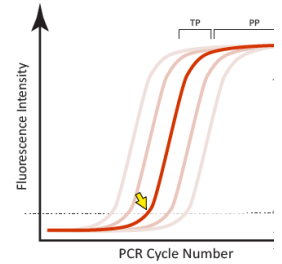




Jun 23, 2020

🌐 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



DOI

<https://dx.doi.org/10.17504/protocols.io.bhpwj5pe>

Judy Northill¹, Ian Mackay¹

¹Public Health Virology, Forensic and Scientific Services

Public Health Virology, F...

Coronavirus Method De...



Judy A Northill

Public Health Virology, Forensic and Scientific Services

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.bhpwj5pe>

Protocol Citation: Judy Northill, Ian Mackay 2020. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR N gene 2020 . protocols.io <https://dx.doi.org/10.17504/protocols.io.bhpwj5pe>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: June 18, 2020

Last Modified: June 23, 2020

Protocol Integer ID: 38358

Keywords: CoV, coronavirus, Wuhan, Real-time, RT-PCR, PCR, virus, China, 2019-nCoV, pneumonia, seafood market, WSMPV, Sarbecovirus, SARS-CoV-2, COVID-19, severe acute respiratory syndrome coronavirus, sars virus, other related sarbecovirus, coronavirus disease, members of the subgenus sarbecovirus, subgenus sarbecovirus, clinical positive cases of coronavirus disease, detecting sar, variant of sar, type sar, sar, low viral load sample, like sar, sequence mn908947, n1 assay, betacov

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)

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

MATERIALS

 SensiFAST™ Probe Lo-ROX Kit **Bioline Catalog #BIO-84002**

STEP MATERIALS

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

Protocol materials

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

 SensiFAST™ Probe Lo-ROX Kit **Bioline Catalog #BIO-84002**

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 **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:

AAAATAATACGACTCACTATAGGGTGAAGAGAATCCACAAGGAATTGAACCGCCTCTGCTCCTTCTGCACAGTGTTTCAGCAGGTCCTGTTGAAAA

Primer control:

AAAATAATACGACTCACTATAGGGTCGTGCTACAACTTCCTCAAGATGATCTGGCACGGGACCCTCCAAYAAGAAATTCAACTCCAGGCAGAAAA

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

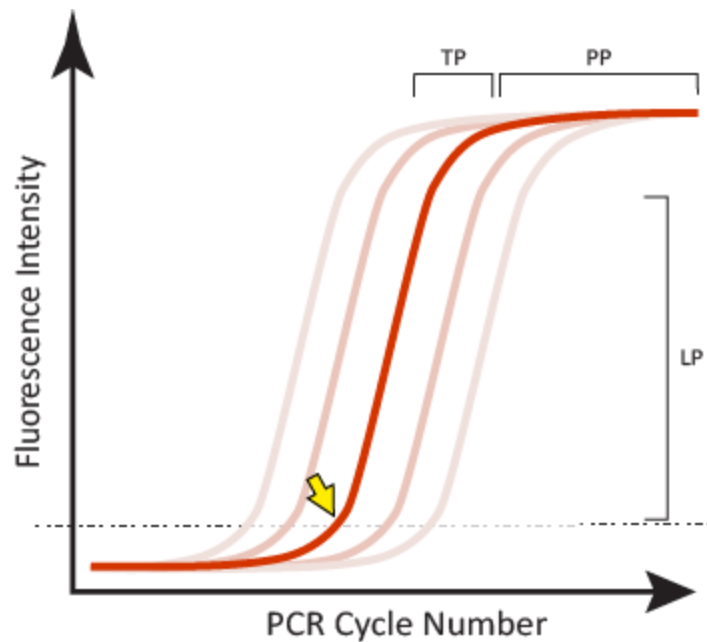


Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary) 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.