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Protocol status: Working
We use this protocol and it's working

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SARS-CoV-2 Receptor Binding Domain Deoxy Fragment Sequencing

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ABSTRACT

Since first reporting of SARS-CoV-2 case in China, there were urgent need to report and monitor emerging variants and keep track circulating mutation, in this methodology, we used simple, cost-effective method to monitor the circulating mutations which could be used to predict the SARS-CoV-2 variants based on accumulating mutations in the receptor binding domain.

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RNA Extraction

1 The RNA of SARS-CoV-2 extracted using Automated Extraction System ExiPrep™ 96 Lite from Bioneer (South Korea) using the Exiprep 96 Viral DNA/RNA kit using the standard recommended procedure by the manufacturer.

cDNA synthesis

2 The extracted RNA used as a template to construct complementary DNA, cDNA synthesis considered using the GoScript™ Reverse Transcription Mix, Random Primers, Promega (USA) using manufacturer recommended procedure. this will ensure synthesis of the first DNA strand only.

3 Concentration of synthesized DNA measured by Quantus Fluorometer (Promega, USA).

Amplicon Amplifications

4 RBD specific primers, amplification regions, and other details listed below:

Intended Procedure	Primers ID	Sequence	Range (From – to)	Amplicon Size bp	References
Set2: Amplification of RBD region of Spike gene	SubA_22440F Forward	TTGACCCTCTCTCAGAAACAAAG	22440 - 22462	836	https://www.protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bh42j8ye
	SubA_23463R Reverse	TGTCAKCAATGTCTCTGCCAAAT	23254 - 23276		https://www.protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bh42j8ye

This set of forward and reverse primers is designed as part of NGS primer pool, the current procedure chooses one set to generate a different length that it designed for that is sufficient to detect all basic mutations in the receptor binding domain that uses frequently for variants discriminations.

5 Reaction setups include:

1- GoTag Green Master Mix (Promega, USA)

2- Ladder gel Marker (100-1500), (Promega, USA)

the following program used for amplification:

Steps	°C	Duration	Cycle
Initial Denaturation	95	5:00 minutes	1
Denaturation	95	30 seconds	40
Annealing	60	30 seconds	40
Extension	72	30 seconds	40
Final extension	72	7:00 minutes	1
Hold	10	10:00 minutes	1

Amplicon Sequencing

6 Amplicon Sequencing referred to Macrogen, South Korea to be sequenced using the ABI3730XL sequencing Machine.