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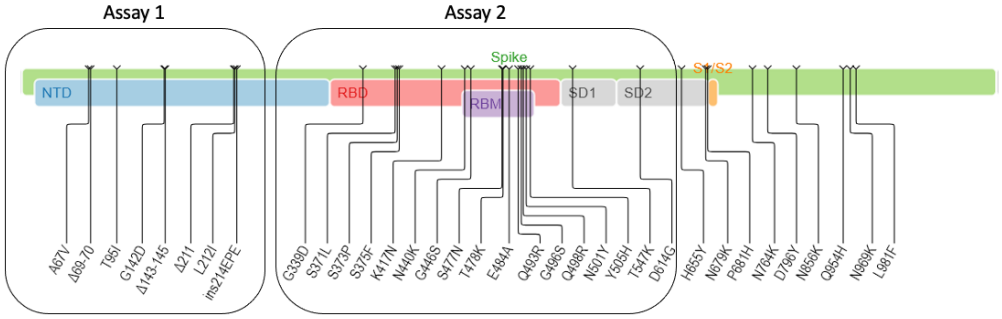
# Protocol for specific RT-PCRs for marker regions of the Spike indicative of the Omicron variant (B.1.1.529)

This is an amplification protocol for a single-round PCR followed by Sanger sequencing to amplify and sequence two partial regions of the S (Spike) gene that covers mutations indicative of the Omicron variant. The protocol can be used to quickly gather information of an Omicron suspicion due to the high number of specific mutations. Optimally, full-genome sequencing should be performed for confirmation of the full-length sequence.




**Overview of Spike deletions/mutations indicative of Omicron covered by this protocol**
Source : Mullen J, Tsueng G, et al. https://covdb.stanford.edu/page/mutation-viewer/#omicron Outbreak.info B.1.1.529 Lineage Report b-1-1-529_1638019891935.svg

| Primer name | Sequence (5'-3') |
|---|---|
| *Assay 1* | |
| F44 | TCTCTTCTTAGTAAAGGTAGACTT |
| R45 | CTAACAATAGATTCTGTTGGTTG |
| *Assay 2* | |
| F46 | CCTTCACTGTAGAAAAAGGAATC |
| R47 | CATATGAGTTGTTGACATGTTCAG |

Table 1. Primer sequences

**PCR assay 1:**
Primer combination **F44-R45** (Product size: around 1070 bp)
Primers used for Sanger sequencing: **F44/R45**

PCR product of assay 1 covers the following deletions/mutations indicative of Omicron:

- A67V
- Del 69-70*
- T95I
- G142D
- Del 143-145
- Del 211
- L212I
- Insert 214 (EPE)

*this mutation is not specific for Omicron but can be found in other currently circulating variants (e.g. Delta VOC)

**PCR assay 2:**
Primer combination **F46-R47** (Product size: around 1070 bp)
Primers used for Sanger sequencing: **F46-R47**

PCR product of assay 2 covers the following mutations indicative of Omicron:

- G339D
- S371L
- S373P
- S375F
- K417N
- N440K
- G446S
- S477N
- T478K
- E484A
- Q493R
- G496S
- Q498R
- N501Y
- Y505H
- T547K
- D614G

**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 PCR**

| | 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

All primer concentrations: 10 µM

Temperature protocol 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 infinite

Sequencing of obtained PCR products by Sanger sequencing

**Sequencing** (ABI 3500XL)

2 ul BigDye sequencing + 1 ul de 5x sequencing buffer (BigDye terminator version 1.1 cycle sequencing kit, Ref : 4336774)
1.6 ul primer 1pmol/ul
1 to 5 ul of purified PCR
H2O Rnase Free up to 10 ul
Sequencing cycles:
1 min 96°C ; 10 sec 96°C, 5 sec 50°C, 35 sec 60°C : 25 cycles ; 4°C forever

A PCR product can be obtained approximately up to a Ct value of 30.

**Important note:**

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

**This protocol can identify several marker mutations for Omicron, but for final confirmation of the variant complete genome sequencing is recommended.**

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**On behalf of the Geneva Centre for Emerging Viruses**

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