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TaqMan Genotyping Assay for Detection of B.1.1.7 SARS-CoV-2

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1 *Works for me* This protocol is published without a DOI.

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SUBMIT TO PLOS ONE

ABSTRACT

At the end of 2020, several new lineages of SARS-CoV-2 were reported that may be more transmissible, less responsive to antibody therapy, or able to “escape” the protection of recently approved vaccines, including B.1.1.7, first reported in the United Kingdom (1). Certain mutations affect functionally important parts of viral proteins and are termed “variants of concern” or VOC. The existing SARS-CoV-2 qPCR and antigen tests cannot identify these new viral variants. Advanced molecular diagnostic assays are needed to understand the spread of VOCs in the state. Viral genome sequencing is the gold standard for characterization mutations, lineage classification, and high-resolution genomic surveillance, but only a small proportion of isolates are currently analyzed by this approach in the USA. Faster and simpler approaches to identifying VOCs based on assays targeting specific mutations represent a complementary approach for monitoring known lineages using assays.

The B.1.1.7 strain exhibits 17 characteristic mutations including a del69-70 mutation in the S-gene binding domain. This mutation can be monitored using S-gene target of the Applied Biosystems TaqPath™ COVID-19 qPCR assay. Samples that are positive for the N-gene and Orf1ab targets of the multiplexed TaqPath™ assay with weak or no S-gene signal are classified as S-gene target failure (SGTF) samples. The del69-70 mutation has been observed in multiple variant lineages and is not unique to B.1.1.7. We report a set of TaqMan™ genotyping assays to identify B.1.1.7 cases based on discrimination between wild-type and mutant alleles that encode three variants in the spike protein. The genotyping assays described here can be used as follow-up assays to determine whether samples share other characteristic mutations that are more indicative of the B.1.1.7 strain – S.A570D and S.D1118H – as well as the S.N501Y mutation that is shared by many VOCs. The targeted genotyping approach can be performed in most labs that conduct SARS-CoV-2 testing using RT-PCR. Broad use of this and similar approaches can provide an expanded perspective on the prevalence of B.1.1.7 at lower cost and faster turn-around time than whole genome sequencing.

(1) https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563

PROTOCOL CITATION

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MATERIALS TEXT

1-Step TaqPath Multiplex Master Mix (No ROX), ThermoFisher CAT# A28523
TaqMan Genotyping Master Mix, ThermoFisher CAT# 4381657
TaqMan Custom Primer/Probe Mix for Alleles of Interest ThermoFisher CAT# 4332077 (See Table)

A	B	C
Base	Amino Acid	Assay ID
A_23063_T	N501Y	ANGZU27
C_23271_A	A570D	ANNK6DJ
G_24914_C	D1118H	ANPRYXG

TaqMan Custom Genotyping Assays

Synthetic RNA Template Controls 100c/uL, Twist Bioscience Cat# 102024 (Control 2; Wuhan-Hu-1) and Cat# 103907 (Control 14; England/205041766/2020 as a B.1.1.7 representative)
Molecular Grade Water (RNase Free)

DISCLAIMER:

This multiplexed protocol is still under development and is for research purposes only. It should not be used for clinical diagnosis. The intention of this assay is to screen for the probable presence of the B.1.1.7. Variant detection should be confirmed by sequencing.

This protocol has not been tested with other real-time PCR instruments and analytical software.

Assay Information

1

Disclaimer - FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Compatible Systems

Protocol has been tested using:
Applied Biosystems Quantstudio™ 7 (384-well format)
Applied Biosystems™ VIIA-7 (96-well format)

Compatible Software

QuantStudio™ Real-Time PCR Software v1.3 or later
Applied Biosystems™ Design and Analysis v2.4.3 or later

Assay Preparation 5m

2 Gather Supplies Needed for the Assay as listed in the Materials tab and:

384-Well PCR Plate
Optical Grade PCR plate adhesive film seal
384-Well cold block
Ice Bucket (ice)
Pipettes and Tips for p20 and p200 pipettes

Sample Preparation 10m

3 Locate and thaw on ice the extracted RNA samples to be tested. 5m

4 Label a sterile PCR Tube for each sample to be tested. 1m

4.1 Aliquot 20uL of Molecular Grade water into each labeled tube. 2m

4.2 Add 5uL of Extracted RNA into each labeled tube and triturate to mix. (avoid bubbles) 2m

Master Mix Preparation 5m

5 Label a sterile PCR Tube for each Allele of Interest. Keep on ice during preparation of Maser Mix. 1m

5.1 For 10 ul reactions performed in a 384-well plate, calculate the number of reactions to be performed including the controls and prepare a Master Mix for each Allele using the following components 4m

Component	Volume Per Sample	Master Mix Volume (+10% Overage)
Custom SNP Primer/Probe	0.5 µL	
TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X)	1 µL	
TaqMan™ Genotyping Master Mix (2X)	4 µL	

TaqMan Custom Primer/Probe mixes are shipped as 40x stocks. Dilute the stocks to a 20x working stock with sterile 1x TE buffer. Store the multiple aliquots at -15C to -25C in the dark. Thaw on ice prior to use.

Loading the PCR Plate 26m

6 Aliquot out 5.5uL of each Allele specific Master Mix into a Specific Quadrant of the 384 well plate for all samples. 10m

6.1 Add 4.5uL of Diluted Sample to each well containing Allele Specific Master Mix 10m

6.2 Aliquot 5.5uL of each Allele specific Master Mix into a Specific Quadrant of the 384 well plate for controls in Columns 23 and 24, leave columns 21 and 22 empty to avoid control contamination if possible. 2m

6.3 Add 4.5uL of Wuhan-Hu-1 control (WT), B.1.1.7 control (VAR), or a 50:50 mixture of the two controls to serve as a "heterozygous" equivalent control (100c/ul) for each Allele specific Master Mix. Use Molecular Grade Water as a no-template control. 2m

6.4

Example Plate Setup

6.5 Seal the PCR plate with an optical adhesive film 2m

Vortex the plate for 3 seconds

Spin down the plate at 2000rpm for 1 minute

Running on QS7 1h 40m

7 Run the PCR with the following Cycling Conditions 1h 40m

PCR Protocol			
Step	**Temperature (°C)**	**Time**	**Number of Cycles**
Reverse Transcription	50	5 min	1
Pre Run Read	60	30 sec	1
Activation	95	10 min	1
Denaturation	95	15 sec	40
Anneal/Extension*	60	1 min	
Post Run Read	60	30 sec	1
*Make sure to select the "data collection" image on the "Anneal/Extension" step.			

Analysis Settings 10m

8 Results are interpreted by the QuantStudio™ Real-Time PCR Software v1.3 10m

Open the run file and Navigate to Analysis/Analysis Settings/ CT Settings

Ensure the following analysis settings are applied

SNP Assay Name	Allele 1	Allele 1 Threshold	Allele 2	Allele 2 Threshold	Baseline Start	Baseline End
N501Y	N501WT	0.04	N501Y	0.04	AUTO	AUTO
D1118H	D1118WT	0.04	D1118H	0.04	AUTO	AUTO
A570D	A570WT	0.04	A570D	0.04	AUTO	AUTO

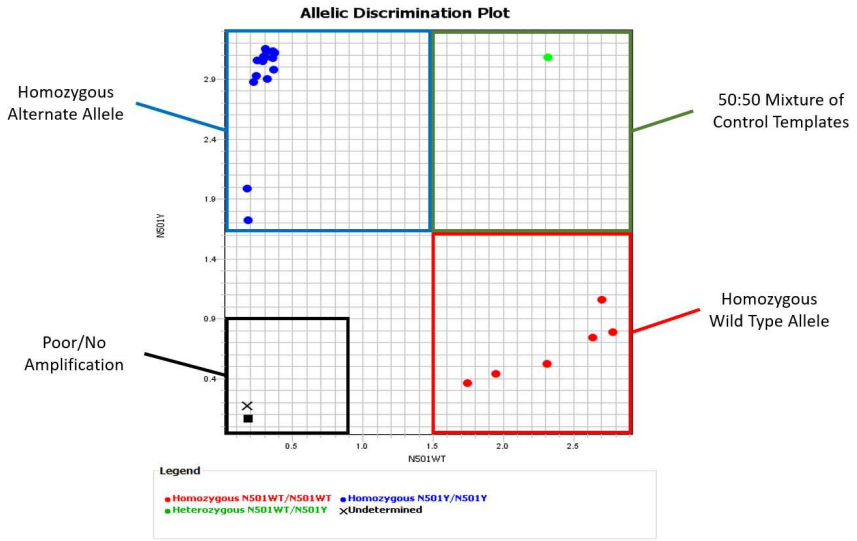
Navigate to Analysis/Analysis Settings/Call Settings

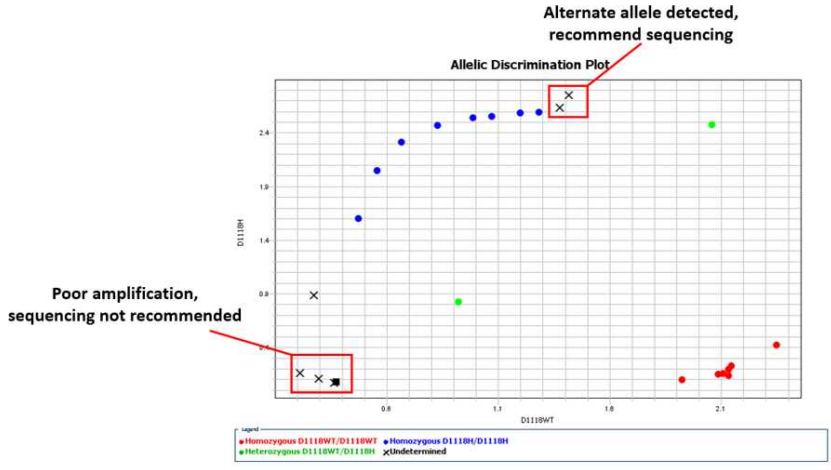
Click "Edit default Settings" and change the "Quality Value" to 90

Interpreting Results 10m

9 10m

Result	Criteria
Likely B.1.1.7	2 or more Alternate Alleles Detected
Possible Other Lineage	N501Y Alternate Allele Detected with remaining Alleles Undetermined/WT
Inconclusive	1 WT Allele with remaining Alleles Undetermined
Likely WT	2 or More WT Alleles Detected
Failed	All Alleles Undetermined





Manual Interpretation of Undetermined Calls.