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Geneva, December 26th, 2020

Protocol for specific RT-PCRs for marker regions of the Spike region indicative of the UK SARS-CoV-2 variant B.1.1.7 and the South African variant 501Y.V2

Primer name	Sequence (5'-3')
F44	TCTCTTCTTAGTAAAGGTAGACTT
F46	CCTTCACTGTAGAAAAAGGAATC
F47	TATCAGGCCGGTAGCACAC
R45	CTAACAATAGATTCTGTTGGTTG
R44	GAATAAACTCTGAACTCACTTTCC
R47	CATATGAGTTGTTGACATGTTCAG
R46	TGGAAACCATATGATTGTAAAGGA

Table 1. Primer sequences

PCR assay 1 (Deletions):

PCR product of the 1st and 2nd round cover the following deletions:

- 69-70 del
- Y144del

1st round assay 1: Primer combination F44-R45 (Product size: 1071 bp)
Primers used for Sanger sequencing: F44/R45

2nd round (hemi-nested) assay 1: Primer combination F44-R44 (Product size: 570 bp)
Primers used for Sanger sequencing: F44/R44

PCR assay 2 (Mutations):

PCR product of the 1st round cover the following mutations:

- K417N*
- E484K*
- N501Y
- A570D
- D614G**

Note: *these mutations are described in the South African SARS-CoV-2 lineage 501Y.V2 but not the in the UK variant VOC 202012/01, B1.1.7.
**this mutation is not specific for the UK variant nor South African variant but found in most recent strains

PCR product of the 2st round (hemi-nested) Nr.1 cover the following mutations:

- K417N
- E484K

PCR product of the 2nd round (hemi-nested) Nr. 2 cover the following mutations:

- N501Y
- A570D
- D614G

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1st round, assay 2:
Primer combination F46-R47 (Product size: 1068 bp)
Primers used for Sanger sequencing: F46/R47

2nd round (hemi-nested) Nr. 1, assay 2:
Primer combination F46-R46 (Product size: 580 bp)
Primers used for Sanger sequencing: F46-R46
*not found in the UK variant but in the SA variant

2nd round (hemi-nested) Nr. 2, assay 2:
Primer combination F47-R47 (Product size: 565 bp)
Primers used for Sanger sequencing: F47-R47

Master Mix RT

	1x	
H2O RNAse free	14.375	
FSB5X	5	
DTT 100mM	2.5	
Random primers (192 ng)	1.375	
dNTP (25mM)	0.5	
Rnasin	0.625	
Superscript II	0.625	
	25.0 µl	**+ 25 µl RNA**

Final concentrations:
75 mM KCL
50 mM Tris-HCL pH 8.3
10 mM DTT
3 mM MgCl2
20 U RNAsine
0.5 mM dNTP
192 ng random primer
100 U SuperScript

Temperature protocol:
5' 50°C
10' 25°C
1 h 42°C
15' 70°C
4°C forever

Master Mix Round 1

	1x	
H2O-Rnase free	26.7	
Buffer PCR II 10X	4.25	
MgCl2 (25mM)	6	
Glycerol 10%	5	
dNTP (25mM)	0.3	
Amplitaq polymerase	0.25	
F primer	1.25	
R primer	1.25	
	45.0 µl	**+ 5µl cDNA**

Final concentrations:
50 mM KCl
13.5 mM Tris-HCl pH 8.3
3.3 mM MgCl2
1% Glycerol
0.2 mM dnTP
250 nM primers
1.25 U Amplitaq

Master Mix Round 2 (hemi-nested PCR)

	1x	
H2O-Rnase free	31.65	
Buffer PCR II 10X	5	
MgCL2 (25 mM)	4	
dNTP (25mM)	0.4	
Amplitaq polymerase	0.25	
F primer	1.25	
R primer	1.25	
	45 µl	**+ 5 µl product PCR 1**

All primer concentrations: 10 µM

Temperature protocol for 1st and 2nd round PCR:

35 cycles : 3 min 95°C
20 sec 95°C
20 sec 55°C
30 sec 72°C

followed by :

3 min 72°C
4°C forever

Sequencing of obtained PCR products by Sanger sequencing

For low Ct values/high viral load, 1st round of PCR is sufficient to obtain a PCR product that is suitable for sequencing, for high Ct values/lower viral load, 2nd round (nested) PCR is necessary.

Important note:

This is a preliminary protocol that has not been validated for optimal PCR conditions.

This protocol can identify several marker mutations of both variants, but for final confirmation of the variant complete genome sequencing should be performed.