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May 30, 2022

VOC and VOI (SARS-Cov-2) identification by Sanger Sequencing V.1

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dx.doi.org/10.17504/protocols.io.ewov1nxqkgr2/v1

Rede Covid FIOCRUZ-PE

Laís Ceschini

The method hereby described is an update of a rapid and accessible protocol based on Sanger sequencing that is able to discriminate the main SARS-CoV-2 VOCs (Variants of Concern) and VOIs (Variants of Interest), according to each characteristic mutational signature at the Spike receptor binding domain (RBD) and an additional mutational profile of the N-terminal domain (NTD) of the Spike protein. Although this approach does not substitute whole-genome sequencing, in a scenario that combines the rapid spread of new VOCs around the world with supply shortages and lack of technical infrastructure, it represents a powerful tool that allows a broader network of laboratories to perform molecular surveillance of SARS-CoV-2 VOCs, improving its capacity to report more results within in a timely manner.

DOI

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Laís Ceschini, Matheus Filgueira Bezerra, Viviane do Carmo Vasconcelos de Carvalho, Cássia Docena, Gabriel Luz Wallau, Marcelo Henrique Santos Paiva 2022. VOC and VOI (SARS-Cov-2) identification by Sanger Sequencing . **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov1nxqkgr2/v1

CNPq
Grant ID: 303902/2019-1

genotyping, RNA virus, Spike protein, molecular assay, screening

protocol ,

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cDNA Synthesis 2h 15m

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The cDNA was prepared according to the manufacturer's instructions: High-Capacity cDNA Reverse Transcription Kit

Applied BiosystemsTM High-Capacity cDNA Reverse Transcription Kit **Applied Biosystems Catalog #4368814**

Mix the following components in an 0.2mL 8-strip tube or 96 well PCR plate;

Component	Value
10X RT B uffer	**2.0 µL**
dNTP Mix (100mM)	**0.8 µL**
10X RT Random primers	**2.0 µL**
MultiScribe Reverse Transcriptase	**1.0 µL**
H20	**4.2 µL**
Template RNA	**10.0 µL**
Total	**20.0 µL**

2h 15m

2

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Incubate the reaction as follows:

Time	Temperature
00:10:00	at 25 °C
02:00:00	at 37 °C ,
00:05:00	at 85 °C
Hold at	4 °C

Primers sequences

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Primer sets targeting the Spike residue binding domain (RBD).

A	B	C	D
Primer set	Flanked region*	Amplicon	Covered mutations
Artic primers 76 Left 5'-AGGGCAAACTGGAAAGATTGCT-3' 77 Right 5'-CAGCCCCTATTAAACAGCCTGC-3'	22819-23500	725 bp	N439K, L452R/Q, Y453F, S477N, T478K, E484K/Q, S494P, N501Y/T/S, A570D, D614G
In house 1MS Fw 5'-TAACGCCACCAGATTTGCAT-3' 2MS Rv 5'-ACACGCCAAGTAGGAGTAAGT-3'	22607-23446	878 bp	K417T/N, N439K, L452R/Q, Y453F, S477N, T478K, E484K/Q, S494P, N501Y/T/S, A570D, D614G

*not including the primer binding site.

Primer set targeting the Spike N-terminal domain (NTD).

A	B	C	D
Primer set	Flanked region*	Amplicon	Covered mutations
Artic primers 71 Left 5'-ACAAATCCAATTCAGTTGTCTTCCTATTC-3' 73 Right 5'- CACCAGCTGTCCAACCTGAAGA-3'	21386-22324	989 bp	Δ69-70/144-145, Δ157-158, 241-243, S13I, L18F, T19R, T20N, P26S, Q52R, A67V, V70F, G75V, T76I, D80A, T95I, D138Y, W152C, E156G, R190S, D215G, A222V, W258L.

*not including the primer binding site.

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PCR amplification 11m 55s

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The PCR was performed under conditions standardized using the
Taq Platinum DNA Polymerase **Invitrogen - Thermo Fisher**

Mix the following components in an 0.2mL 8-strip tube or 96 well PCR plate;

Component	Value
10x Buffer	**2.5 µL**
MgCl2	**0.5 µL**
dNTP (10 mM)	**1.0 µL**
Forward primer (10uM)	**0.5 µL**
Reverse primer (10uM)	**0.5 µL**
Taq Polymerase	**0.25 µL**
H2O	**18.25 µL**
cDNA input	**1.5 µL**
Total	**25 µL**

5 Incubate the reaction as follows: 11m 55s

Step	Time	Temperature	Cycle
Initial denaturation	**00:05:00**	**98 °C**	1x
Denaturation	**00:00:30**	**98 °C**	35x
Annealing	**00:00:35**	**59 °C**	35x
Extension	**00:00:50**	**72 °C**	35x

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Final extension	00:05:00	72 °C	1x
Hold	Indefinite	4 °C	

Electrophoresis, quantification and Sequencoing

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- Agarose gel was prepared at [M]0.1 mg/mL and stained with Sybr Safe (Sigma-Aldrich).

- PCR products was quantified using

Nanodrop 2000C

Thermo Scientific **TSC-ND2000C**

(1uL per sample) and diluited to a final concentration of 30 ng/uL.

- Sequencing reaction is performed with BigDye Terminator v3.1 (Applied Biosystems) and run in capillary electrophoresis (ABI 3500, Applied Biosystems), according to the manufacturer's instructions.

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