



Version 2

May 05, 2021

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

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Works for me

dx.doi.org/10.17504/protocols.io.bur7nv9n

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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), B.1.351 (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: $\Delta 106S$, $\Delta 107G$, and $\Delta 108F$. This NSP6 signature is present in the variants of concern (VOC) described above (B.1.1.7; B.1.351; P.1) and has not been detected in other SARS-CoV-2 lineages.

DOI

dx.doi.org/10.17504/protocols.io.bur7nv9n

PROTOCOL CITATION

Pedro Cardoso, Fernando do Couto Motta, Marilda Agudo Mendonça Teixeira de Siqueira, Daniela Tupy de Godoy, Rodrigo Brindeiro, Monica Barcellos Arruda, Elisabete Andrade, Marisa Ribeiro, Marcela Fontana-Maurell, Elaine Costa, Daniele Rocha, Patricia Alvarez 2021. SYBR green RT-PCR assay for the surveillance of SARS-CoV-2 variants of concern. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bur7nv9n>
Version created by Patricia Alvarez

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CREATED

May 05, 2021

LAST MODIFIED

May 05, 2021

PROTOCOL INTEGER ID

49695

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)
-

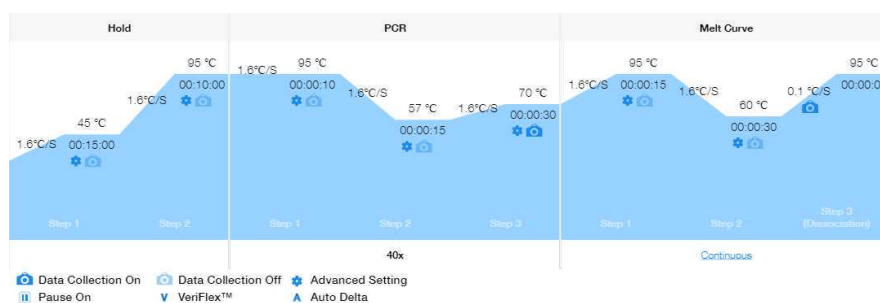
DISCLAIMER:

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

- Briefly vortex and centrifuge reagents before use.
- Prepare 10 μM working stocks of the primers.
- Thaw the GoTaq® qPCR Master Mix (Promega), Nuclease-Free Water, and primers working stocks.
- Use the 10 μM working stocks to prepare the mix, containing:

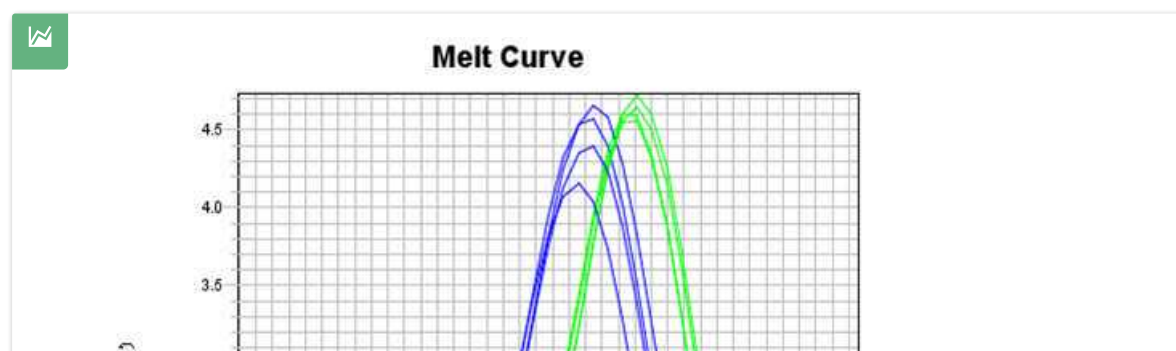
| A | B | C |
|---|-----------------------|---------------------|
| | Volume (per reaction) | final concentration |
| GoTaq® qPCR Master Mix, 2X | 7.5 μl | 1x |
| GoScript™ RT Mix for 1-Step RT-qPCR (50X) | 0.3 μl | 1x |
| Mix of Primers Forward and Reverse | 2.2 μl | 0.1 μM |

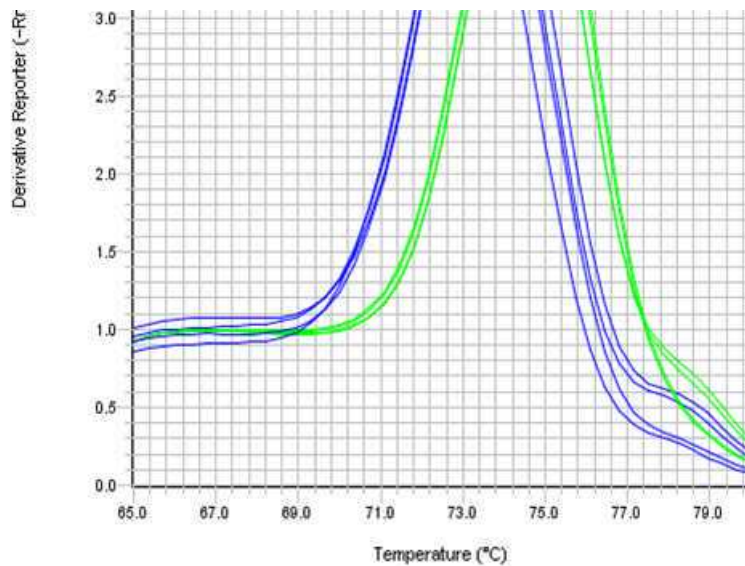
- 5 Add 10µl to each well or PCR tube.
- 6 Add 5 µl of RNA from positive controls 1 and positive control 2 to the correspondent well or PCR tube. Add 5 µl nuclease-free water to the negative control well or PCR tube. Mix by pipetting.
- 7 Add 5µl of extracted RNA (unknown sample) to the designated wells 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

10 Data Evaluation





Blue - mutant $\Delta S106$; $\Delta G107$ e $\Delta F108$
 Green - wild type

Results Interpretation:

| A | B | C |
|---|----------------------|----------|
| | Size of the fragment | Tm |
| NSP6: $\Delta S106$; $\Delta G107$ e $\Delta F108$ | 60 bp | <73,99°C |
| NSP6: Wild type | 69bp | ≥74°C |