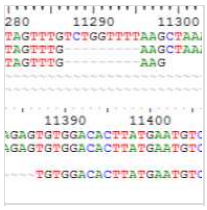

Nov 30, 2021

RT–PCR protocol for the detection ORF1 11288–11296 deletion (NSP6 106-108del) in SARS-CoV-2 genome

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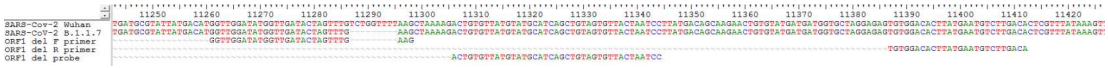
ORF1 deletion is observed in some new SARS-CoV-2 lineages and is lineage defining for B.1.1.7 and P.1. Biological effect of this mutation is still unknown. Developed RT-PCR was tested on numerous samples of lineages B.1.1.7, B.1.351, P.1 (validated by sequencing) and samples without this mutation with no false-postive results.



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SARS-CoV-2, ORF1 deletion, 11288–11296, ORF1del PCR

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Order oligonucleotides with following sequences:

A	B
Name	Sequence (5’ –> 3’)
ORF1-del-F	GGTTGGATATGGTTGATACTAGTTTGAAG
ORF1-del-R	TGTCAAGACATTCATAAGTGTCCACA
ORF1-del-P	Cy5.5-ACTGTGTTATGTATGCATCAGCTGTAGTGTTACTAATCC-BHQ3

2 Briefly vortex and centrifuge reagents before use.
Prepare the PCR reaction mixture following the specifications below:

2X RT-PCR Buffer	12.5 µl
25X RT-PCR Enzyme Mix	1 µl
ORF1-del primers	600 nM each
ORF1-del probe	400 nM
RNA template	5 µl
Nuclease-free water	to 25 µl

AgPath-ID™ One-Step RT-PCR Reagents was used in our case

Use SARS-CoV-2 positive samples as the template. In the case of screening of unknown samples use multiplex RT-PCR with some oligonucleotides for detection SARS-CoV-2 as a control reaction. This primer set for SARS-CoV-2 detection can be added to the test without decrease of sensitivity of the ORF1 deletion test:

A	B
Name	Sequence (5’ – 3’)
2-SARS-CoV-2-ORF-1-F	AGAGCTATGAATTGCAGAC
2-SARS-CoV-2-ORF-1-R	GGGAAATACAAAATTTGGACA
2-SARS-CoV-2-ORF-1-P	FAM-AATTGGCAAAGAAATTTGACACCTTCA-BHQ-1

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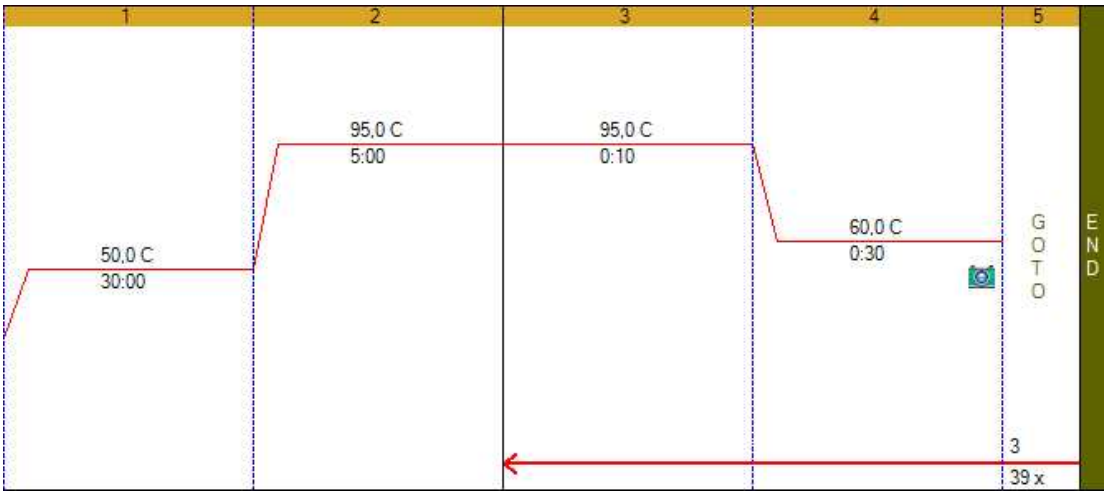
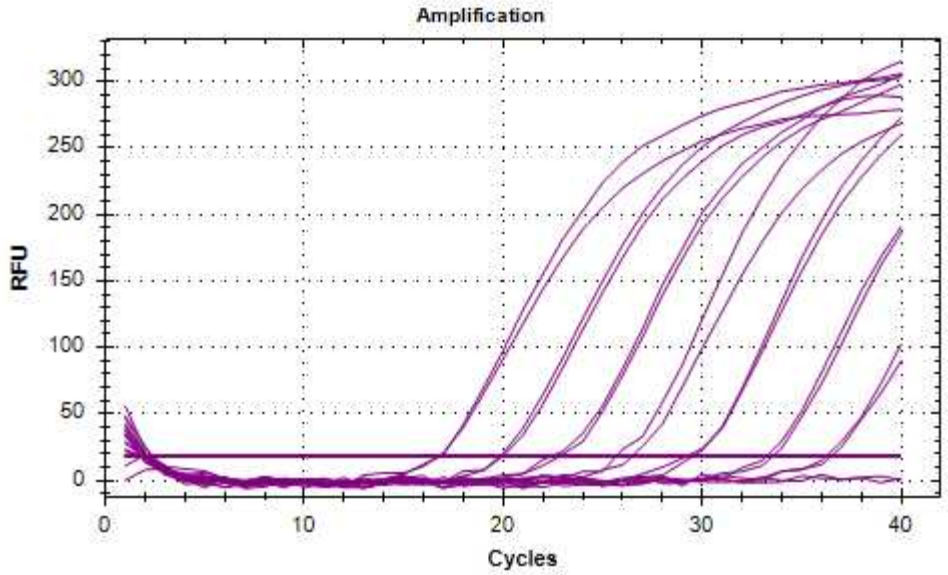
3 Perform the amplification in a general thermocycler with appropriate temperature profile:



Read plate at the annealing and elongation step. Developed PCR was validated for Bio-Rad CFX96, but is believed to work well at any thermocycler.

4 Interprete the results: detection of fluorescence probe “ORF1-del-P” (Cy5 channel in our case) means presence of ORF deletion 11288–11296



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