SARS-CoV-2 Variants Tracking using Thermo Fisher Scientific qPCR Technology

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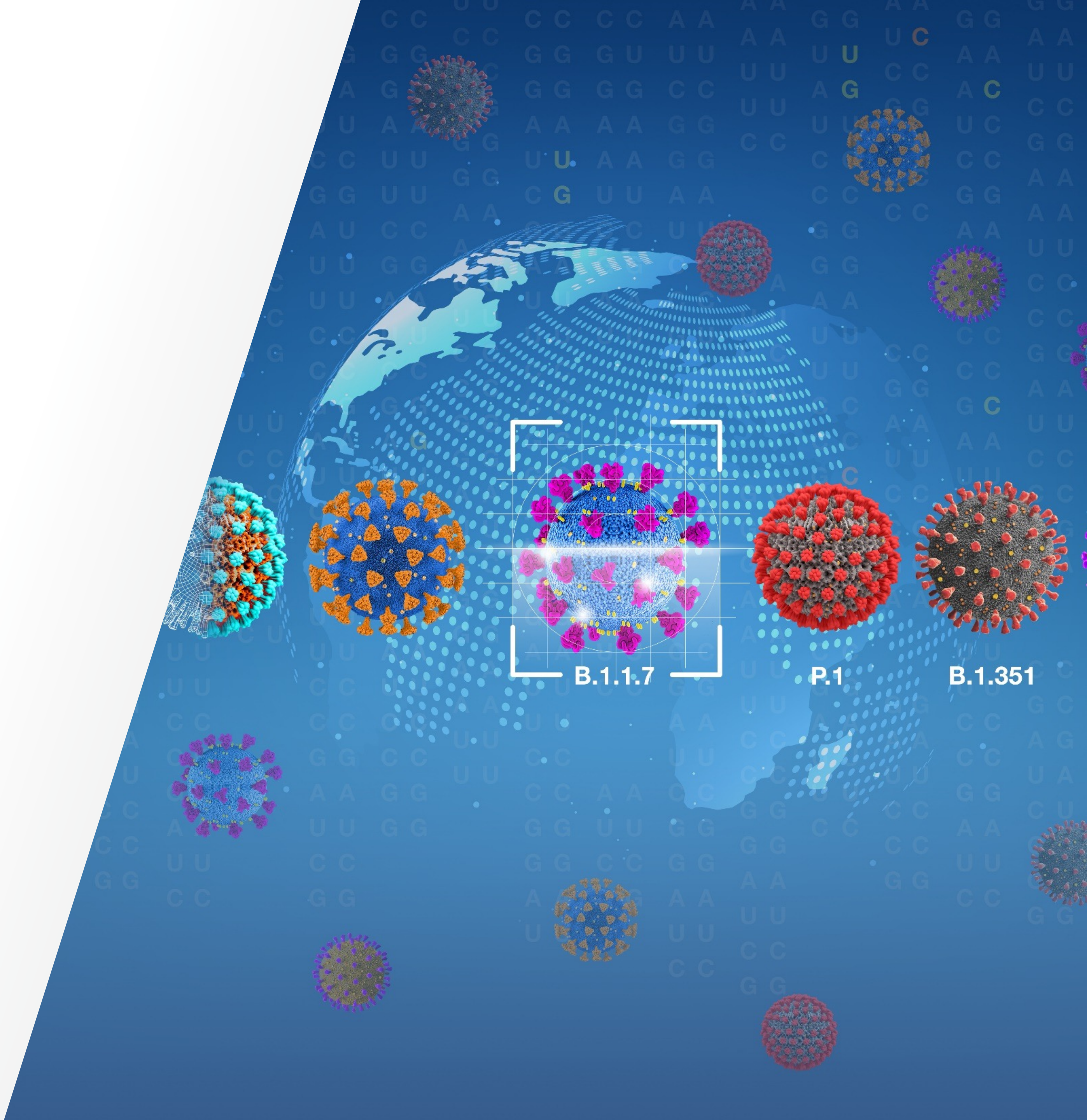


The world leader in serving science

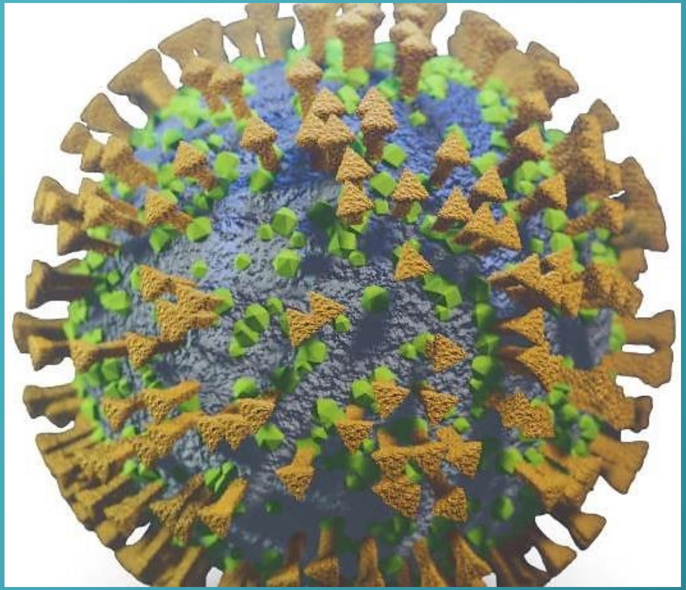
Agenda

1. Background: Potential Variant Impact
2. Introducing: SNPs detection using TaqMan SARS-CoV-2 Mutation Panel
3. Features and qPCR Workflow
4. Technical Data
5. Questions

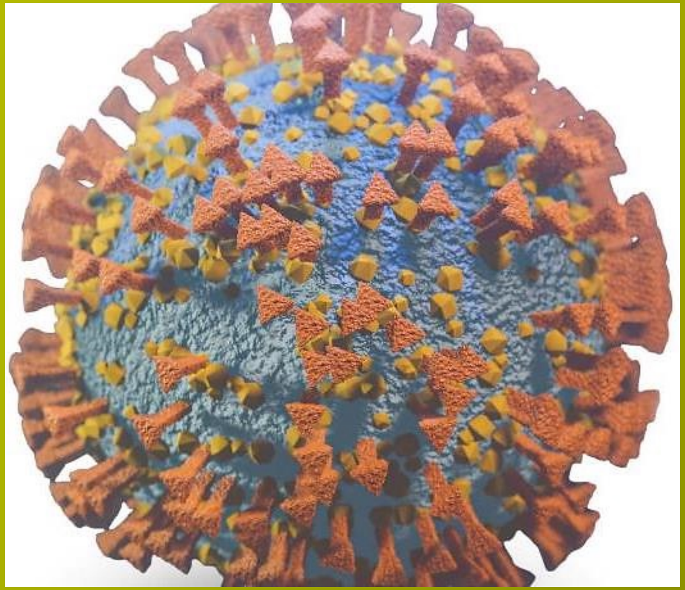
SARS-CoV-2 Viral Mutations

Viruses mutate. RNA viruses, like SARS-CoV-2, mutate at high rates in response to selective pressures

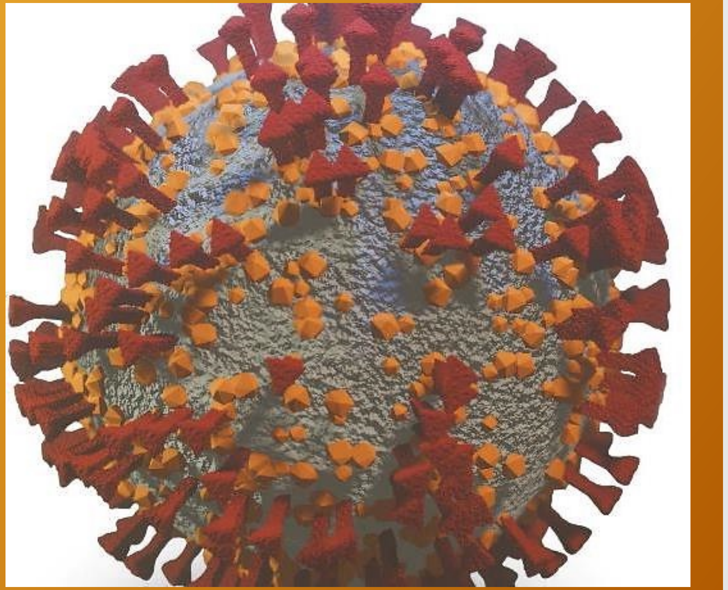
Continued uncontrolled transmission of SARS-CoV-2 in many parts of the world is creating conditions for significant virus evolution



SARS-CoV-2 has been mutating at a rate of about one to two mutations per month*



Some recently identified variants, however, have acquired mutations much more rapidly than scientists expected



*Nature https://www.nature.com/articles/d41586-020-02544-6

Potential Implications of New SARS-CoV-2 Variants

Potential Variant Impacts

- Speed of human-to-human transmission
- Change disease severity
- Susceptibility to therapeutic agents (i.e., monoclonal antibodies)
- Evade vaccine-induced immunity
- Impact on detection tests

Source: US CDC https://www.cdc.gov/coronavirus/2019-ncov/more/science-and-research/scientific-brief-emerging-variants.html

SNPs detection in the current multi-variant analysis

Overview

- SNPs – Single Nucleotide polymorphisms detection of gene variations in a well.
- RUO Reflex technology; samples initially processed using TaqPath COVID-19 CE-IVD assay kits can be reflex tested using the same workflow. (LoD: Ct value of 30 or less)
- If the sample is positive and the S-gene is undetectable (S-gene “dropout”) the sample can be reflex-tested to RT PCR
- Multiple primer/ probe sets available to confirm mutations associated with 69/70del and B.1.1.7 or B.1.351, B.1.617.2 strains
- GenoTyping software using qPCR platforms (Any real-time PCR instrument such as the Applied Biosystems 7500, 7500 Fast, 7500 Fast Dx, QuantStudio5 and QuantStudio7 systems)

Global Surveillance of SARS-CoV-2 Variants

Multiple **surveillance solutions to identify, detect, and confirm** new and emerging SARS-CoV-2 variants and strains.

Public health partners

- Monitor viral genome
- Detect & Inform on emerging mutations
- Determine impact on detection, spread and vaccine / therapy effectivity

Research

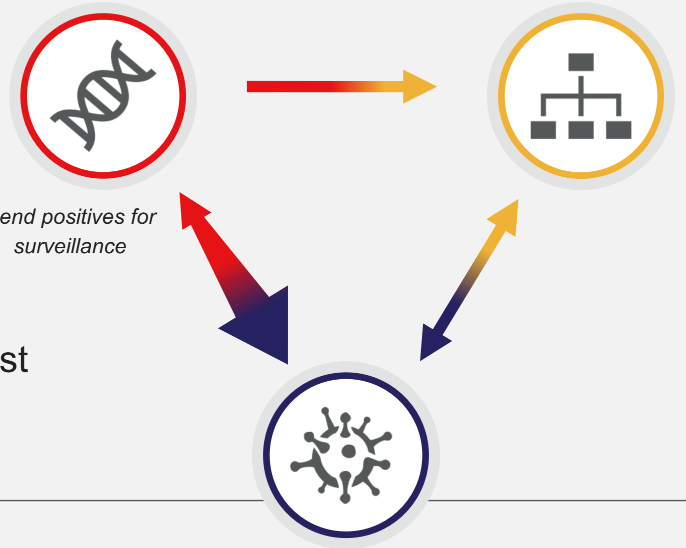
Genetic Surveillance

Next Gen Sequencing (NGS)

- Sequence full viral genome
- Detect new and emerging mutations

Capillary Electrophoresis

- Sequence targeted genes / areas of interest on viral genome (i.e., S-gene)



Send positives for surveillance

Mutation Verification

Capillary Electrophoresis

- Sequence verification of unexpected results
- Confirmation of mutations, strains or lineages

RT-qPCR

- Convenience of using same instrument for detection and mutation confirmation
- Customizable solution with a menu of assays

Clinical

RT qPCR Detection

RT-qPCR

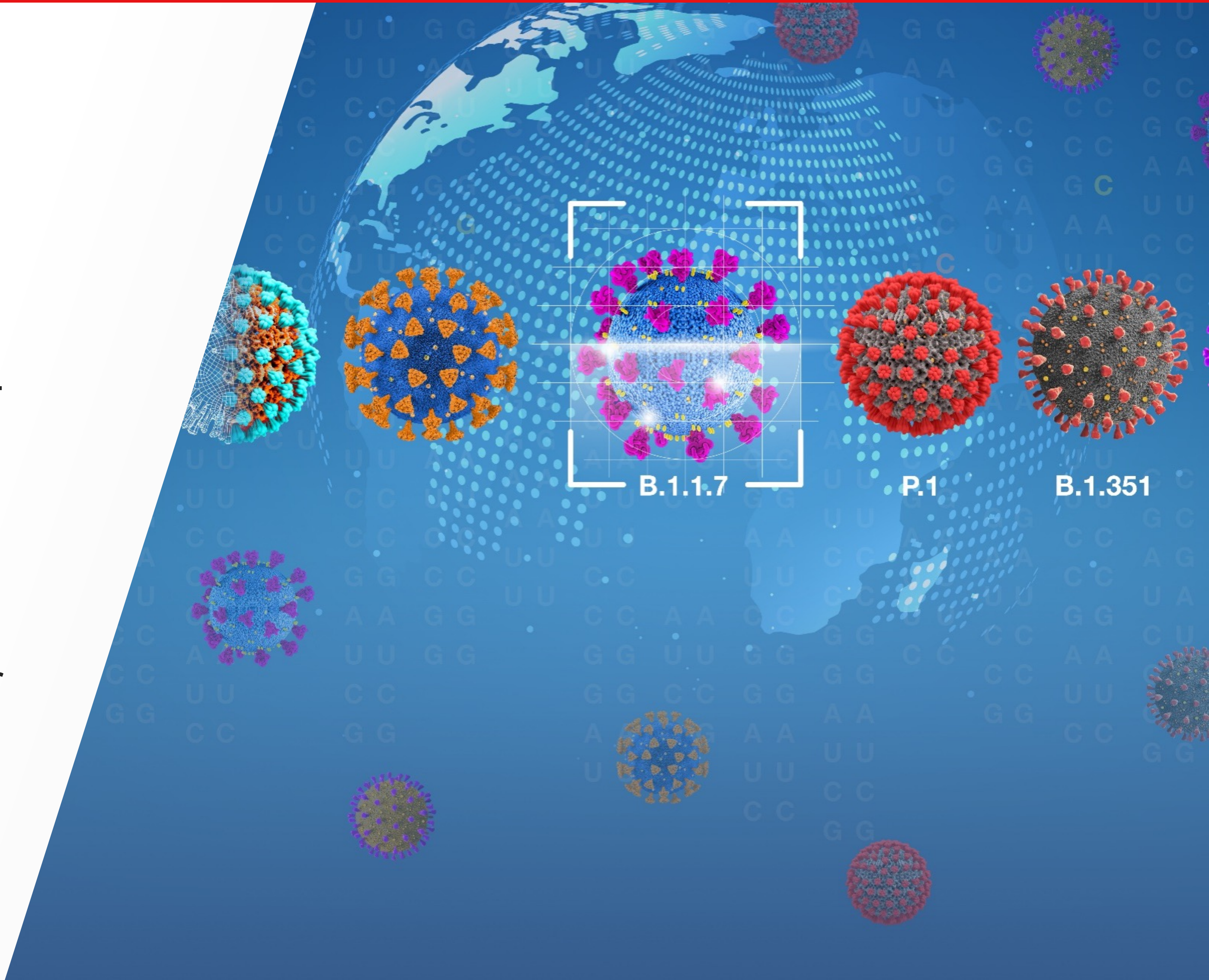
- Assess emerging mutations impact on performance claims
- Informs product development
- Drives rapid, proactive product modifications and communications

TaqMan® SARS-CoV-2 Mutation Panel

Thermo Fisher SCIENTIFIC

Convenient, customizable **real-time PCR** research solution for detection analysis and surveillance

- **Customizable panel**—build your own custom panel from a menu of verified assays to identify currently relevant SARS-CoV-2 mutations and adapt quickly as others emerge
- **Convenient**—use your current real-time PCR instrument to conduct follow up testing of SARS-CoV-2 samples
- **Scalable**—run a few or hundreds of samples to identify for one or many mutations
- **Unique, streamlined workflow**—combining our gold-standard TaqMan SNP Genotyping Assays with a 1-step RT-PCR reaction, go from RNA to results in just over 1 hour



TaqMan® SARS-CoV-2 Mutation Panel

PRODUCT DETAILS

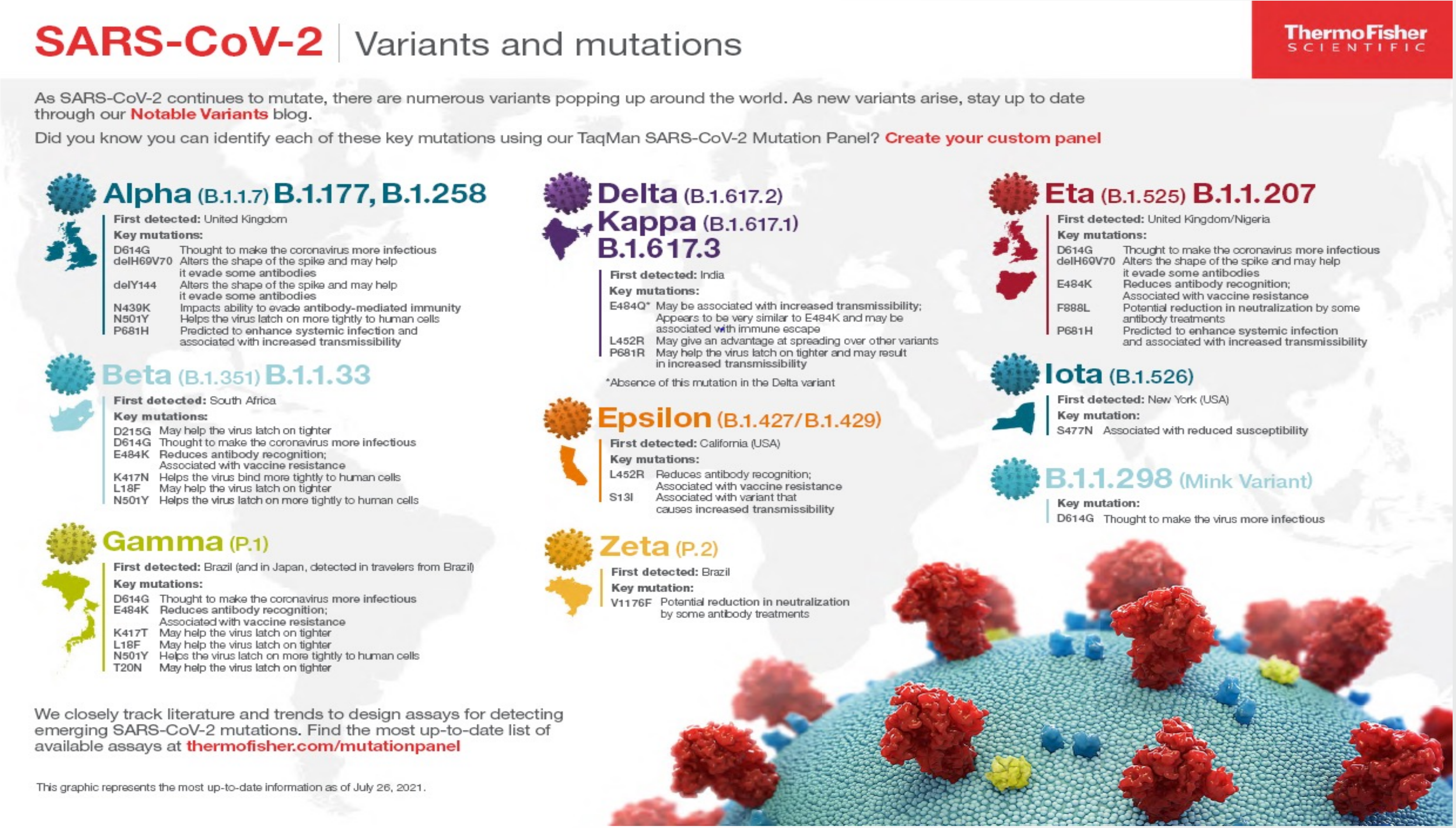
Targets	• Choose from a menu of verified real-time PCR SNP assays to build your custom panel
Assay design	• Each assay includes two TaqMan minor groove binder (MGB) probes with nonfluorescent quenchers (NFQ): • One VIC™ dye-labeled probe to detect the reference sequence • One FAM™ dye-labeled probe to detect the mutation sequence
Available sizes	• 374 reactions • 1,250 reactions
Format	• 1 mutation assay per well
Sample input	• RNA extracted from SARS-CoV-2 samples with a CT value of less or equal to 30
Turnaround time	• 1 hour and 10 min from extracted RNA to results
Recommended instruments	• Any real-time PCR instrument such as the Applied Biosystems 7500, 7500 Fast, 7500 Fast Dx, QuantStudio5 and QuantStudio7 systems.
Recommended analysis software	• QuantStudio™ Design and Analysis Software v2.5 or later with the Genotyping Analysis Module
Optional controls	• AcroMetrix™ Coronavirus 2019 RNA Control • GeneArt™ DNA string or plasmid controls

Build a custom panel by choosing from our menu of over 50 verified SNP assays

Mutation	Gene	Associated Variants	MTO/MTS
A1708D	ORF1	B.1.1.7	MTO
A222V	S	B.1.177	MTO
A570D	S	B.1.1.7	MTO
A701V	S	B.1.351	MTO
D215G	S	B.1.351	MTO MTS
D614G	S	B.1.1.207, P.1, B.1.1.33, B.1.1.7, B.1.177, B.1.258, B.1.351, B.1.525, Mink Variant	MTS
D80A	S	B.1.351	MTO
delH69V70	S	B.1.1.7, B.1.258, B.1.525	MTS
delY144	S	B.1.1.7	MTS
E484K	S	P.1 ,B.1.1.33, B.1.351, B.1.525	MTS
E484Q	S	B.1.617.1, B.1.617.3	MTS
K417N	S	B.1.351	MTS
K417T	S	P.1	MTS

Mutation	Gene	Associated Variants	MTO/MTS
L18F	S	P.1, B.1.351	MTO MTS
L242_244L	S	B.1.351	MTO
L452R	S	B.1.617, B.1.429	MTS
N439K	S	B.1.258	MTO MTS
N501Y	S	P.1, B.1.1.7, B.1.351	MTS
P681H	S	B.1.1.207, B.1.1.7	MTS
P681R	S	B.1.617	MTS
Q27stop	ORF8	B.1.1.7	MTO
R246I	S	B.1.351	MTO
S982A	S	B.1.1.7	MTO
T20N	S	P.1	MTS
T716I	S	B.1.1.7	MTO
Y453F	S	Mink Variant	MTO

Key mutations and their distribution



SARS-CoV-2 | Variants and mutations

As SARS-CoV-2 continues to mutate, there are numerous variants popping up around the world. As new variants arise, stay up to date through our **Notable Variants** blog.

Did you know you can identify each of these key mutations using our TaqMan SARS-CoV-2 Mutation Panel? **Create your custom panel**

Alpha (B.1.1.7) B.1.177, B.1.258

First detected: United Kingdom

Key mutations:

D614G	Thought to make the coronavirus more infectious
delH69V70	Alters the shape of the spike and may help it evade some antibodies
delY144	Alters the shape of the spike and may help it evade some antibodies
N439K	Impacts ability to evade antibody-mediated immunity
N501Y	Helps the virus latch on more tightly to human cells
P681H	Predicted to enhance systemic infection and associated with increased transmissibility

Beta (B.1.351) B.1.1.33

First detected: South Africa

Key mutations:

D215G	May help the virus latch on tighter
D614G	Thought to make the coronavirus more infectious
E484K	Reduces antibody recognition; Associated with vaccine resistance
K417N	Helps the virus bind more tightly to human cells
L18F	May help the virus latch on tighter
N501Y	Helps the virus latch on more tightly to human cells

Gamma (P.1)

First detected: Brazil (and in Japan, detected in travelers from Brazil)

Key mutations:

D614G	Thought to make the coronavirus more infectious
E484K	Reduces antibody recognition; Associated with vaccine resistance
K417T	May help the virus latch on tighter
L18F	May help the virus latch on tighter
N501Y	Helps the virus latch on more tightly to human cells
T20N	May help the virus latch on tighter

Delta (B.1.617.2) Kappa (B.1.617.1) B.1.617.3

First detected: India

Key mutations:

E484Q*	May be associated with increased transmissibility; Appears to be very similar to E484K and may be associated with immune escape
L452R	May give an advantage at spreading over other variants
P681R	May help the virus latch on tighter and may result in increased transmissibility

*Absence of this mutation in the Delta variant

Epsilon (B.1.427/B.1.429)

First detected: California (USA)

Key mutations:

L452R	Reduces antibody recognition; Associated with vaccine resistance
S13I	Associated with variant that causes increased transmissibility

Zeta (P.2)

First detected: Brazil

Key mutation:

V1176F	Potential reduction in neutralization by some antibody treatments

Eta (B.1.525) B.1.1.207

First detected: United Kingdom/Nigeria

Key mutations:

D614G	Thought to make the coronavirus more infectious
delH69V70	Alters the shape of the spike and may help it evade some antibodies
E484K	Reduces antibody recognition; Associated with vaccine resistance
F888L	Potential reduction in neutralization by some antibody treatments
P681H	Predicted to enhance systemic infection and associated with increased transmissibility

Iota (B.1.526)

First detected: New York (USA)

Key mutation:

S477N	Associated with reduced susceptibility

B.1.1.298 (Mink Variant)

Key mutation:

D614G	Thought to make the virus more infectious

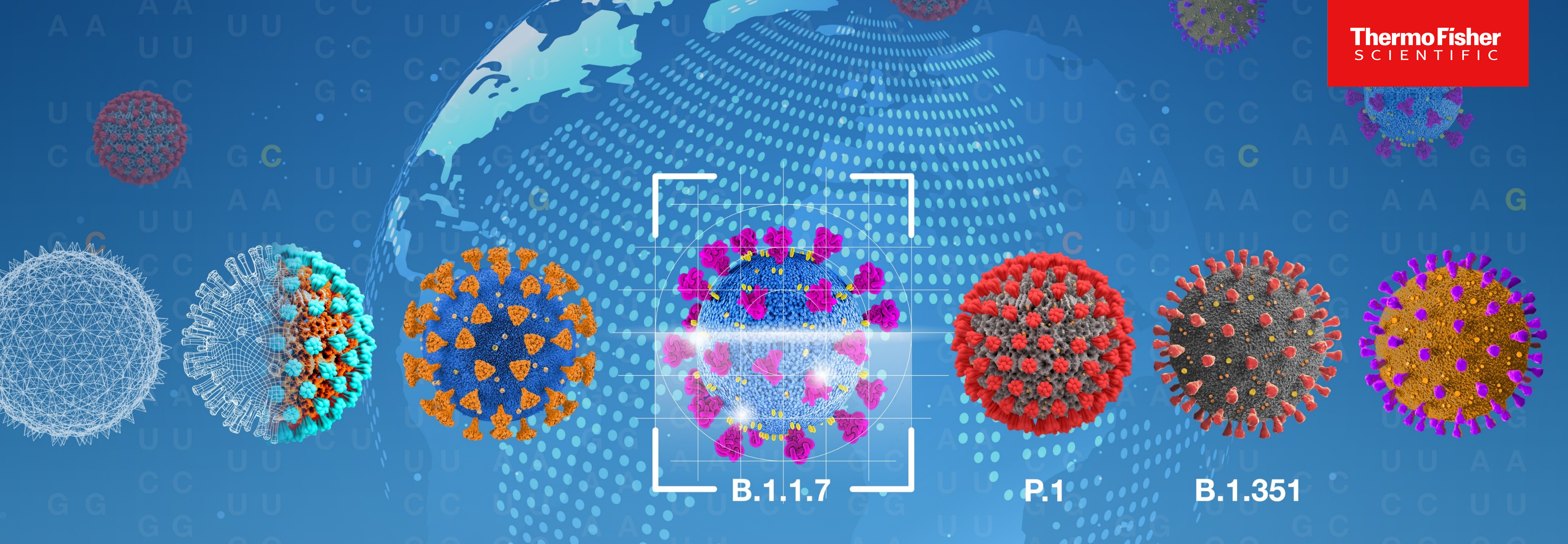
We closely track literature and trends to design assays for detecting emerging SARS-CoV-2 mutations. Find the most up-to-date list of available assays at **thermofisher.com/mutationpanel**

This graphic represents the most up-to-date information as of July 26, 2021.

Catalog numbers for reaction volumes

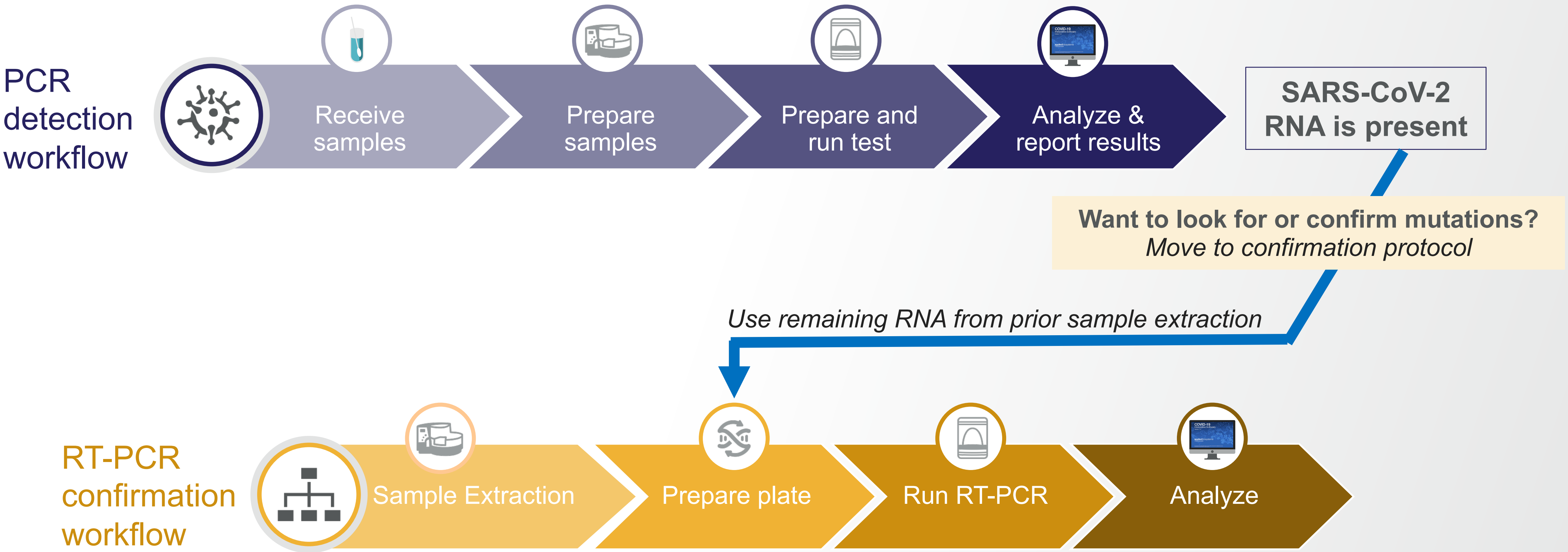
Size	No. of 10µL reactions (384-well plate)	No. of 20µL reactions (96-well plate)	Cat. No.
Small	750	374	A49785
Medium	2,500	1,250	A49786

Product	Size	Order No.
Master Mix		
TaqPath™ 1-Step RT-qPCR Master Mix, CG	5 x 1 mL	A15299
	1 x 10 mL	A15300
Recommended Controls		
AcroMetrix™ Coronavirus 2019 RNA Control	Each	954519
GeneArt™ DNA string or plasmid controls	Varies	https://www.thermofisher.com/geneart



Workflow

Mutation Confirmation Workflow



PCR detection workflow

Receive samples

Prepare samples

Prepare and run test

Analyze & report results

SARS-CoV-2 RNA is present

Want to look for or confirm mutations?
Move to confirmation protocol

Use remaining RNA from prior sample extraction

RT-PCR confirmation workflow

Sample Extraction

Prepare plate

Run RT-PCR

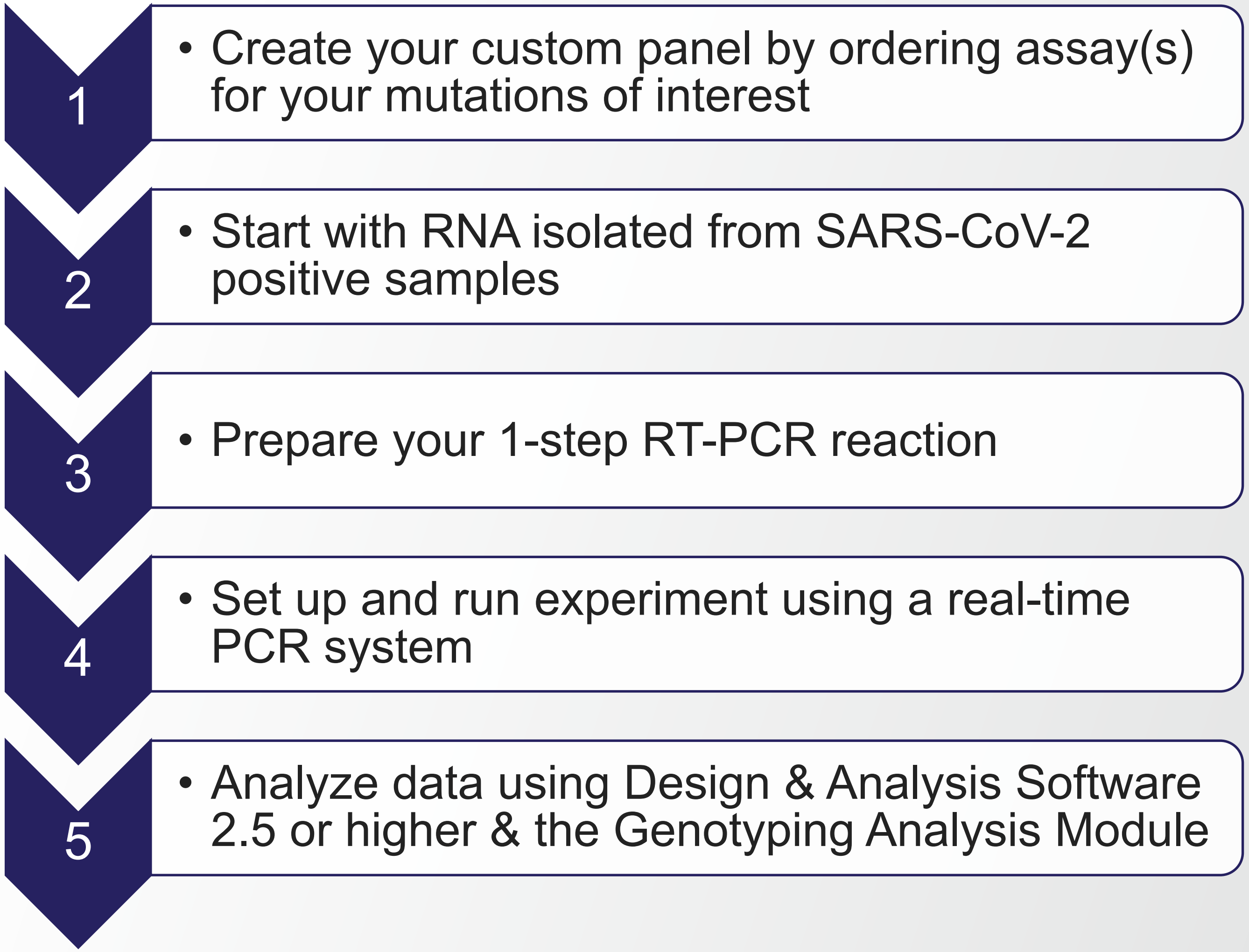
Analyze

Protocol using SNP assays to confirm mutations associated with SARS-CoV-2 emerging variants

Streamlined Workflow Using Gold Standard Technology

Get robust mutation detection from a simplified workflow: **1-step RT-PCR with TaqMan SNP assays**

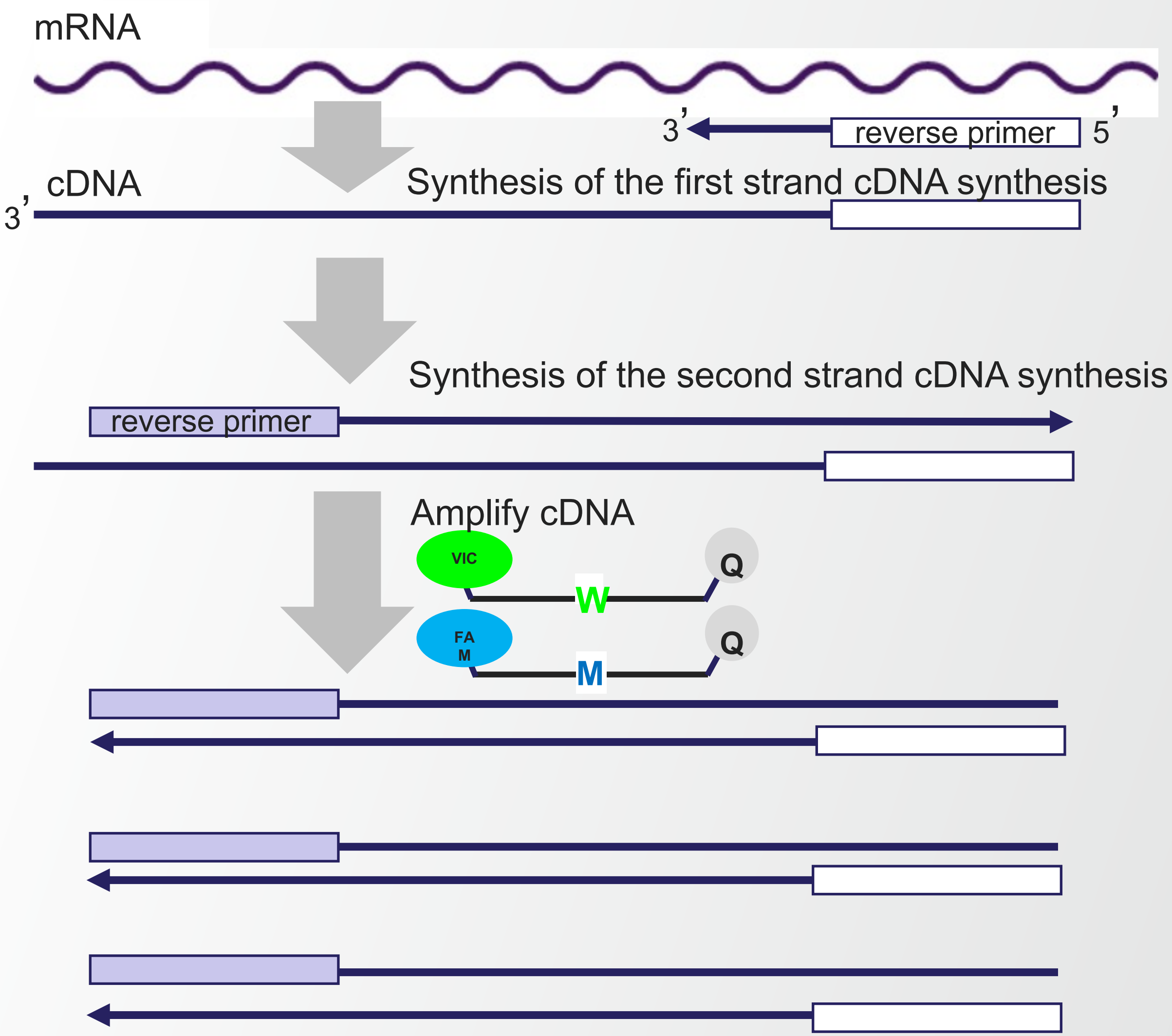
- Use **TaqMan SNP Assays to detect mutations in RNA** extracted from SARS-CoV-2 positive samples
- Compared to typical SNP workflow this includes a **reverse-transcription (RT) step** and is done using the **Real Time setting**
- Results are analyzed in cluster plots which clearly indicate which samples have the mutation of interest and which do not

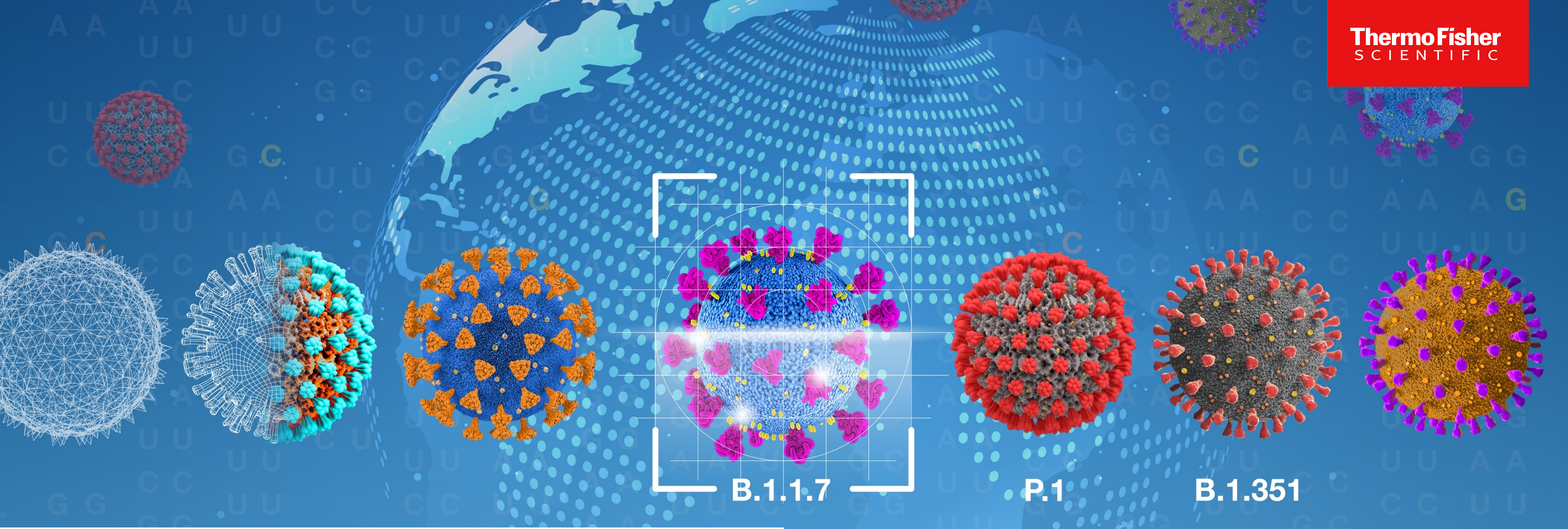


1. Create your custom panel by ordering assay(s) for your mutations of interest
2. Start with RNA isolated from SARS-CoV-2 positive samples
3. Prepare your 1-step RT-PCR reaction
4. Set up and run experiment using a real-time PCR system
5. Analyze data using Design & Analysis Software 2.5 or higher & the Genotyping Analysis Module

One-Step RT-PCR Using SNP Genotyping Assays

- **Start with RNA** extracted from SARS-CoV-2 positive samples
- During reverse transcription (RT), RNA is reversed transcribed to cDNA
- After reverse transcription, the PCR amplification of the cDNA template begins.
  - Reaction contains a forward primer, a reverse primer, a FAM-labeled TaqMan probe, and a VIC-labeled TaqMan probe
- The fluorescence occurs only if the target sequence is complementary to the probe and amplified during PCR
- Mutation allele detected by FAM dye & reference allele by VIC dye



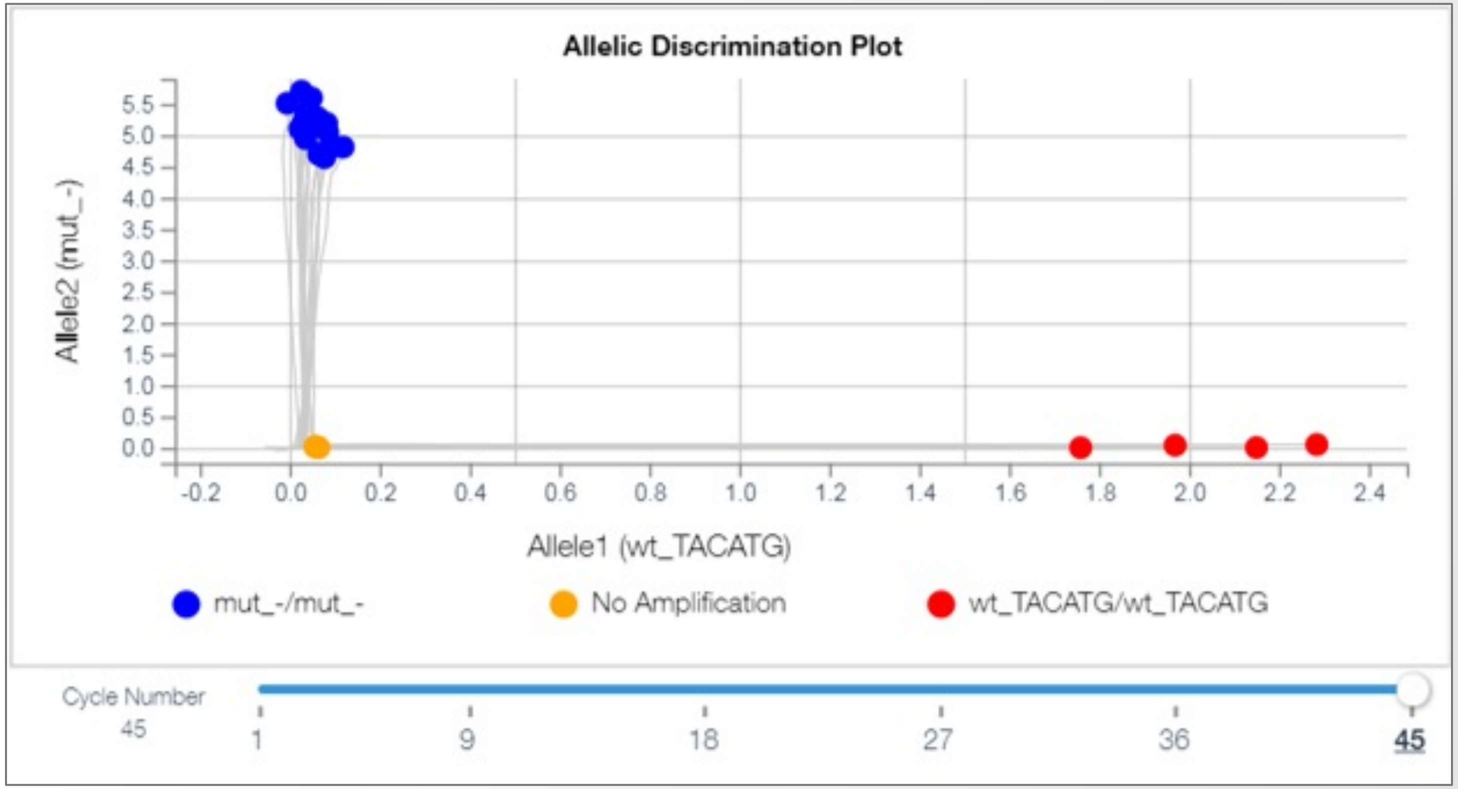


Technical Data

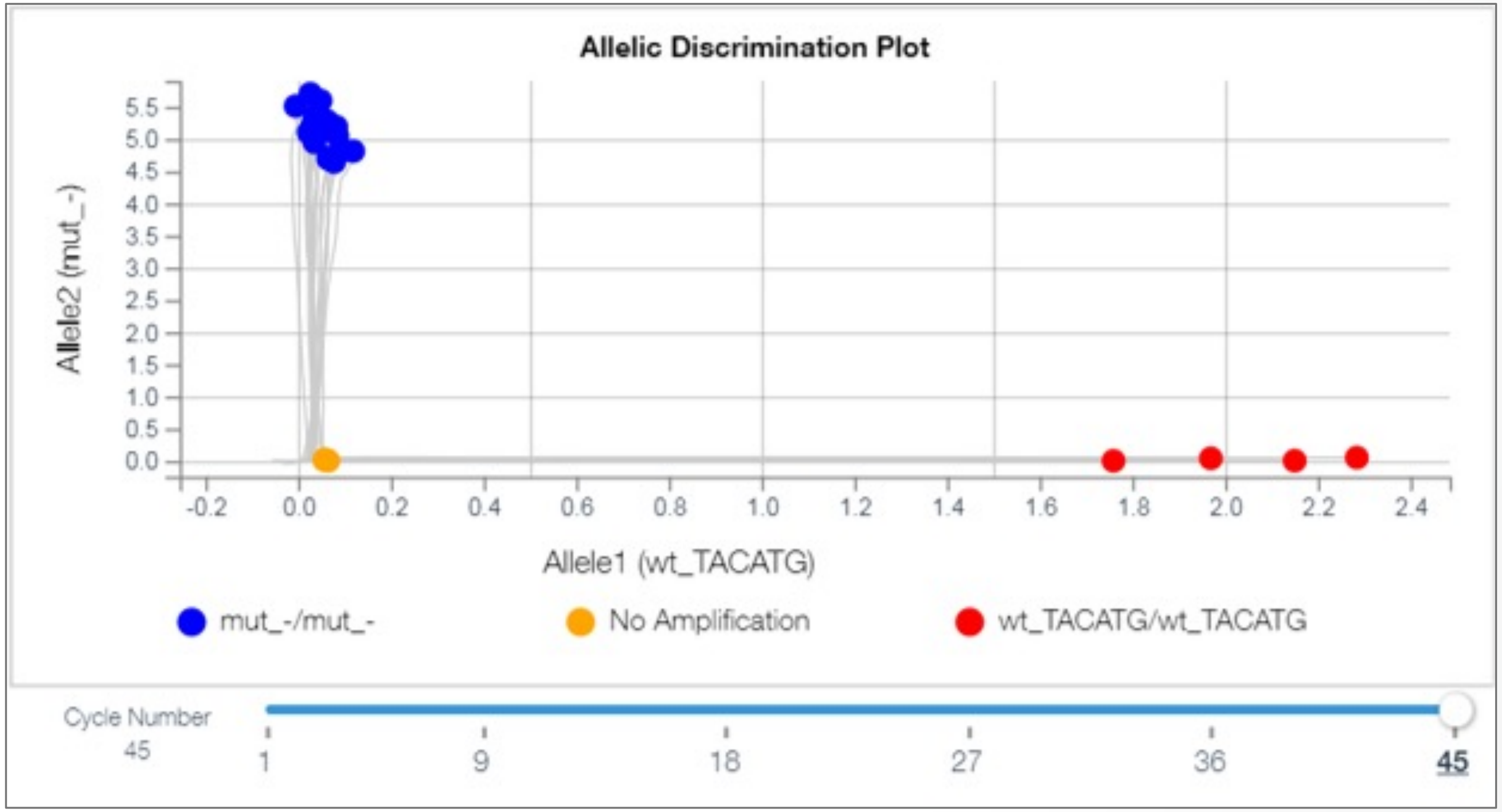
Reading the Results: Cluster Plot

How to interpret a cluster plot

- Cluster plots help to enable clear discrimination between reference and mutant
- Real time traces help clearly identify clusters as well as non-amplified samples
- Samples carrying reference allele will cluster along the X-axis (VIC dye; shown here in red).
- Samples carrying mutant allele will cluster along the Y-axis (FAM dye; shown here in blue).



Robust detection of S gene mutation delH69V70

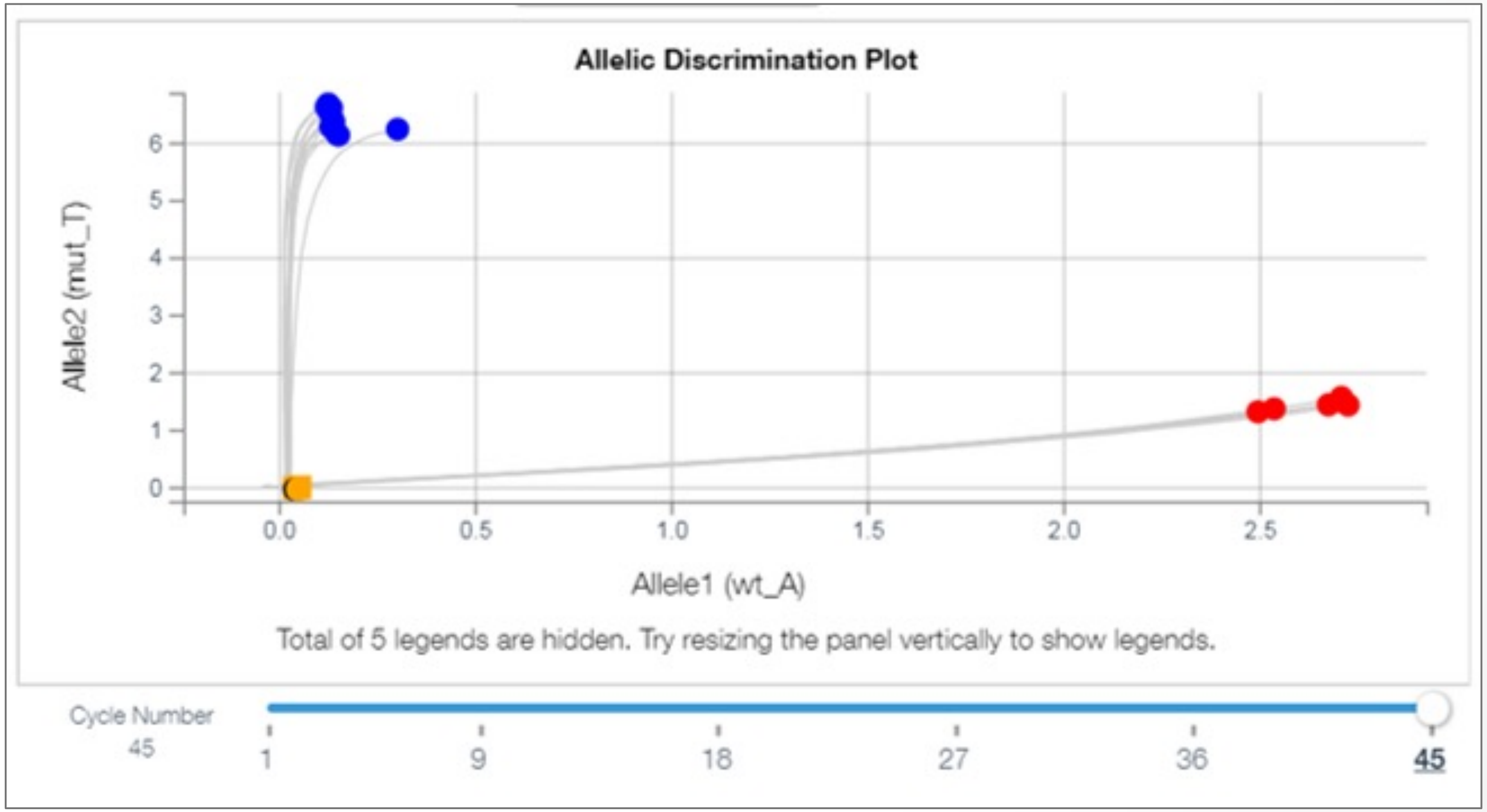


# of samples: 18

- delH69V70 (also known as *69-70del*) is a known mutation of B.1.1.7 (501Y.V1 or UK variant)
  - delH69V70 is not exclusive to B.1.1.7

  - **Red**: Wild type detected
  - **Blue**: delH68V70 mutation detected

Robust detection of S gene mutation N501Y



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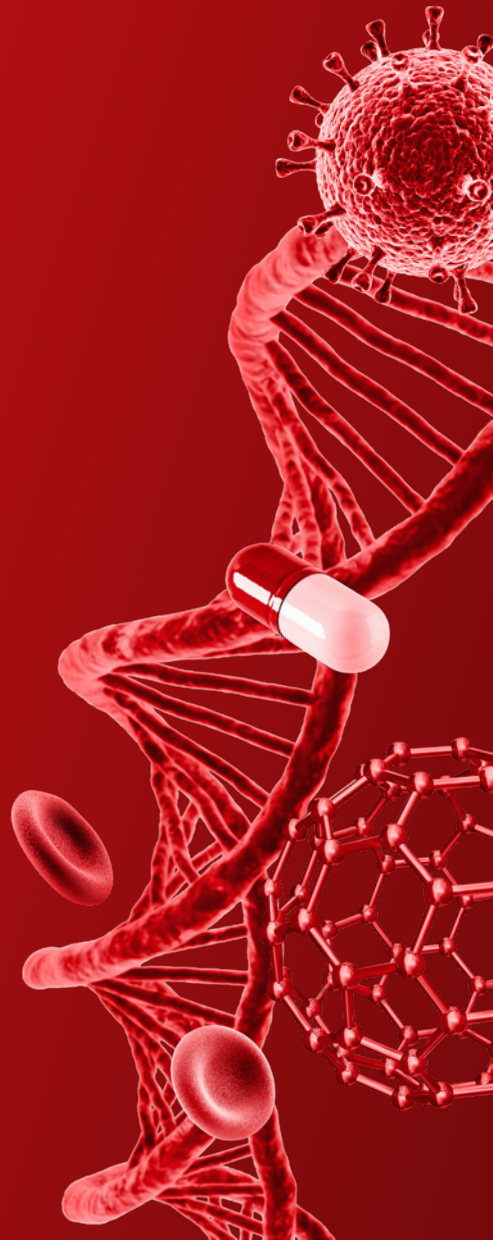
# of samples: 18

- N501Y is commonly found in:
  - B.1.1.7 (UK variant)
  - B.1.351 (South African variant)
  - P.1 (Brazilian variant)

- **Red**: Wild type detected
- **Blue**: N501Y mutation detected



Thank you

Learn more and equip your lab for what's next

thermofisher.com/mutationpanel