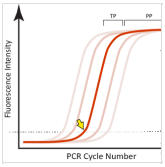

Jun 24, 2020

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR N gene 2020

Forked from Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR N gene 2020 (Wuhan-N; 2019-nCoV-related test) -NOT RECOMMENDED

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1 Works for me dx.doi.org/10.17504/protocols.io.bhpwj5pe

Public Health Virology, Forensic and Scientific Services | Coronavirus Method Development Community

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ABSTRACT

- A real-time RT-PCR to designed to detect SARS-CoV-2 and other related sarbecoviruses. Based on sequence MN908947 made available by Professor Yong-Zhen Zhang, Fudan University, Shanghai, China.
- The target region encodes the nucleocapsid (N).
- Tested on wild-type SARS-CoV-2 virus , it is expected to be capable of detecting SARS-CoV-2, bat-like SARS and SARS virus (members of the subgenus *Sarbecovirus*).
- Limit of detection not yet determined.
- The performance of the assay has not been tested with low viral load samples or samples from patients who are clinically well.
- The sensitivity of this assay was improved with the use of the SensiFast™Probe Lo-ROX One-step kit.
- A single 1 mismatch at probe-binding site identified with the *BetaCoV/USA/CA1/2020|EPI_ISL_406034* (GenBank MN994467.1) variant of SARS-CoV-2 (as of 23JUNE2020).
- Probe is in the 3'-5' (reverse complement) direction.
- Reverse primers were replaced in March 2020.
- We also recommend the ORF1ab assay (Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR ORF1ab 2020), US-CDC-N1 assay or the E gene assay by Corman *et al.* (Protocol v2-1)

Notes:

- Assay is optimised.
- This test has identified clinical positive cases of coronavirus disease (COVID-19)

DOI

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PROTOCOL CITATION

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FORK FROM

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KEYWORDS

CoV, coronavirus, Wuhan, Real-time, RT-PCR, PCR, virus, China, 2019-nCoV, pneumonia, seafood market, WSMPV, Sarbecovirus, SARS-CoV-2, COVID-19

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GUIDELINES

- If using a different brand or model of real-time thermocycler, check the concentration of ROX is adequate.
- Method assumes the user is familiar with the thermocycler and software used to run the protocol.

MATERIALS

NAME	CATALOG #	VENDOR
SensiFAST™ Probe Lo-ROX Kit	BIO-84002	Bioline

STEPS MATERIALS

NAME	CATALOG #	VENDOR
SensiFAST™ Probe Lo-ROX One-Step Kit	BIO-78001	Bioline

Mix

1 Oligonucleotides

Oligo Name	Sequence 5'-3'	Location based on NC_045512.2*
Wuhan-TM2020For	TCGTGCTACAACTTCCTCAAG	28743-28763
Wuhan-TM2020Probe	6FAM-CCGCCTCTGCTCCCTTCTGC-BHQ1	28809-28790
SARS2-28875R-G	CTGCCTGGAGTTGAATTTCTTG	28875-28854
SARS2-28875R-A	CTGCCTGGAGTTGAATTTCTTA	28875-28854

*GenBank accession NC_045512.2 Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1.

2 Reagents

SensiFAST™ Probe Lo-ROX One-Step Kit
by Bioline
Catalog #: BIO-78001

3 Synthetic controls

Synthetic controls are produced using the binary synthetic template oligonucleotide positive control for in-house diagnostic real-time RT-PCR method.

The oligonucleotide sequences required to make controls for this assay are:
Probe control:
AAAATAATACGACTCACTATAGGGTGAAGAGAATCCACAAGGAATTGAACCGCCTCTGCTCCCTTCTGCACAGTGTTCAGC
AGGTCCTGTTGAAAA
Primer control:

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AAAATAATACGACTCACTATAGGGTCGTGCTACAACTTCCTCAAGATGATCTGGCACGGGACCCTCCAAYAAGAAATTCAAC TCCAGGCAGAAAA

4 Reaction Set-up

- Assay has been designed to be used on both a Rotor-Gene 6000 / Rotor-Gene Q 5-plex using 100-place rotor discs and an ABI 7500 Fast real-time machine.
- Total reaction volume is 20µL.
- Prepare sufficient for number of reaction plus a 'dead volume' usually 2 extra. Adjust as necessary if using a robotic dispenser.

Reagent	Volume (µL) x1	Final reaction concentration
Nuclease free water	4.2	
Wuhan-TM2020F (200µM)	0.05	500nM
SARS2-28875R-G (200µM)	0.05	500nM
SARS2-28875R-A (200µM)	0.09	900nM
Wuhan-TM2020Probe (100µM)	0.01	50nM
2 X SensiFast Probe Lo-Rox One-Step mix*	10	1X
RiboSafe RNase Inhibitor*	0.4	
Reverse transcriptase*	0.2	
TOTAL VOLUME	15	

*SensiFast™ Probe Lo-ROX One-Step kit (BIO-78005)

Dispense 15µl to each reaction well.
Add 5µl of template, extracted RNA, controls or NTC (nuclease-free water).
Total reaction volume is 20µl.

Amplification

5 PCR amplification

1 cycle	**40 cycles**
50°C 5min	95°C 3 seconds
95°C 2min	60°C 30 seconds*

*Florescence acquisition step

Result Analysis

6 The definition used for a satisfactory positive result from a real-time fluorogenic PCR should include each of the following:

1. A sigmoidal curve – the trace travels horizontally, curves upward, continues in an exponential rise and followed by a curve towards a horizontal plateau phase
2. A suitable level of fluorescence intensity as measured in comparison to a positive control (y-axis)
3. A defined threshold (C_T) value which the fluorescent curve has clearly exceeded (Fig.1 arrow) and which sits early in the log-linear phase and is <40 cycles
4. A flat or non-sigmoidal curve or a curve that crosses the threshold with a C_T value >40 cycles is considered a negative result
5. NTCs should not produce a curve

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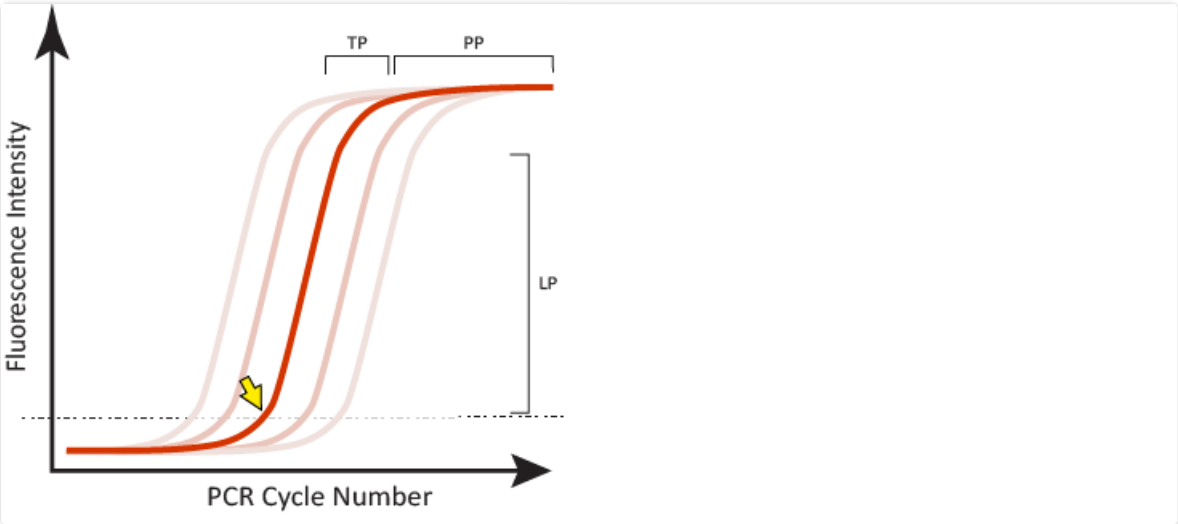

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary) threshold level as shown in this normalized linear scale depiction. LP-log-linear phase of signal generated during the exponential part of the PCR amplification; TP-a slowing of the amplification and accompanying fluorescence signal marks the transition phase; PP-the plateau phase is reached when there is little or no increase in fluorescent signal despite continued cycling.

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