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

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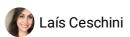
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The method hereby described is an update of a rapid and accessible protocol based on Sanger sequencing that is able to discriminate the main SARS-CoV-2 VOCs (Variants of Concern) and VOIs (Variants of Interest), according to each characteristic mutational 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.

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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

Incubate the reaction as follows:

Temperature Time

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2

2

© 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).

Α	В	С	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).

Α	В	С	D
Primer set	Flanked	Amplicon	Covered mutations
	region*		
Artic primers 71 Left 5'-	21386-	989 bp	∆ 69-70/144-145, ∆ 157-158, ⋈
ACAAATCCAATTCAGTTGTCTTCCTATTC-3' 73	22324		№241-243, S13I, L18F, T19R,
Right 5'- CACCAGCTGTCCAACCTGAAGA-3'			T20N, P26S, Q52R, A67V,
			V70F, G75V, T76I, D80A, T95I,
			D138Y, W152C, E156G,
			R190S, D215G, A222V,
			W258L.

^{*}not including the primer binding site.

PCR amplification

11m 55s

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The PCR was performed under conditions standardized using the

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
H20	⊒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	8 98 °C	1x
Denaturation	© 00:00:30	8 98 °C	35x
Annealing	© 00:00:35	8 59 °C	35x
Extension	© 00:00:50	8 72 °C	35x
Final extension	© 00:05:00	8 72 °C	1x
Hold	Indefinite	8 4 °C	

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Electrophoresis, quantification and Sequencing

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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.