SYBR green RT-PCR assay for the surveillance of SARS-CoV-2 variants of concern V.3

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Version 3

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2 *Works for me* dx.doi.org/10.17504/protocols.io.butznwp6

Coronavirus Method Development Community LATED

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ABSTRACT

The emergence of SARS-CoV-2 variants with multiple shared mutations has been described to be more transmissible and could affect COVID-19 morbidity and mortality. Some of these variants, known as B.1.1.7 (originally described in the United Kingdom), B1.351 (originally described in South Africa), and P.1 (originally described in Brazil) have rapidly become dominant within their countries and require a vigorous public health response. Whole Genomic sequencing remains the gold standard method to identify the SARS-CoV-2 variant, even though this approach is laborious, time-consuming, and expensive. Here, we have developed a fast and simple SYBR green-based real-time RT-PCR assay that identifies a distinct signature affecting the Non-Structural Protein 6 (NSP6), a nine-nucleotides deletion leading to amino acid losses: Δ106S, Δ107G, and Δ108F. This NSP6 signature is present in the variants of concern (VOC) described above (B.1.1.7; B1.351; P.1) and has not been detected in other SARS-CoV-2 lineages. The new version has corrections made on the master mix and authorship.

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PROTOCOL CITATION

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MATERIALS TEXT

- Go Taq 1-step RT-qPCR System (Promega) A2060

GoTaq OneStep RT qPCR System Quick Protocol FB127.pdf

- Nuclease-free water
- Primers

A	B
	NSP6: ΔS106; ΔG107 e ΔF108
Primer name	Sequence
Forward	5' -TGGTTGGATATGGTTGATACTAGTT-3'
Reverse	5'-AGCTGATGCATACATAACACAGT-3'

Primers details

- Positive controls: (1) SARS-CoV-2 RNA from virus culture of the Brazilian variant P2 and (2) SARS-CoV-2 RNA from virus culture of the Brazilian variant P1. The SARS-CoV-2 RNA was kindly provided by the Respiratory Virus and Measles Laboratory – IOC (LVRS-IOC).
- Applied Biosystems™ 7500 Real-Time PCR Systems (Thermo Fischer Scientific)
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DISCLAIMER:

> This SYBR green RT-PCR protocol is for research purposes only. It should not be used for clinical diagnosis. The intention of this assay is to screen for the presence of a distinct signature affecting the Non-Structural Protein 6 (NSP6) ΔS106; ΔG107 e ΔF108, present in high-virulent SARS-CoV-2 variants

1 Briefly vortex and centrifuge reagents before use.

2 Prepare 10 µM working stocks of the primers.

3 Thaw the GoTaq® qPCR Master Mix (Promega), Nuclease-Free Water, and primers working stocks.

4 Use the 10 µM working stocks to prepare the mix, containing:

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A	B	C	D
	Volume (per reaction)	final concentration	
GoTaq® qPCR Master Mix, 2X	7.5 µl	1x	
GoScript™ RT Mix for 1-Step RT-qPCR (50X)	0.3 µl	1x	
Primer Forward	0.15 µl	0.1 µM	
Primer Reverse	0.15 µl	0.1 µM	
Nuclease-free water	1.9 µl		

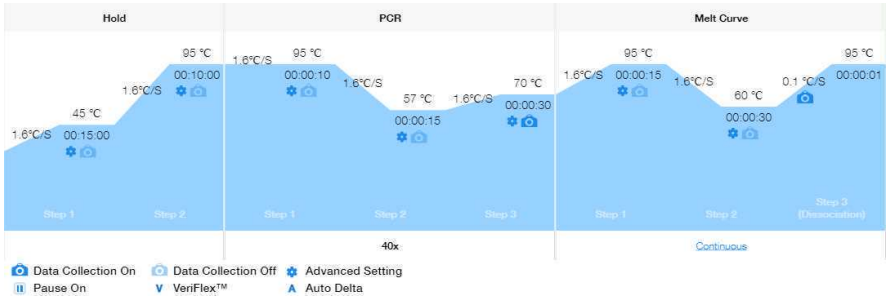
5 Add 10µl to each weel or PCR tube.

6 Add 5 µl of RNA from positive controls 1 and positive control 2 to the correspondent well or PCR tube. Add 5 µl nuclease-free water to the negative control well or PCR tube. Mix by pipetting.

7 Add 5µl of extracted RNA (unknown sample) to the designated wells and mix by pipetting.

8 Seal plates and tubes.

9 Run the PCR with the following cycler conditions



7500 Real Time PCR System

ABI **4351104**

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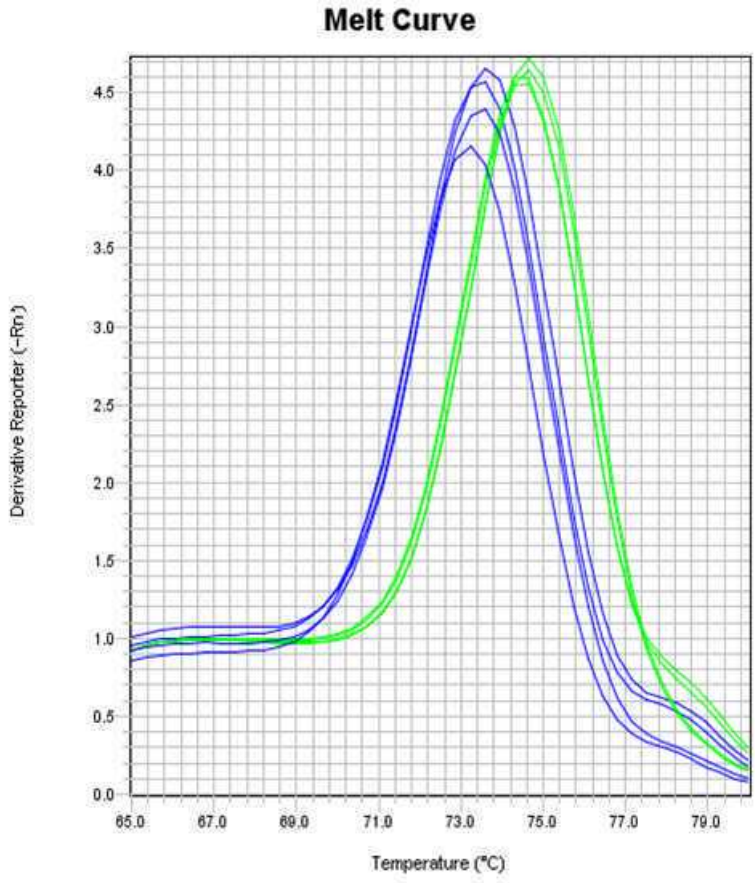
10 Data Evaluation



Blue - mutant ΔS106; ΔG107 e ΔF108
Green - wild type

Results Interpretation:

A	B	C
	Size of the fragment	**Tm**
NSP6: ΔS106; ΔG107 e ΔF108	60 bp	<73,99°C
NSP6: Wild type	69bp	≥74°C

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