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SARS-CoV-2 Receptor Binding Domain Deoxy Fragment Sequencing

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Noor Saber Jawad[1], Nuha Joseph Kandala[1]

[1]University of Baghdad

Noor Saber Jawad: Currently Mustansiriyah University

Noor Saber Jawad
Mustansiriyah University

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

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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.

Troubleshooting

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.