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We use this protocol and it's working

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SARS-CoV-2 Spike Gene N terminal Domain targeted Sequencing

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ABSTRACT

We use a simple and effective method for generating 757bp of the N terminal domain of thetheSARS-CoV-2 Spike gene for variant surveillance,

RNA Extraction

1 The Automated extraction was handled using the ExiPrep™ 96 Lite (A-5250, BIONEER) with the ExiPrep™ Viral DNA/RNA extraction kit (K-4614, BIONEER).

RT-PCR Amplification

2 TaqPath™ COVID-19 CE-IVD RT-PCR Kit (Multiplex real-time RT-PCR test intended for qualitatively detecting nucleic acid from SARS-CoV-2) used for viral detection. follow the user manual recommendation as listed in the following link: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0019215_TaqPathCOVID-19_CE-IVD_RT-PCR%20Kit_IFU.pdf

3 Results can be distinguished to:
1- samples with three positive targets for (ORF1ab, N, and S genes)
2- sample with two positive targets for (ORF1ab and N genes), negative for theS gene. Failure of Spike gene amplification is referred to as S gene target failure (SGTF) or S gene signal dropdown.
3- SGTF resulted due to 69/70 codons deletion of Valine and Histidine, respectively.

C-CDNA Synthesis and Quality checking

4 Promega GoScript™ Reverse Transcription Mix with Random Primers system (A2800) is used to generate complementary DNA. following the same kit-recommended procedure.

5 Quality checking is considered for all steps using the Fluorometer Quantus using Quantifluor dye

Primers

6 the forward primer is: SubA_21587F: CCACTAGTCTCTAGTCAGTGTGTT

Reverse primer: SubA_22344R: CCAGCTGTCCAACCTGAAGA

these primers generate an amplicon of 757bp.

6.1 Primer's preparation:
These primers were supplied by Macrogen Company in a lyophilized form. Lyophilized primers were dissolved in nuclease-free water to give a final concentration of 100pmol/µl as a

stock solution. A working solution of these primers was prepared by adding 10µl of primer stock solution (stored at freezer -20 C) to 90µl of nuclease-free water to obtain a working primer solution of 10pmol/µl.

Reagent preparation for Amplicon synthesis

7 Amplification reaction carried on using the flowing calculations:

1- 10 ul of GoTag Green Master Mix, Promega (**M7122**).
2- 1 ul of Forward primer
3- 1ul of Reverse primer
4- 6 ul of nuclease-free water
5- 2ul of cDNA template.

PCR adopted program and

8 The following program was considered for amplification:

Steps	°C	m: s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	40
Annealing	60	00:30	
Extension	72	01:00	
Final extension	72	07:00	1
Hold	10	10:00	

Gel visualization

9 We use the classic gel visualization method through gel electrophoresis (100-1500 bp ladder gel marker) and gel documentation.

Sequencing

10 We referred our amplicons to a sequencing company (Macrogen, South Korea).