Mutation & Variant Surveillance of SARS-CoV-2

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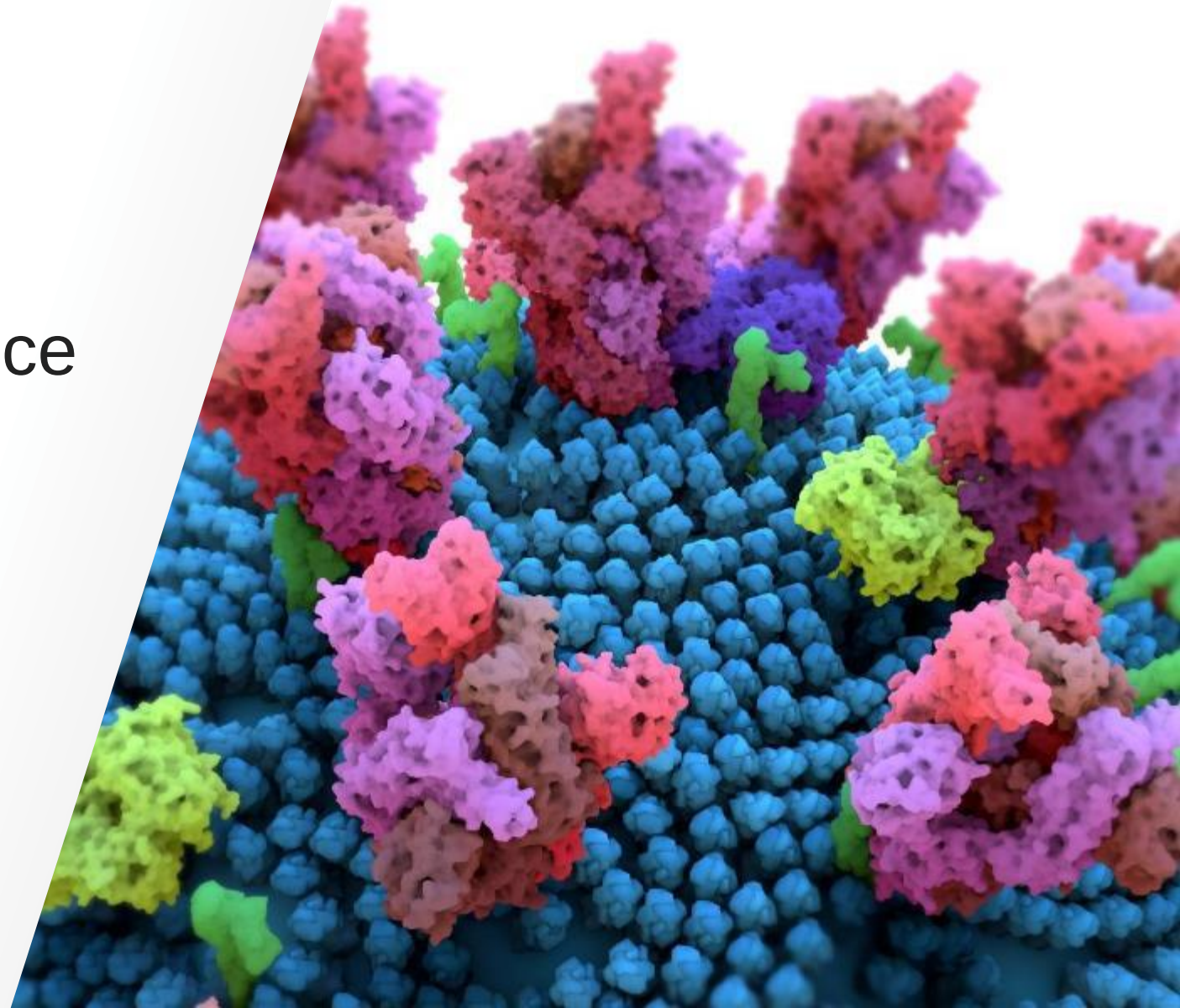
Field Application Scientist

ASLM Presentation

7 April 2021



The world leader in serving science

Who we are

Thermo Fisher SCIENTIFIC

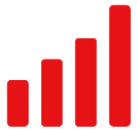
Thermo SCIENTIFIC | applied biosystems | invitrogen | Fisher Scientific | Unity Lab Services

Thermo Fisher Scientific is the Global Leader in Coronavirus Testing

thermo scientific | applied biosystems | invitrogen | fisher scientific | unity lab services | patheon

>50%

Coronavirus testing performed using Thermo Fisher technology

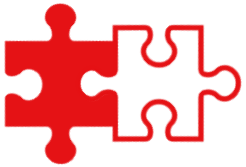


→ 20M

Coronavirus tests manufactured per week

Supply Chain

Secured supply chain to reliably meet global testing demands



One Trusted Source

24/7 service & support; consumables, instruments, software

Sample-to-Answer Workflow for TaqPath™ COVID-19 CE-IVD RT-PCR Kit

COVID-19

Same Day Turnaround Time

Service and support available throughout the entire process

Sample collection

Sample Preparation

Plate Setup

Perform RT-PCR

~115 min

Transfer Data

SDS or EDS run file

Analysis and Reporting

Applied Biosystems™
COVID-19 Interpretive Software

Please Note: Authorized laboratories using the TaqPath COVID-19 CE-IVD Kit will perform the TaqPath COVID-19 CE-IVD Kit as outlined in the Instructions for Use.

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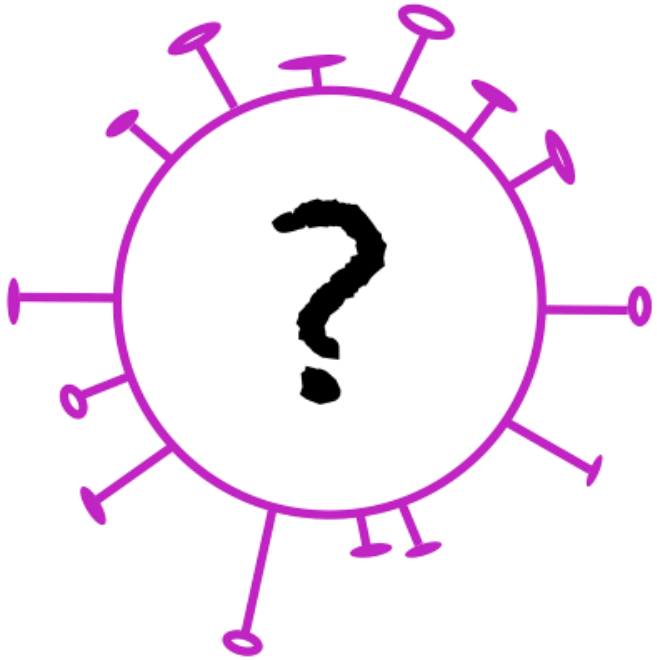
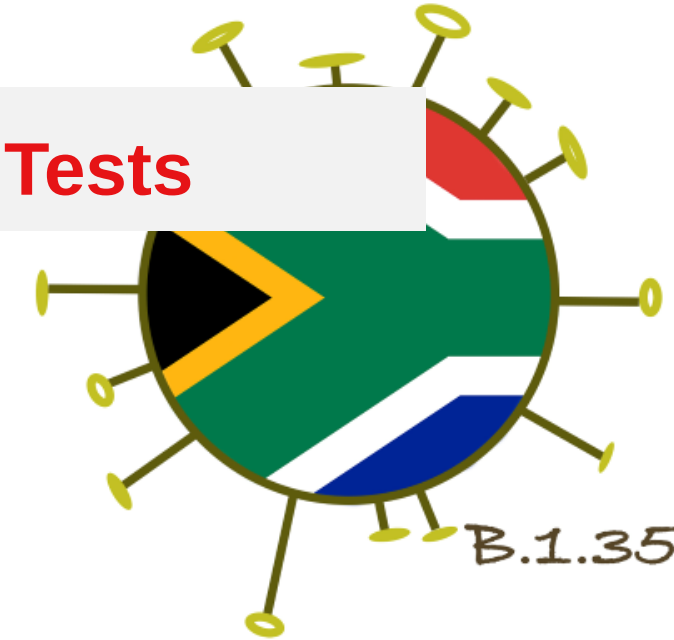
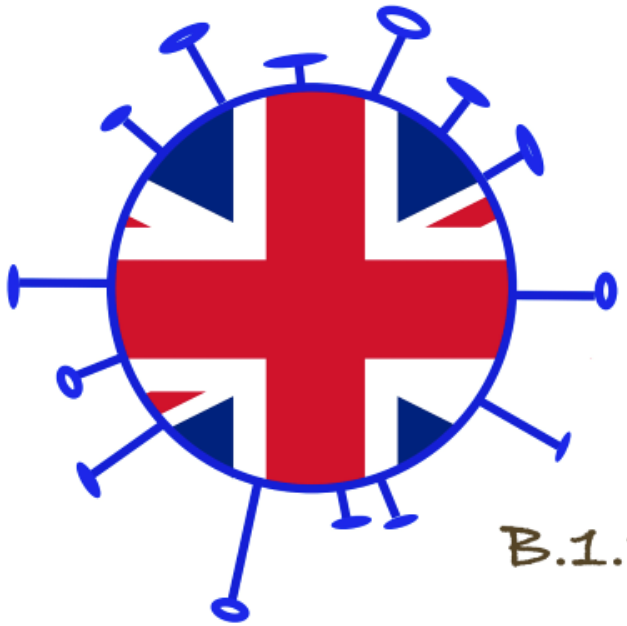


COVID Variants & Our Tests

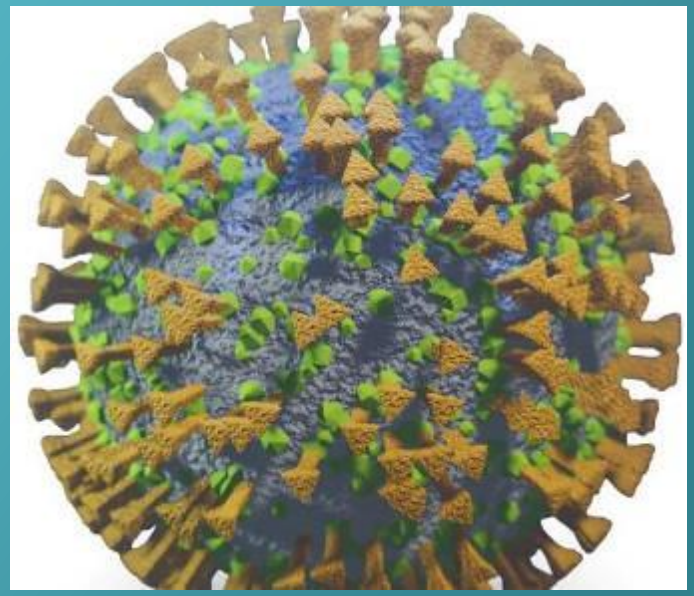




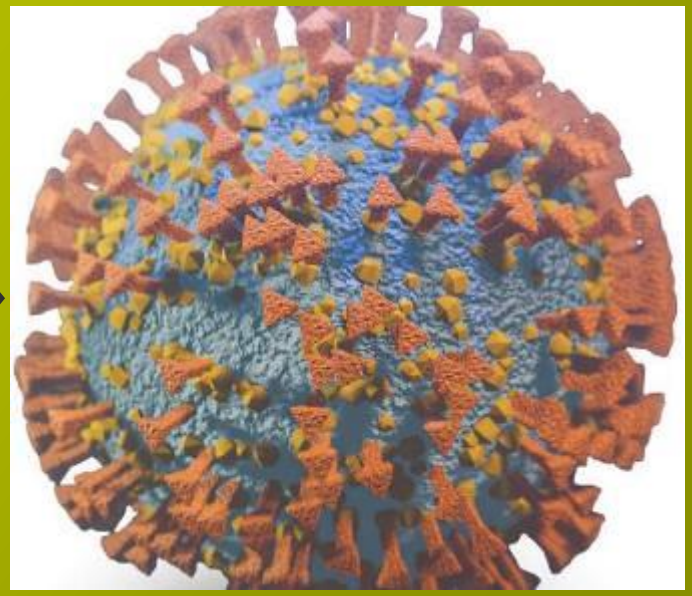
SARS-CoV-2 Viral Mutations

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

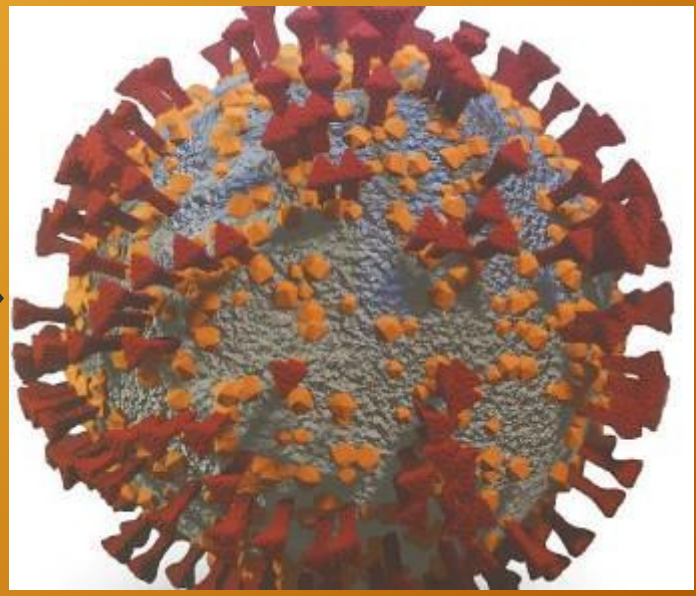
Continued uncontrolled transmission of SARS-CoV-2 in many parts of the world is creating conditions for significant virus evolution



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

Global Surveillance to Monitor Mutations

Purpose

Epidemiological surveillance

- Ensure viral diseases match the reference strain
- Monitor possible mutations
- Monitor viral evolution

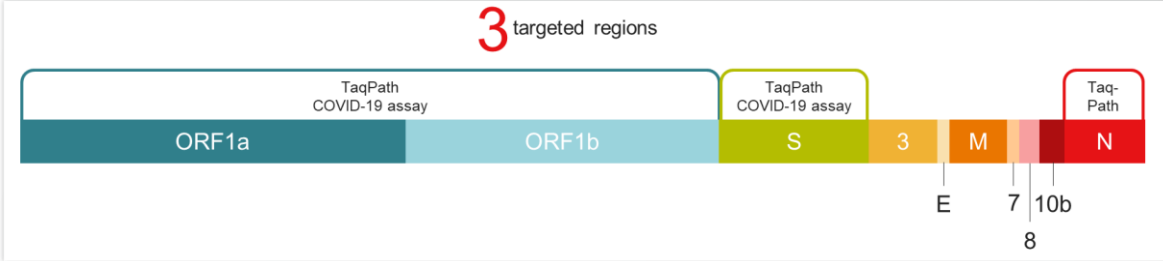
Why is this important

Changes in the viral genome impact public health policies and options, how the illness spreads in the population, potential study of treatment options, and vaccine development and vaccine efficacy

Unknowns

Focused on better predicting virus strain evolution

- What is variant origin and prevalence?
- Will current tests detect variants?
- Does variant spread more quickly?
- Does variant increase disease severity?
- What is vaccine efficacy against new variant?
- How to detect new variants?



Potential Implications of New SARS-CoV-2 Variants

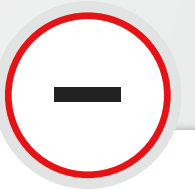
Potential Variant Impacts

Speed of human to human transmission

Change disease severity

Susceptibility to therapeutic agents (i.e., monoclonal antibodies)

Evade vaccine-induced immunity



Impact detection by clinical tests

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

Global Surveillance of SARS-CoV-2 Variants

Your Trusted Partner in SARS-CoV-2 Surveillance

Public health partners

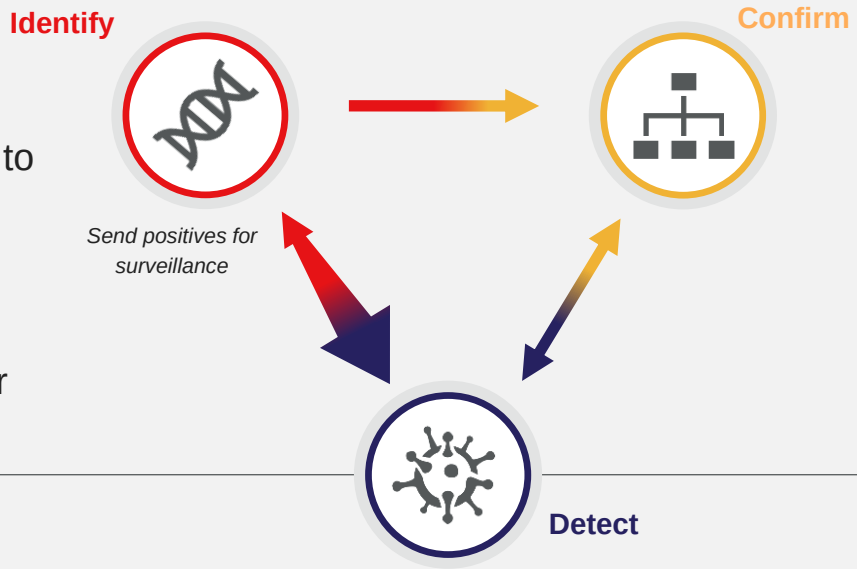
- Monitor viral genome
- Detect & Inform on emerging mutations
- Determine impact on detection, spread and vaccine / therapy effectivity

Research

Strain Surveillance

Monitor the SARS-CoV-2 virus to discover novel changes, detect emerging mutations to determine their effect on disease spread, diagnostic detection, and vaccine effectiveness

- **How**: Sequence the full viral genome or specific sections / genes of interest



Strain and Mutation Verification

Conduct strain identification by Interrogating positive samples to confirm known mutations associated with specific strains or linages

- How: Interrogate specific sections or areas of the viral genome by sequencing or genotyping

Clinical

RT PCR Detection

- Diagnostic assays that are confirmed to remain efficacious with evolution of SARS-CoV-2
- Understand how single or multiple mutations affect kit performance

Global Surveillance of SARS-CoV-2 Variants

Prepared with 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

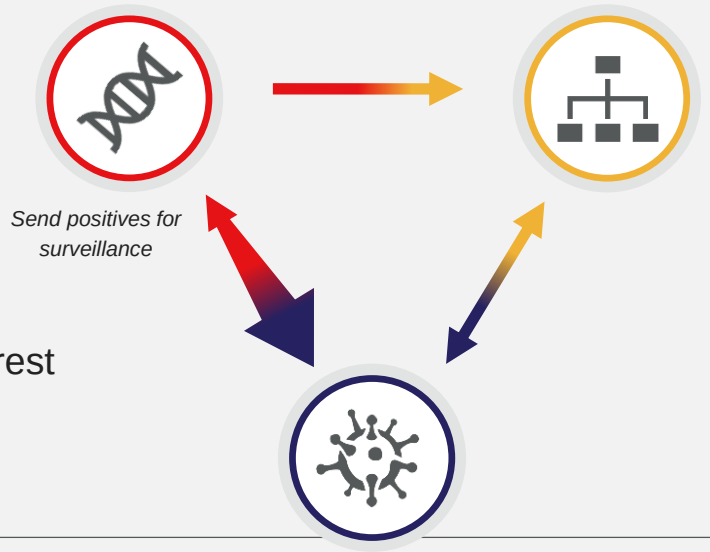
Strain Surveillance

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)



Strain and Mutation Verification

Capillary Electrophoresis

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

RT-qPCR (In Development)

- Detection and mutation Verification on the same instrument
- Confirm known mutations associated with specific strains or linages

Clinical

RT PCR Detection

RT-qPCR

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

General Molecular Biology Workflow

Sample Preparation/ Nucleic Acid Extraction

- Wide variety of kits available
- Flexible sample input types

Next-generation sequencing

- Whole genome sequencing
- New Variant detection
- Microbiome/ Transcriptome analysis

Sanger sequencing by capillary electrophoresis

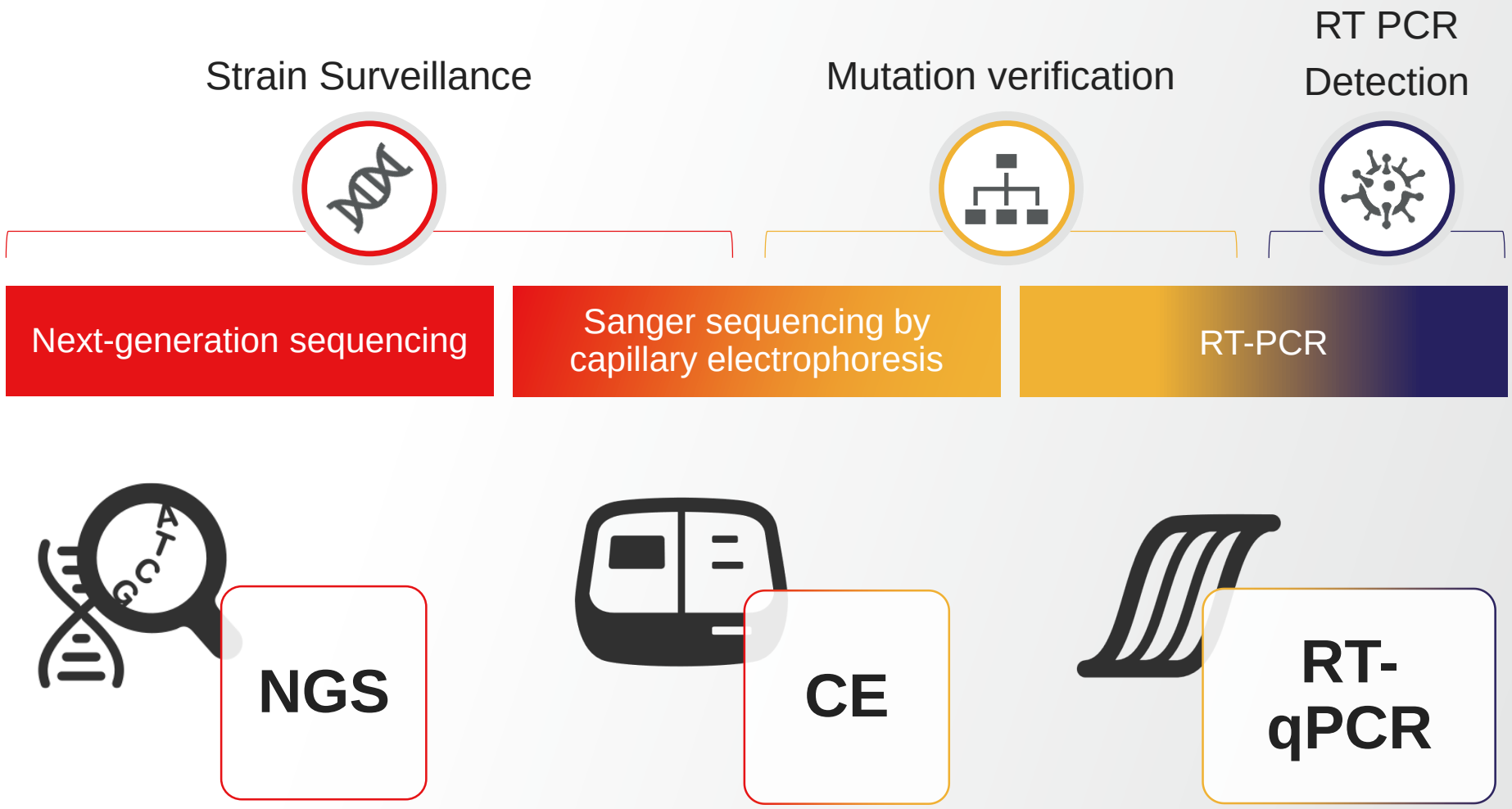
- COVID Mutation Scanning
- HIV-DR
- Microbial Fingerprinting
- NGS Confirmation
- Species identification

RT-PCR

- Viral load quantification
- Gene Expression
- Presence/ Absence Analysis
- SNP Genotyping

Applied Biosystems and Ion Torrent Genetic Analysis Technologies

Multiple research solutions support SARS-CoV-2 genetic surveillance and variant Verification



Research Solutions for Strain Surveillance

Value	• Confirm the presence of known or emerging mutations • Use molecular fingerprints to assign genetic clusters and build more definitive transmission chains • Help understand how the virus is spreading within local communities, across a nation, and globally	
Goal	Sequence full viral genome	Targeted (sanger) sequencing
Methodology	Next Gen Sequencing (NGS)	Capillary Electrophoresis (CE)
Customer Utility	• Interrogate for all mutations present across the genome • Strain Surveillance to determine new strains or linages • Sequence large numbers of samples or mutations in a single run	• Sequence targeted genes / areas of interest on viral genome (i.e., S-gene) • Targeted sequencing of short stretches of the viral genome to confirm known variants • Single sample sequencing to identify mutations and to confirm data from NGS

NGS Assay for Genome Sequencing of SARS-CoV-2

AmpliSeq™ SARS-CoV-2 Research Assay

- One assay surveys the complete genome
  - >99% genome coverage (~30 kb)
  - Covers all potential serotypes
- Use with biological research samples
- Viral loads as low as 20 copies
- Confirm individual amplicons with Sanger sequencing*

*Using Applied Biosystems Genetic Analyzer

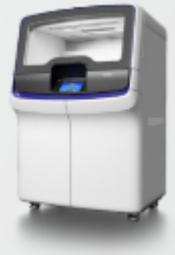
Intelligent design

Exceptional protection against naturally occurring variation

Robust performance even as the virus rapidly mutates

TARGETED NGS WORKFLOWS

Ion GeneStudio™ S5 Series



Genexus™ Integrated Sequencer

Complementary Ion Torrent NGS Systems for SARS-CoV-2 Research

ThermoFisher SCIENTIFIC

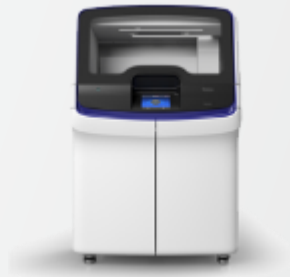
Fast, automated, and accurate targeted NGS workflows for coronavirus typing in <24 hours

Targeted NGS workhorse

High throughput and broad assay utility

From nucleic acid to report in a single day

Hands-off and automated workflow



Ion GeneStudio™ S5 Series

Single platform versatility for infectious disease and broader outbreak research applications

- Immune response
- Microbiome analysis
- Vaccine research and development

Cost-effective and scalable for high throughput sample processing

Genexus™ Integrated Sequencer

Single platform with a highly automated workflow and broad research capabilities

- Automated cDNA synthesis
- Library prep
- Template prep
- Sequencing and analysis

Setup-and-go, automated workflow for ease of adding in-house NGS

Sanger Sequencing

Strain Surveillance and Verification of SARS-CoV-2 Mutations

Gold standard Sanger sequencing to sequence short stretches of the viral genome, detect mutations and confirm NGS results

Sequencing Surveillance Protocols

- Cost effective sequencing of targeted genes or emerging mutations
- Sanger sequencing protocols and primer sequence sets available for surveillance
  - Protocol and primer set for full S gene sequencing
  - Protocol and primer sequences B.1.1.7 and 501Y.V2

Applied Biosystems Genetic Analyzers

A trusted standard for targeted sequencing

SeqStudio

3500xL

3730xl

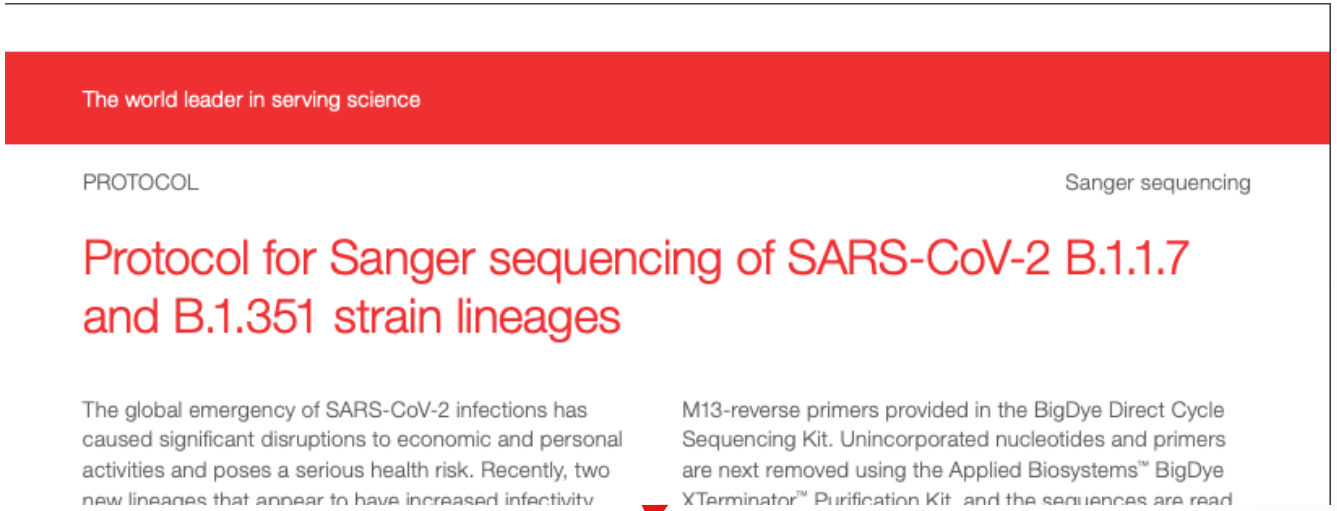
Low to high throughput options

Simple workflows

Sample to answer in as little as 1 day

Protocol for Sanger sequencing of SARS-CoV-2 B.1.1.7 and B.1.351 strain lineages

ThermoFisher SCIENTIFIC



The world leader in serving science

PROTOCOL

Sanger sequencing

Protocol for Sanger sequencing of SARS-CoV-2 B.1.1.7 and B.1.351 strain lineages

The global emergency of SARS-CoV-2 infections has caused significant disruptions to economic and personal activities and poses a serious health risk. Recently, two new lineages that appear to have increased infectivity

M13-reverse primers provided in the BigDye Direct Cycle Sequencing Kit. Unincorporated nucleotides and primers are next removed using the Applied Biosystems™ BigDye XTerminator™ Purification Kit, and the sequences are read

Detailed protocol

Excel sheet with primer sequences preloaded for direct input into custom ordering page (this is not the sheet)

Table 1. Sequences of M13-tagged primers for detecting mutations found in the lineage B.1.1.7. A subset of primer pairs that focus on specific regions of the SARS-CoV-2 genome can be chosen according to researchers' needs; a complete list that covers all regions mutated in the B.1.1.7 and South African lineages is provided here. Mutations that are synonymous and produce no amino acid change have a ":syn" suffix (i.e., T26801C:syn). Mutations that are associated with the South African B.1.351 lineage are indicated with (SA). The M13 sequence tags are highlighted in red.

Mutation covered by primer pair	Forward primer name	Forward primer sequence	Reverse primer name	Reverse primer sequence
C913T	SC2M1-3_LEFT_M13	TGTAAAACGACGGCCAGTAACAACTTCTGTGGCCCTGATG	SC2M1-3_RIGHT_M13	CAGGAAACAGCTATGACCTCTGAATTGTGACATGCTGGACA
C3267T:T1001I	SC2M1-8_LEFT_M13	TGTAAAACGACGGCCAGTACTTACACCACTGGGCATTGATT	SC2M1-8_RIGHT_M13	CAGGAAACAGCTATGACCCTGCAACACCTCCTCCATGTTT
C5388A:A1708D	SC2M1-14_LEFT_M13	TGTAAAACGACGGCCAGTACTTCTATTAAATGGGCAGATAACAACTGT	SC2M1-14_RIGHT_M13	CAGGAAACAGCTATGACCAGCACCGTCTATGCAATACAAAGT
C5986T:syn	SC2M1-15_LEFT_M13	TGTAAAACGACGGCCAGTTGTTATGATGTCAGCACCACCTG	SC2M1-15_RIGHT_M13	CAGGAAACAGCTATGACCAGCCACCACATCACCATTTAAGT
T6954C:I2230T	SC2M1-18_LEFT_M13	TGTAAAACGACGGCCAGTAAACCGTGTTTGTACTAATTATATGCCTT	SC2M1-18_RIGHT_M13	CAGGAAACAGCTATGACCTGCCAAAAACCACTCTGCAACT
del11288-11296i: delSGF3675-3677	SC2M1-29_LEFT_M13	TGTAAAACGACGGCCAGTAGTCCAGAGTACTCAATGGTCTTTGT	SC2M1-29_RIGHT_M13	CAGGAAACAGCTATGACCACAATACCTCTGGCCAAAAACATGA
C14676T:syn	SC2M1-38_LEFT_M13	TGTAAAACGACGGCCAGTACTTCAGAGAGCTAGGTGTTGTACA	SC2M1-38_RIGHT_M13	CAGGAAACAGCTATGACCTGCGAAAAGTGCATCTTGATCCT
C15279T:syn	SC2M1-39_LEFT_M13	TGTAAAACGACGGCCAGTACGATGGTGGCTGTATTAATGCT	SC2M1-39_RIGHT_M13	CAGGAAACAGCTATGACCGGTGTGACAAGCTACAACACGT
C16176T:syn	SC2M1-42_LEFT_M13	TGTAAAACGACGGCCAGTGGAGTATGCTGATGTCTTTCATTTGTAC	SC2M1-42_RIGHT_M13	CAGGAAACAGCTATGACCGCGTTTCTGCTGCAAAAAGCTT
del21765-21770: delHV69-70 C21614T_S:L18F (SA) A21801C_S:D80A (SA)	SC2M1-55_LEFT_M13	TGTAAAACGACGGCCAGTAGGGGTACTGCTGTTATGTCTTTAAA	SC2M1-55_RIGHT_M13	CAGGAAACAGCTATGACCAAGTAGGGACTGGGTCTTCGAA
del21991-21993:delY144 A22206G_S:D215G (SA) S:delL242_244L (SA) T22287A_S:L242H (SA) G22299T_S:R2461 (SA)	SC2M1-56-LEFT_M13	TGTAAAACGACGGCCAGTTGGGACCAATGGTACTAAGAGGT	SC2M1-56_RIGHT_M13	CAGGAAACAGCTATGACCACCAGCTGTCCAACCTGAAGAA
A23063T:N501Y C23271A:A570D G23012A_S:E484K (SA) A23063T_S:N501Y (SA)	SC2M1-59_LEFT_M13	TGTAAAACGACGGCCAGTCCGGTAGCACACCTTGTAATGG	SC2M1-59_RIGHT_M13	CAGGAAACAGCTATGACCCCCCTATTAAACAGCCTGCACG
C23604A:P681H C23709T:T716I C23664T_S:A701V (SA)	SC2M1-60_LEFT_M13	TGTAAAACGACGGCCAGTACCAGGTTGCTGTTCTTTATCAGG	SC2M1-60_RIGHT_M13	CAGGAAACAGCTATGACCCAGCTATTCCAGTTAAAGCACGGT
T24506G:S982A	SC2M1-62_LEFT_M13	TGTAAAACGACGGCCAGTGCTGCTAGAGACCTCATTTGTGC	SC2M1-62_RIGHT_M13	CAGGAAACAGCTATGACCAAGCTCTGATTTCTGCAGCTCT
G24914C:D1118H G22813T_S:K417N (SA)	SC2M1-64_LEFT_M13	TGTAAAACGACGGCCAGTGCACACACTGGTTTGTAACACAA	SC2M1-64_RIGHT_M13	CAGGAAACAGCTATGACCTTTGACTCCTTTGAGCACTGGC
T26801C:syn	SC2M1-68_LEFT_M13	TGTAAAACGACGGCCAGTTCCTGATCTTCTGGTCTAAACGAACT	SC2M1-68_RIGHT_M13	CAGGAAACAGCTATGACCATGTCACAGCGTCCTAGATGGT
C27972T:Q27stop G28048T:R521 A28111G:Y73C	SC2M1-71_LEFT_M13	TGTAAAACGACGGCCAGTTGTTCATCAGACAAGAGGAAGTTCA	SC2M1-71_RIGHT_M13	CAGGAAACAGCTATGACCACGAACAACGCACTACAAGACT
GAT_CTA:D3L	SC2M1-72_LEFT_M13	TGTAAAACGACGGCCAGTTTGAATTGTGCGTGGATGAGGC	SC2M1-72_RIGHT_M13	CAGGAAACAGCTATGACCTAGCACCATAGGGAAGTCCAGC
C28977T:S235F	SC2M1-74_LEFT_M13	TGTAAAACGACGGCCAGTTGATGCTGCTCTTGCTTTGCTG	SC2M1-74_RIGHT_M13	CAGGAAACAGCTATGACCTCTGCAGCAGGAAGAAGAGTCA

The function of the primer sequences described in this document are in the process of being verified.

Research Solutions for Strain and Mutation Verification

Value	• Confirm the presence of known mutations in diagnostic samples • Identify if a sample is of a specific strain or linage • Verify mutations to determine source of discordant results	
Goal	Targeted (Sanger) sequencing	RT-QPCR
Methodology	Capillary Electrophoresis (CE)	RT-qPCR (in development)
Customer Utility	• Sequence to verify unexpected Dx sample results (i.e., S-gene “dropout”) • Confirm multiple mutations, strains or lineages	• Customizable – choose from a menu of verified real-time PCR assays that allows you to identify currently relevant SARS-CoV-2 mutations and adapt quickly as additional mutations and variants emerge • Convenient – use your current real-time PCR instrumentation to conduct reflex analysis of SARS-CoV-2 samples

Real-time PCR for Genetic Verification of SARS-CoV-2 Emerging Mutations

ThermoFisher SCIENTIFIC

Real-time PCR for reflex testing for clinical samples with known SARS-CoV-2 mutations

Mutation Targets	Over 22 clinically relevant mutations including 69/70 deletion, UK (B.1.1.7), South African (B.1.351), and Brazilian (P.1, P.2) variants
Workflow	Reflex from positive COVID-19 test or COVID 19 test with s gene dropout using TaqPath
Assay	Customizable menu of genotyping assays to confirm SARS-CoV-2 mutations
Rxn Vols	300 Rxn, 1000 Rxn, 2400 Rxn
Instruments	Use existing qPCR platforms including 7500Dx , QS7, QS5
Status	RUO

QuantStudio 5 Real-time PCR System

7500 Fast Dx Real-time PCR System

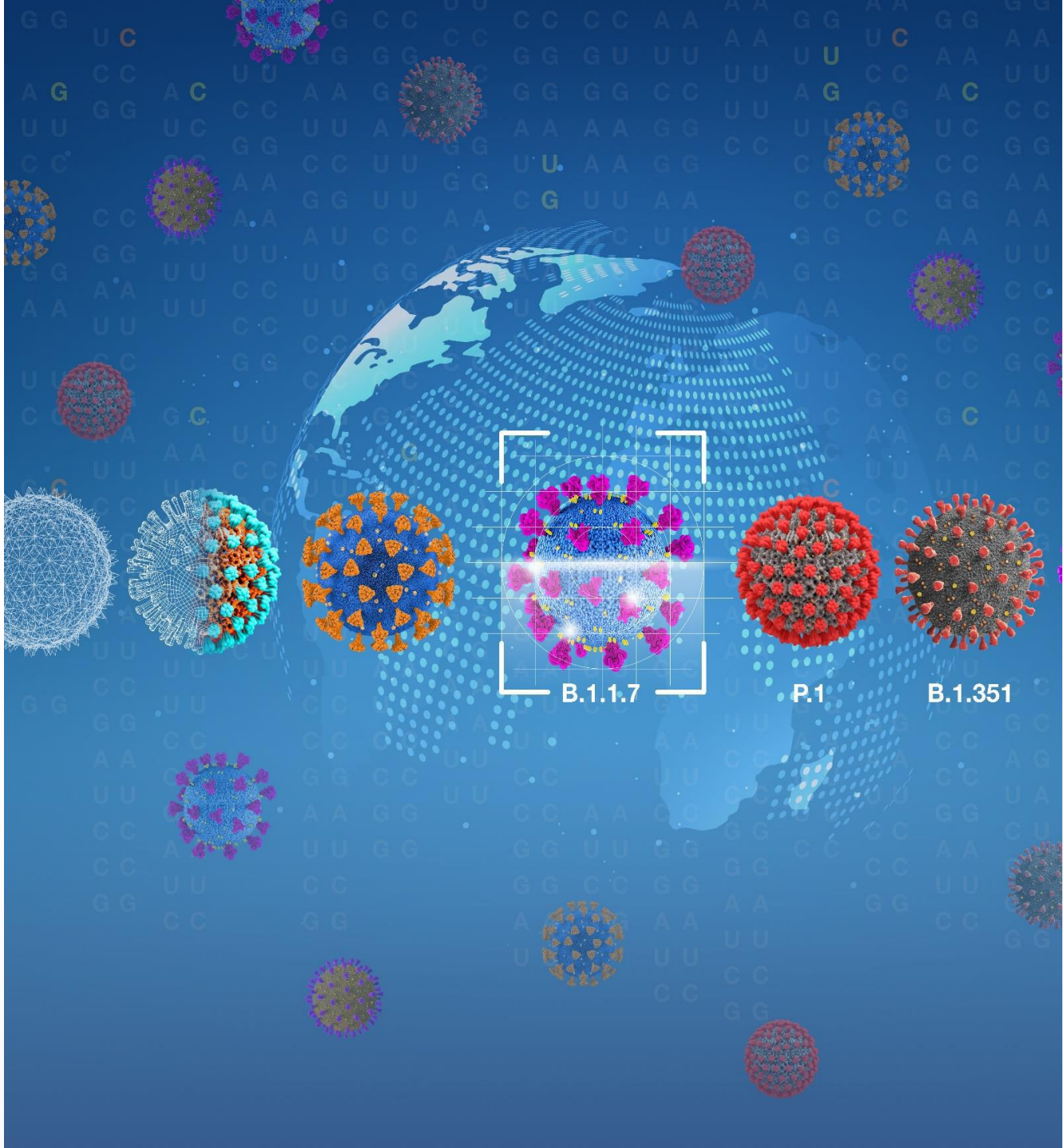
QuantStudio 7 Real-time PCR System (384-well block)

Rapid, cost effective and scalable laboratory solution ready to adapt to future detection needs as more mutations emerge

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

TaqMan® SARS-CoV-2 Mutation Panel

- **Customizable panel**—choose from a menu of 22 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 analysis 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



Applied Biosystems TaqMan Genotyping Assays

ThermoFisher SCIENTIFIC

Distinguish SNPs to better understand their associations and roles in gene function

TaqMan Assays

- Efficient and cost-effective method for SNP mutation research in large populations

Genotyping Assays

- Discriminate between sequences, rather than measure the level of a particular genomic sequence.

Minor Groove Binder Technology (MGB)

- Enables TaqMan probes to discriminate between highly homologous allele sequences

TaqMan Performance Guarantee*

- Ensures the assay will work as described or we'll replace it

Multiple Research Solutions for Surveillance and Verification

ThermoFisher SCIENTIFIC

	Surveillance			Verification	
	Next-generation sequencing (whole viral genome)		Sanger sequencing by capillary electrophoresis		RT-PCR (coming soon)
Use Case	Surveillance of the full viral genome to discover and identify new and emerging variants and mutations.	Surveillance to identify novel variants.	Rapid, surveillance of targeted genes / areas of interest	Rapid Verification of genes / areas of interest with known mutations, strains or lineages	Mutation Verification of known mutations
Lab profile	Manual prep resources and skills Experienced with NGS	Limited resources or skills, need automation or fast turn around time New to NGS	Cost effective and able to se	Cost effective and able to see full sequence of mutations	Use the same PCR instrument for both clinical detection and mutation Verification Detection of mutations
Number of targets	>20 - 10000	>20 - 10000	1-20	1-20	1-20
Solution Complexity	***	**	**	**	*
Instruments	Genestudio & Chef	Genexus	SeqStudio, 3500, 3730	SeqStudio, 3500, 3730	Quantstudio 5, Quantstudio 7, 7500Dx
Surveillance Research Solutions	Ampliseq SARS Cov2 Panel for full length sequence		Primer sets for full S-gene sequencing	Primer sets for 69/70del, B.1.1.7 and B.1.351 Variants	Primer / Probe sets 69/70del, B.1.1.7, B.1.351 and P.1, P.2 Variants ***
Sample Prep	Manual or Automated (Chef)	Automated	Manual	Manual or Automated	Manual or Automated
Samples/day (incl. sample prep & data analysis)	160 (Ion 540™ chip) 260 (Ion 550™ chip) - if 2 chip runs back-to-back	16 per day (2 lanes of GX5™ chip)	286* protocol 69/70del 12** protocol B117	24** protocol S-full	TBD
Sensitivity	***	***	**	**	*
Time to Result	2 days	1 day	hours	hours	hours
Cost/sample (USD List)	$108/ on 540 chip (manual library prep) $94/ on 550 chip (manual library prep)	$411 (automated)	$16 (69/70del Verification) $241 (full B.1.1.7 with 38 Sanger rxns)	Whole S genome: $241 for full with 24 Sanger rxns	TBD

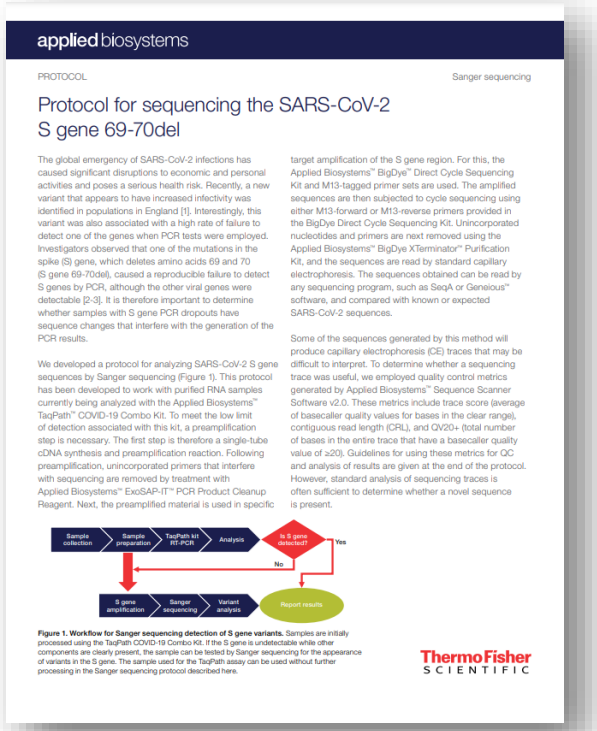
*1 plate with 46 samples (plus controls), 5 plates with 48 samples (no controls)
** if use all sequencing primers outlined in the protocol (38 primers for B.1.1.7, 24 for S-full)
*** Key mutations known to be in UK (B.1.1.7), South African (B.1.351), and Brazilian (P.1, P.2) variants

Verification Educational Content

Guidance and best practices to facilitate testing

Protocol

Sequencing the SARS-CoV-2 S gene 69-70del


applied biosystems

PROTOCOL Sanger sequencing

Protocol for sequencing the SARS-CoV-2 S gene 69-70del

The global emergency of SARS-CoV-2 infections has caused significant disruptions to economic and personal activities and poses a serious health risk. Recently, a new variant that appears to have increased infectivity was identified in populations in England [1]. Interestingly, this variant was also associated with a high rate of failure to detect one of the genes when PCR tests were employed. Investigators observed that one of the mutations in the spike (S) gene, which deletes amino acids 69 and 70 (S gene 69-70del), caused a reproducible failure to detect S genes by PCR, although the other viral genes were detectable [2-3]. It is therefore important to determine whether samples with S gene PCR dropouts have sequence changes that interfere with the generation of the PCR results.

We developed a protocol for analyzing SARS-CoV-2 S gene sequences by Sanger sequencing (Figure 1). This protocol has been developed to work with purified RNA samples currently being analyzed with the Applied Biosystems™ TaqPath™ COVID-19 Combo Kit. To meet the low limit of detection associated with this kit, a preamplification step is necessary. The first step is therefore a single-tube cDNA synthesis and preamplification reaction. Following preamplification, unincorporated primers that interfere with sequencing are removed by treatment with Applied Biosystems™ ExoSAP-IT™ PCR Product Cleanup Reagent. Next, the preamplified material is used in specific target amplification of the S gene region. For this, the Applied Biosystems™ BigDye™ Direct Cycle Sequencing Kit and M13-tagged primer sets are used. The amplified sequences are then subjected to cycle sequencing using either M13-forward or M13-reverse primers provided in the BigDye Direct Cycle Sequencing Kit. Unincorporated nucleotides and primers are next removed using the Applied Biosystems™ BigDye XTerminator™ Purification Kit, and the sequences are read by standard capillary electrophoresis. The sequences obtained can be read by any sequencing program, such as SeqA or Geneious™ software, and compared with known or expected SARS-CoV-2 sequences.

Some of the sequences generated by this method will produce capillary electrophoresis (CE) traces that may be difficult to interpret. To determine whether a sequencing trace was useful, we employed quality control metrics generated by Applied Biosystems™ Sequence Scanner Software v2.0. These metrics include trace score (average of basecaller quality values for bases in the clear range), contiguous read length (CRL), and QV20+ (total number of bases in the entire trace that have a basecaller quality value of ≥20). Guidelines for using these metrics for QC and analysis of results are given at the end of the protocol. However, standard analysis of sequencing traces is often sufficient to determine whether a novel sequence is present.

Sample collection | Sample preparation | TaqPath kit RT-PCR | Analysis | Is S gene detected? | Yes | No | S gene amplification | Sanger sequencing | Variant analysis | Report results

Figure 1. Workflow for Sanger sequencing detection of S gene variants. Samples are initially processed using the TaqPath COVID-19 Combo Kit. If the S gene is undetectable while other components are clearly present, the sample can be tested by Sanger sequencing for the appearance of variants in the S gene. The sample used for the TaqPath assay can be used without further processing in the Sanger sequencing protocol described here.

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Protocol

SARS-CoV-2 B.1.1.7, B.1.351, or B.1.1.28 strain lineages verification


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PROTOCOL Sanger sequencing

Protocol for Sanger sequencing of SARS-CoV-2 B.1.1.7 and B.1.351 strain lineages

The global emergency of SARS-CoV-2 infections has caused significant disruptions to economic and personal activities and poses a serious health risk. Recently, two new lineages that appear to have increased infectivity have been identified. The B.1.1.7 lineage, first identified in England and subsequently in the US, contains 23 mutations scattered across the SARS-CoV-2 genome [1]. The other lineage, B.1.351, first identified in South Africa, contains a set of mutations clustering in the SARS-CoV-2 spike (S) gene [2]. Since both lineages are thought to have increased transmissibility relative to other lineages, it is important to determine whether samples contain the mutations identified in the two lineages [2,3].

We developed a Research Use Only protocol for detecting these lineages by Sanger sequencing. The primer sequences used here are based on those published by the Centers for Disease Control and Prevention (CDC) [4]. Briefly, cDNA synthesis is performed on a sample containing viral RNA. Next, the cDNA is used in specific regions of target amplification using primers that have been selected to cover the novel mutations. For this, the Applied Biosystems™ BigDye™ Direct Cycle Sequencing Kit and M13 sequence–tagged primer sets are used. The amplified sequences are then subjected to cycle sequencing using either M13-forward or M13-reverse primers provided in the BigDye Direct Cycle Sequencing Kit. Unincorporated nucleotides and primers are next removed using the Applied Biosystems™ BigDye XTerminator™ Purification Kit, and the sequences are read by standard capillary electrophoresis (CE). The sequences obtained can be read by any sequencing program, such as SeqA or Geneious™ software, and compared with known or expected SARS-CoV-2 sequences (Figure 1).

Some of the sequences generated by this method will produce CE traces that may be difficult to interpret. To determine whether a sequencing trace was useful, we employed quality control metrics generated by Applied Biosystems™ Sequence Scanner Software v2.0. These metrics include trace score (average of basecaller quality values for bases in the clear range), contiguous read length (CRL), and QV20+ (total number of bases in the entire trace that have a basecaller quality value of ≥20). Guidelines for using these metrics for QC and analysis of results are given at the end of the protocol. However, standard analysis of sequencing traces is often sufficient to determine whether a novel sequence is present.

Note: This protocol and the reagents described within are for Research Use Only. Not for use in diagnostic procedures.

RNA purification | Amplicon generation | Cycle sequencing | Capillary electrophoresis | Analysis

Figure 1. Workflow for detection of SARS-CoV-2 lineages using Sanger sequencing. RNA is purified from samples using standard techniques. cDNA is synthesized from the RNA, and specific M13 sequence–tagged amplicons are generated by PCR. The amplicons are sequenced in the forward and reverse directions using universal M13 primers and the BigDye Direct Cycle Sequencing Kit. The sequencing reactions are cleaned using the BigDye XTerminator kit and subjected to CE. The resulting sequencing traces can be analyzed and compared to reference SARS-CoV-2 sequences to determine if the lineages are present.

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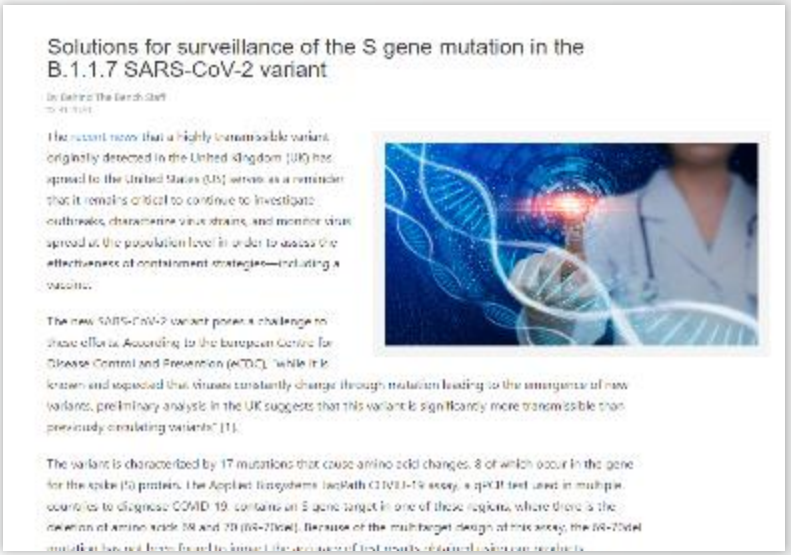
Blog

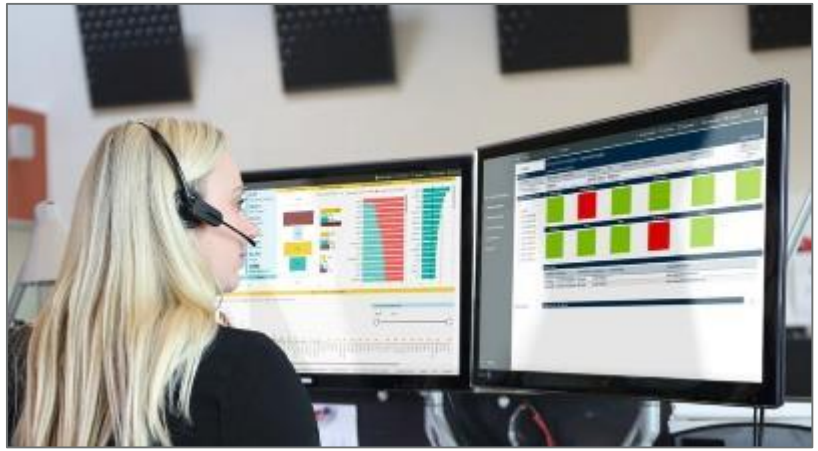
Solutions for Surveillance


Solutions for surveillance of the S gene mutation in the B.1.1.7 SARS-CoV-2 variant

Includes links to protocols and primer ordering lists

Global Surveillance Can’t Wait on Instrument Downtime

***AR support availability varies by region.**



AR & desktop remote support* – real-time collaboration tools enable remote identification and resolution of issues in minutes, helping to eliminate on-site repairs that keep you idle for days

Remote monitoring and diagnostics service – problem detection and faster service resolution improves instrument uptime by resolving issues proactively

Interactive, self-paced education – having a great instrument is good. Knowing how to use it—and getting up to speed quickly—is paramount. Formats include eLearning and 3D Experiences

Instrument Connect – use Connect, our cloud-based platform, to get connected to your data more easily and quickly than ever before

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Thank you

Any further questions?