping Analysis Setting** dialog box, select **Real Time Data**, then click **Apply**.
This setting enables real-time tract data to be viewed in the **Allelic Discrimination Plot**.

7. View the **Allelic Discrimination Plot**.

Note: The genotype calling algorithm is designed for diploid organism genotype calling. For the TaqMan™ SARS-CoV-2 Mutation Panel assays, the reference allele cluster should be called as **HOMOZYGOUS_ALLELE1** and the mutant allele cluster should be called as **HOMOZYGOUS_ALLELE2**.

If the clustering algorithm calls one of the expected two clusters as **HETEROZYGOUS**, select the cluster samples and manually adjust the call using the dropdown list in the upper right hand corner of the results table.

Samples that are not called due to weak amplification and that lie along the real-time trajectory of one of the two clusters can be called manually.

Samples may run off the main 'homozygous' clusters if they contain one of the following items:

- A mutation that underlies the assay binding sites
- A mixture of viruses that have both reference and mutant alleles

For such samples, further characterization of the sample by sequence analysis is recommended.

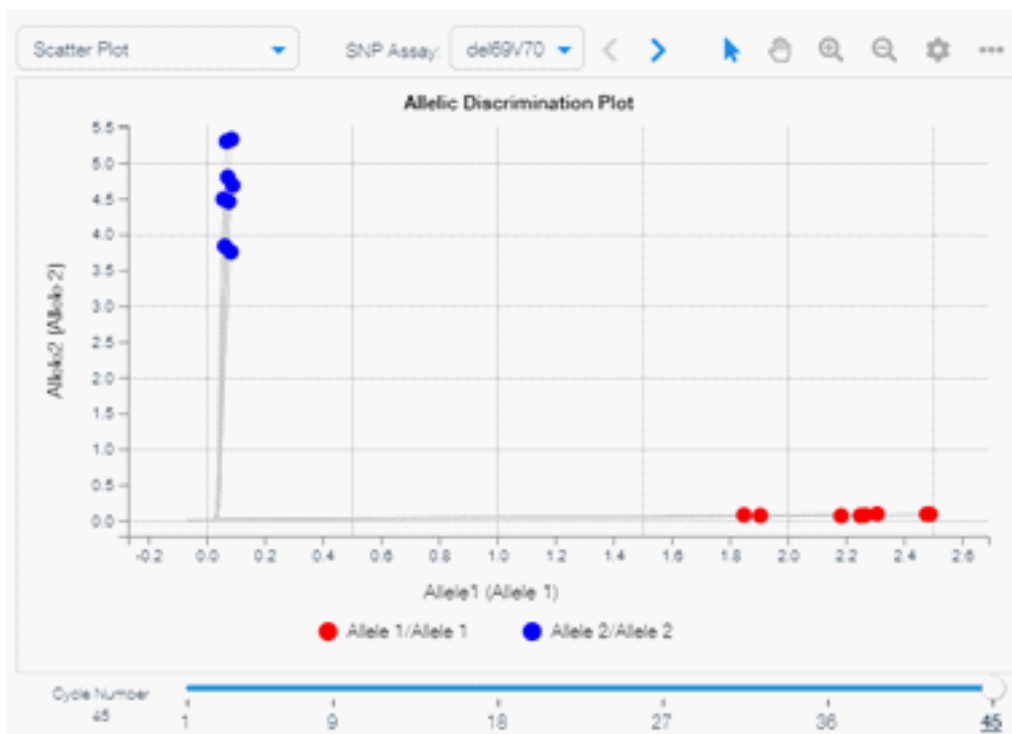
8. Select a well from the plate layout view or the table view to display on the plot.

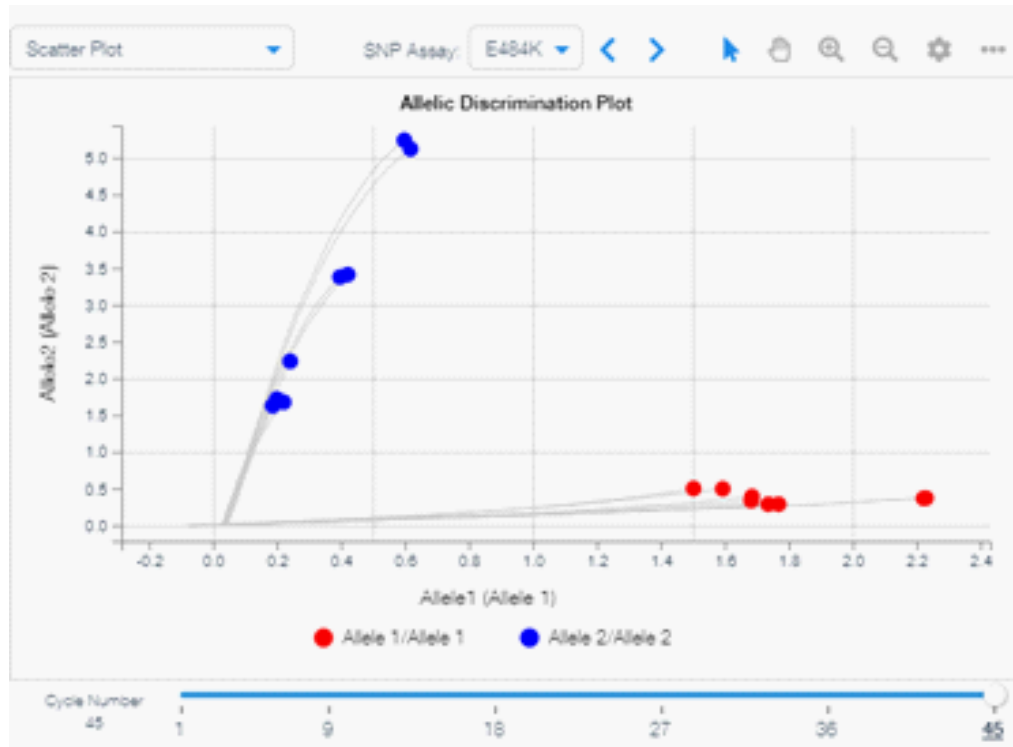
Samples that carry the reference allele will cluster along the X-axis (Allele 1 VIC™ dye). Samples that carry the mutant alleles will cluster along the Y-axis (Allele 2 FAM™ dye).

The following figures show genotyping data plots for two TaqMan™ SARS-CoV-2 Mutation Panel assays.

IVT-RNA control samples for reference and mutant alleles were plated at the following concentrations:

- 100,000 copies/20 µL reaction
- 10,000 copies/20 µL reaction
- 1,000 copies/20 µL reaction
- 250 copies/20 µL reaction





Analyze the data (for experiments not set up with QuantStudio™ Design and Analysis Software v2.5)

This section provides guidelines for data analysis if an experiment was not set up with the QuantStudio™ Design and Analysis Software v2.5. This includes the 7500 Fast Dx Real-Time PCR instrument and other instruments in the 7500 Real-Time PCR Instrument series.

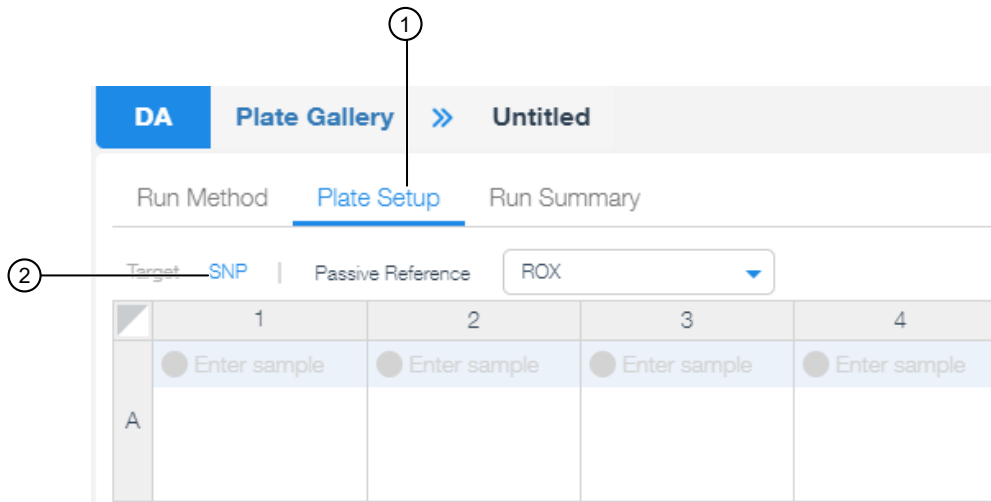
A data file is generated in EDS or SDS format, depending on the real-time PCR instrument.

After the experiment has been run, use the analysis software associated with your real-time PCR instrument to process and analyze your data.

1. Open a data file.

Note: QuantStudio™ Design and Analysis Software v2 requires data files created on the compatible instruments to be saved as a new data file. Click **Actions** ▶ **Save As**, then save the data file with a new name.

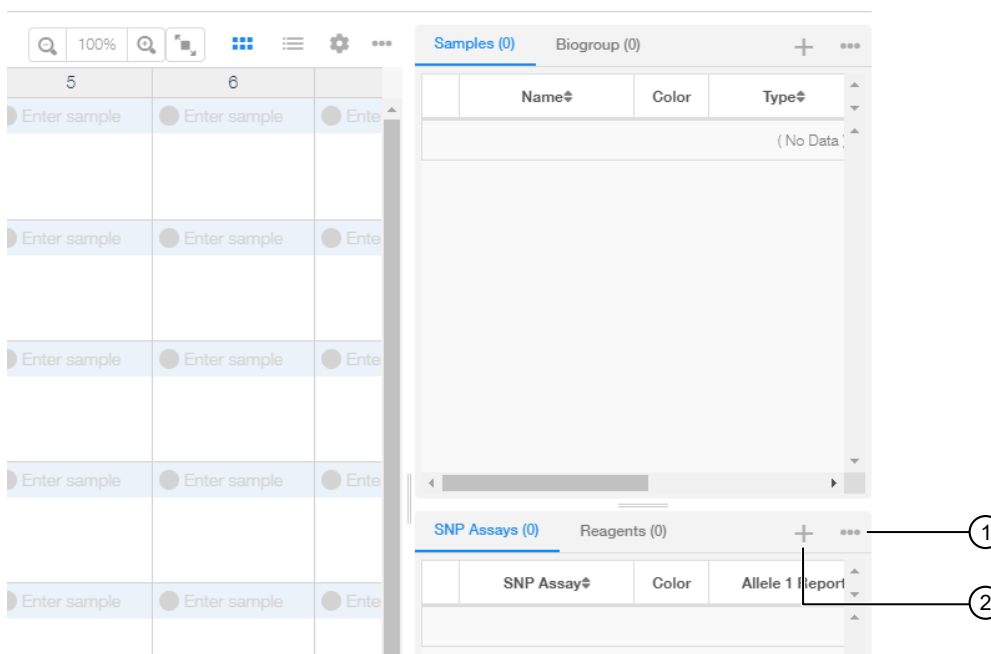
2. In the **Plate Setup** tab, select **SNP**.



- ① Plate Setup tab
- ② SNP

3. Add SNP assays.

Option	Action
Import an assay	Above the SNP Assays table, click ⋮ ▶ Import SNP Assays .
Add assays individually	Above the SNP Assays table, click + (Add).



- ① Import an assay
- ② Add assay individually

An imported file must be a CSV file. The file must contain the headings listed, with the required cells populated.

- *Required Name*
- *Color*
- *Required Allele 1 Name (Name-Allele 1)*
- *Allele 1 Color*
- *Required Allele 1 Reporter (VIC)*
- *Required Allele 1 Quencher (NFQ-MGB)*
- *Required Allele 2 Name (Name-Allele 2)*
- *Allele 1 Color*
- *Required Allele 2 Reporter (FAM)*
- *Required Allele 2 Quencher (NFQ-MGB)*
- *Assay ID*
- *Gene Symbol*
- *Gene Name*
- *NCBI SNP Reference*
- *Context Sequence*
- *Comments*

4. If the assays are added individually, add a name to the **SNP Assay** field, then define the following parameters.
 - **Allele 1 Reporter: VIC**
 - **Allele 1 Quencher: NFQ-MGB**
 - **Allele 2 Reporter: FAM**
 - **Allele 2 Quencher: NFQ-MGB**

Note: The mutant allele is detected by FAM™ dye. The reference allele is detected by VIC™ dye.

5. In the **Plate Setup** tab, select **Target**.
6. Select a well or multiple wells.
7. In the **Targets** table on the lower right, deselect the assigned target.
This is the target that was assigned in the instrument software, for example SDS Software for the 7500 Fast Dx Real-Time PCR instrument.
8. In the **Plate Setup** tab, select **SNP**, then assign the correct mutation assay by selecting the assay in the **SNP Assays** table.
This is the target that was added in step 3.
9. Click **Actions** ▶ **Analysis Modules**, then select **Genotyping**.
If the **Genotyping** tab is not available, click **Analyze** ▶ **Analysis Modules**.
10. In the **Analysis Modules** dialog box, select **Genotyping**, then click **OK**.
11. Navigate to the **Genotyping** tab.
12. In the **Actions** dropdown list, click **Genotyping Analysis Setting**.
13. In the **Genotyping Analysis Setting** dialog box, select **Real Time Data**, then click **Apply**.
This setting enables real-time tract data to be viewed in the **Allelic Discrimination Plot**.
14. Click **Analyze**, then click **Save** to save the analyzed file.

15. View the Allelic Discrimination Plot.

Note: The genotype calling algorithm is designed for diploid organism genotype calling. For the TaqMan™ SARS-CoV-2 Mutation Panel assays, the reference allele cluster should be called as **HOMOZYGOUS_ALLELE1** and the mutant allele cluster should be called as **HOMOZYGOUS_ALLELE2**.

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- A mixture of viruses that have both reference and mutant alleles

For such samples, further characterization of the sample by sequence analysis is recommended.

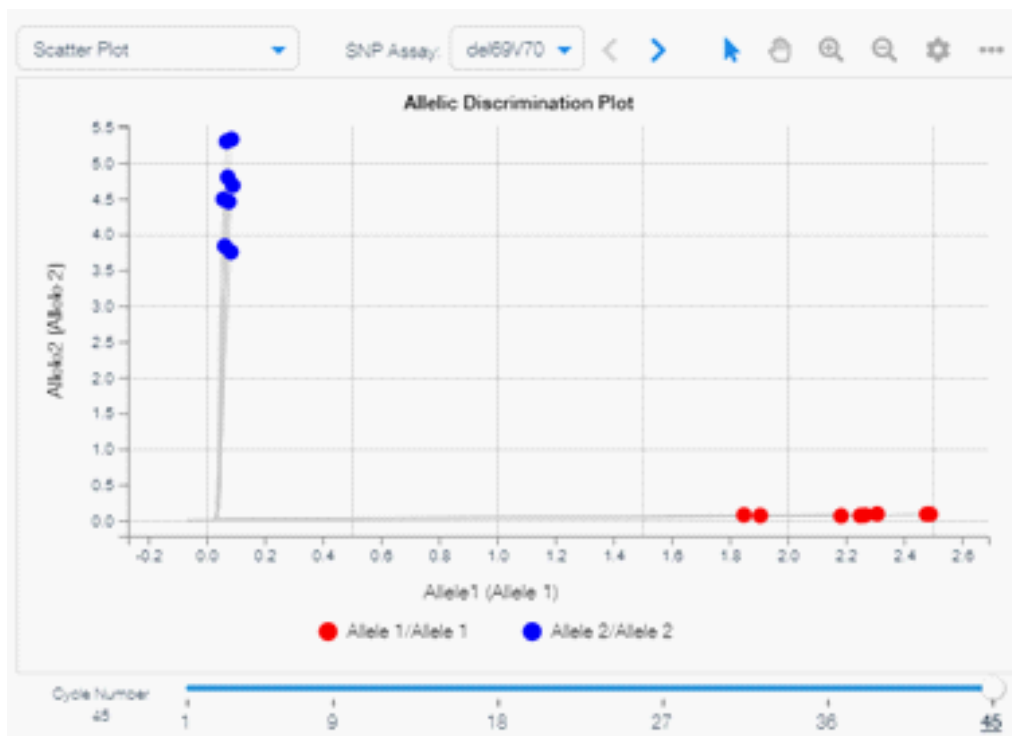
16. Select a well from the plate layout view or the table view to display on the plot.

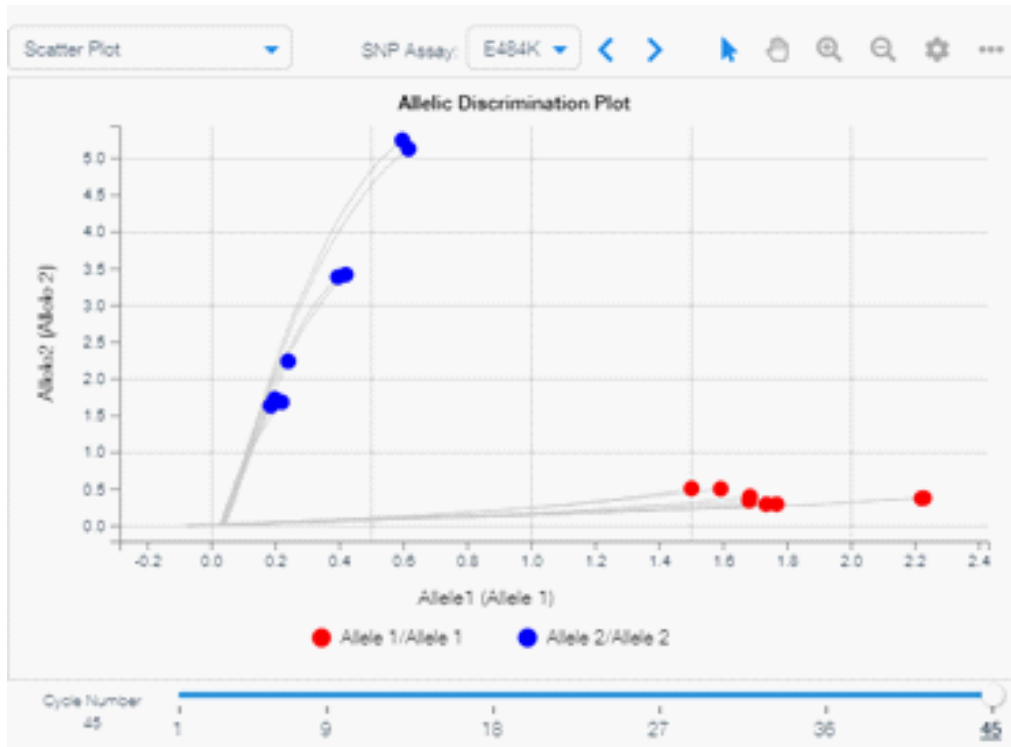
Samples that carry the reference allele will cluster along the X-axis (Allele 1 VIC™ dye). Samples that carry the mutant alleles will cluster along the Y-axis (Allele 2 FAM™ dye).

The following figures show genotyping data plots for two TaqMan™ SARS-CoV-2 Mutation Panel assays.

IVT-RNA control samples for reference and mutant alleles were plated at the following concentrations:

- 100,000 copies/20 µL reaction
- 10,000 copies/20 µL reaction
- 1,000 copies/20 µL reaction
- 250 copies/20 µL reaction





K417T and K417N mutation assay analysis

K417T (A22812C) and K417N (G22813T) are independent mutations that are located next to one another in the SARS-CoV-2 virus genome. This can complicate genotype analysis as the probes of an assay to one mutation will fail to bind to viral sequences that contain the other, adjacent mutation.

The context sequences for the K417T and K417N assays are shown below.

K417T (A22812C) assay context sequence

AGGGCAAACCTGGAA [A/C] GATTGCTGATTATAAT

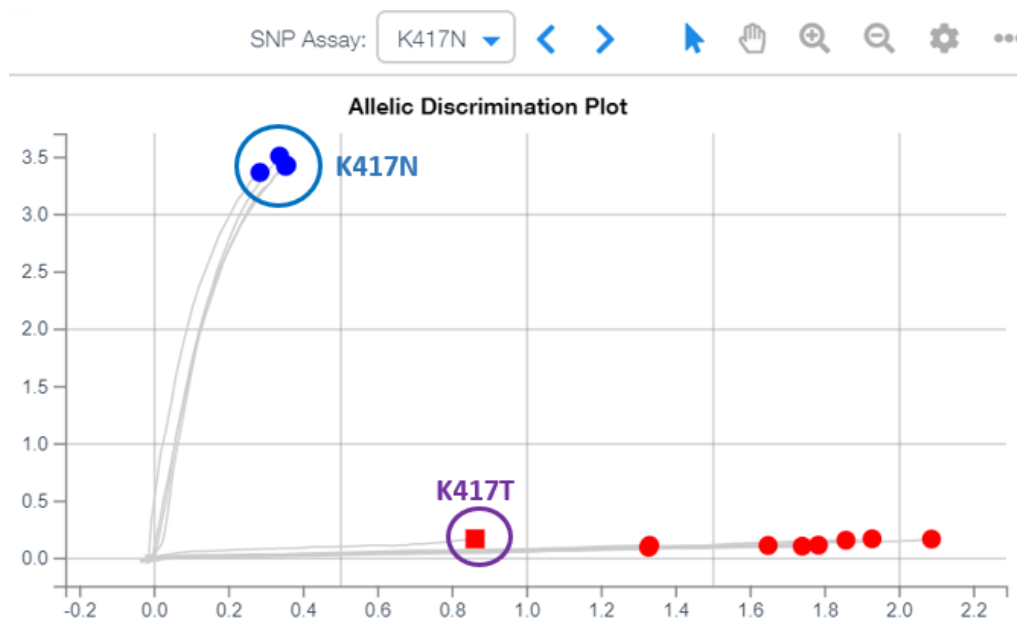
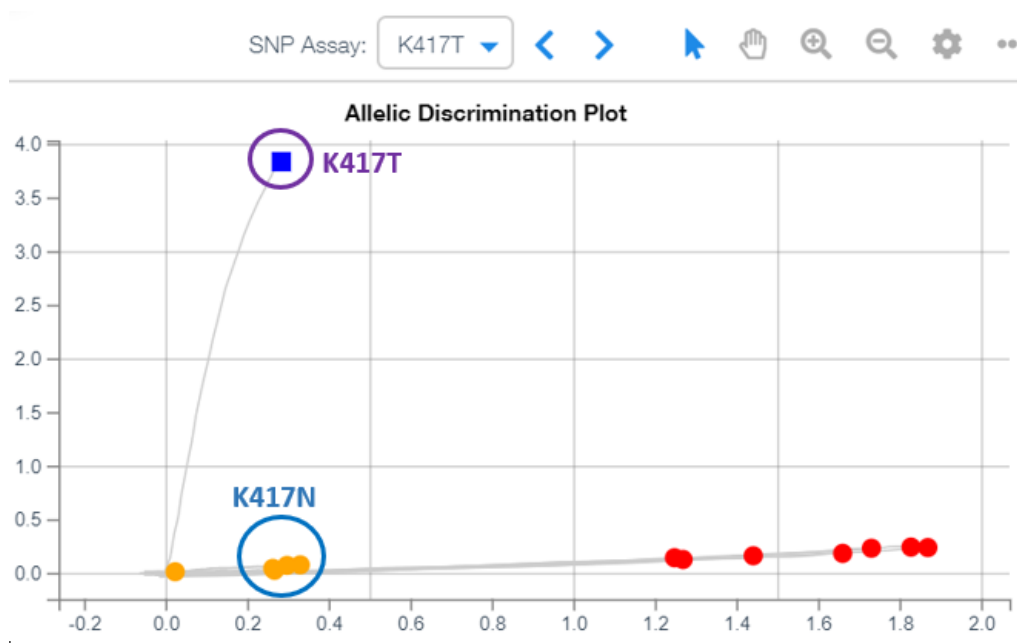
K417N (G22813T) assay context sequence

AGGGCAAACCTGGAAA A [G/T] ATTGCTGATTATAAT

K417T (A22812C) - K417N (G22813T)

AGGGCAAACCTGGAA [A/C] [G/T] ATTGCTGATTATAAT

Example data plots for the K417T and K417N assays are shown in the figures below. Samples that contain K417T or K417N mutations are identified and circled.



Samples that are run with the K417T assay and that contain the K417N mutation, or samples run with the K417N assay and that contain the K417T mutation, can cluster near the NTCs or exhibit weak amplification due to probe nonspecific activity. If a mutation containing sample is called as wild type with the adjacent SNP assay, the call can be manually adjusted to "no amp".

We highly recommend that both K417T and K417N mutation assays are run on the same samples, and that the results of both assays are compared, to facilitate accurate genotype analysis.

If it is not possible to make a clear genotype call, rerun the experiment or further characterize the sample by sequence analysis.

P681H and P681R mutation assay analysis

P681H (C23604A) and P681R (C23604G) are independent mutations that are located at the same position in the SARS-CoV-2 virus genome. This can complicate genotype analysis as the probes of an assay to one mutation will fail to bind to viral sequences that contain the other.

The context sequences for the P681H and P681R assays are shown below.

P681H (C23604A) assay context sequence

CTCAGACTAATTCTC [C/A] TCGGCGGGCACGTAG

P681R (C23604A) assay context sequence

CTCAGACTAATTCTC [C/G] TCGGCGGGCACGTAG

Example data plots for the P681H and P681R assays are shown in the figures below. Samples that contain P681H or P681R mutations are identified and circled.



Samples that are run with the P681H assay and that contain the P681R mutation, or samples run with the P681R assay and that contain the P681H mutation, can cluster near the NTCs or exhibit weak amplification due to probe nonspecific activity. If a mutation containing sample is called as wild type with the adjacent SNP assay (red), the call can be manually adjusted to "no amp".

We highly recommend that both P681H and P681R mutation assays are run on the same samples, and that the results of both assays are compared, to facilitate accurate genotype analysis.

If it is not possible to make a clear genotype call, rerun the experiment or further characterize the sample by sequence analysis.

Export the data

1. Click **Actions** ▶ **Export**.

The **Export Plate** dialog box is displayed.

2. (Optional) Edit the following fields: .

- **Export Name**
- **File Format**
- **Destination**
- **Export Setting**

3. Click **Export**.



Supplemental information

Assay description

The assay is a one-step RT-PCR to detect reference and mutation sequences.

Note: The following figures are general representations of RT-PCR with TaqMan™ MGB probes and the TaqMan™ SARS-CoV-2 Mutation Panel assays. The sequence regions are not necessarily drawn to scale.

- | | |
|----------------------------|---------------------------------|
| ■ RNA | ● V VIC™ reporter dye |
| ■ cDNA template | ● F FAM™ reporter dye |
| ● RT Reverse transcriptase | ● FAM™ reporter dye fluorescing |
| ■ Forward primer | ● NFO Quencher dye |
| ■ Reverse primer | ● MGB MGB |
| ■ Probe | |

During reverse transcription (RT), the RNA is reversed transcribed to cDNA (Figure 1 and Figure 2). The reverse transcription product is single-stranded.



Figure 1 Reverse transcription



Figure 2 Reverse transcription product

After RT, the PCR amplification of the cDNA template begins.

The master mix contains a DNA polymerase. The reaction also contains a forward primer, a reverse primer, a FAM™-labeled TaqMan™ probe, and a VIC™-labeled TaqMan™ probe (Figure 3).

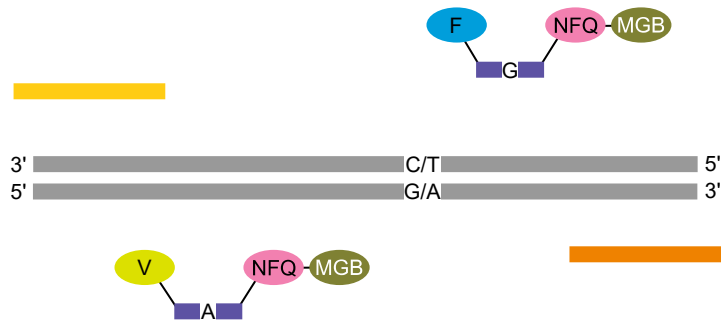


Figure 3 Components

The cDNA templates are denatured. The probe anneals to a complementary sequence between the forward and reverse primer sites (Figure 4).

When the probe is intact, the proximity of the reporter dye to the quencher results in suppression of the fluorescent signal.

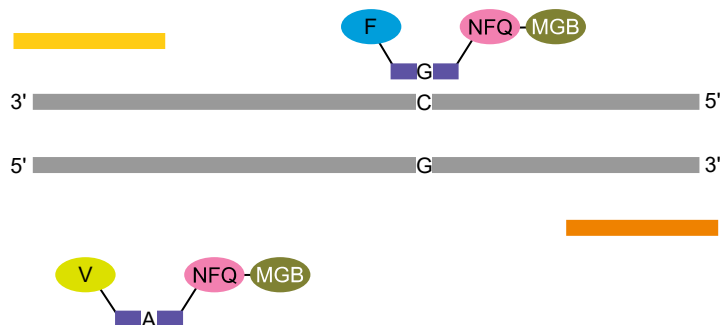


Figure 4 Probe anneal

The forward and reverse primers anneal to complementary sequences along the denatured cDNA strands (Figure 5).

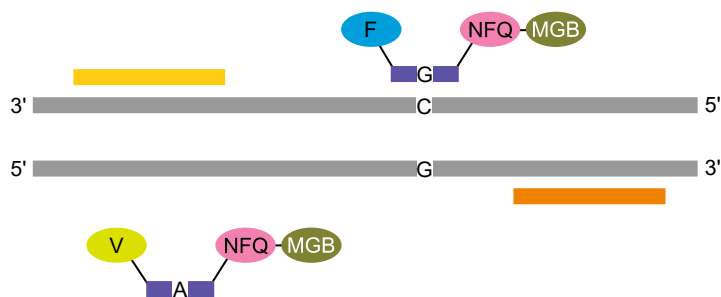


Figure 5 Primer anneal

During polymerization, the DNA polymerase only cleaves probes that hybridize to the target sequence. Cleavage separates the reporter dye from the probe. The separation of the reporter dye from the quencher dye results in increased fluorescence by the reporter (Figure 6).

The increase in fluorescence occurs only if the target sequence is complementary to the probe and amplified during PCR. Because of these requirements, nonspecific amplification is not detected, and the fluorescence signal indicates which alleles are in the sample

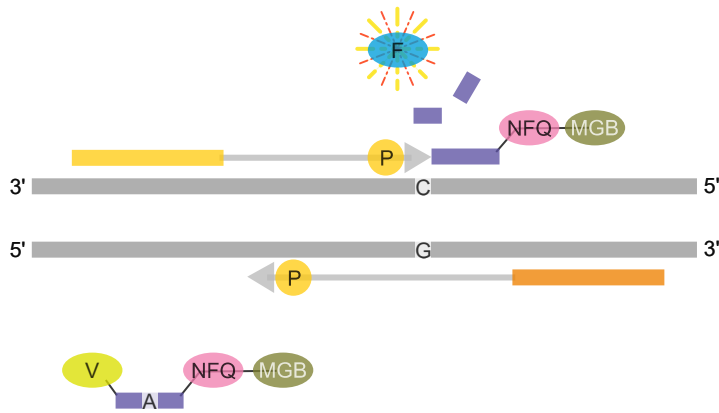


Figure 6 Probe cleavage

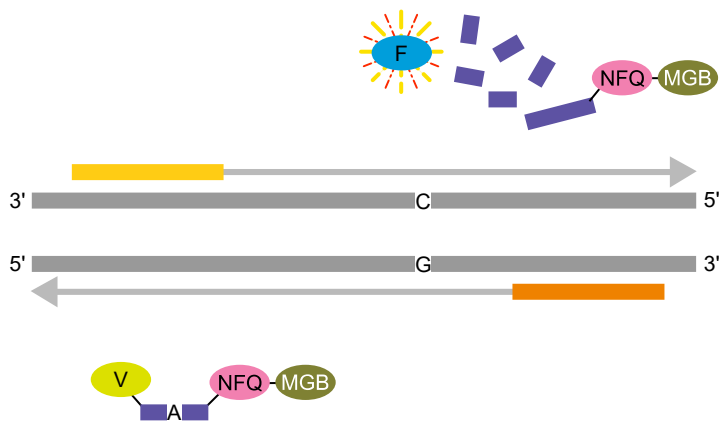


Figure 7 Completion

Signals and sample genotype

Signal	Sample genotype
VIC™ dye	Reference sequence
FAM™ dye	Mutation sequence

Overview of TaqMan™ MGB probes

TaqMan™ MGB probes consist of target-specific oligonucleotides with:

- A reporter dye at the 5′ end of the probe:
 - VIC™ dye for Allele 1 probe
 - FAM™ dye for Allele 2 probe
- A non-fluorescent quencher (NFQ) dye at the 3′ end of the probe.
- A minor groove binder (MGB) at the 3′ end of the probe that:
 - Increases the melting temperature (T_m) without increasing probe length.
 - Allows for the design of shorter probes.

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
 - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
 - Sample preparation and reaction setup.
 - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.



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

Chemical safety



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

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



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

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

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

Biological hazard safety



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

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020; found at:
<https://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); found at:
www.who.int/publications/i/item/9789240011311



Documentation and support

Related documentation

Document	Publication number
<i>TaqPath™ 1-Step RT-qPCR Master Mix, CG User Guide</i>	MAN0007959
<i>QuantStudio™ Design and Analysis Software v2 User Guide</i>	MAN0018200
<i>QuantStudio™ Design and Analysis Software v2 Genotyping Analysis Module User Guide</i>	MAN0018749
<i>Understanding Your Shipment</i>	MAN0017153

Customer and technical support

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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

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

