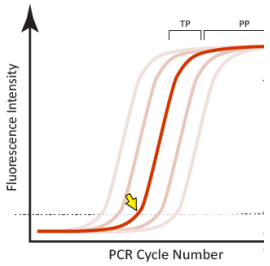
Feb 02, 2020 Version 2

Novel coronavirus (2019-nCoV) real-time RT-PCR ORF1ab 2020 (Wuhan-ORF1ab; nCoV-specific test) V.2



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Protocol status: In development
We are still developing and optimizing this protocol

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Keywords: CoV, coronavirus, Wuhan, Real-time, RT-PCR, PCR, virus, China, 2019-nCoV. ORF1ab, pneumonia, seafood market, WSMPV, wuhan coronavirus, wuhan seafood market pneumonia virus, novel coronavirus, betacoronavirus, final name for this virus, pcr, type virus, sequence mn908947, orf1ab sequence, novel wuhan, wuhan

Abstract

- A real-time RT-PCR to detect the "novel Wuhan" betacoronavirus or Wuhan seafood market pneumonia virus. Based on sequence **MN908947** made available by Professor Yong-Zhen Zhang, Fudan University, Shanghai, China.
- The target region is within the ORF1ab sequence.
- Not tested on wild-type virus (as of 25Jan2020), it is expected to be capable of only detecting the Wuhan coronavirus.
- ·Limit of detection not yet determined.

Notes

1. Assay is fully optimised (as of 24Jan2020).
2. A final name for this virus has not been decided (as of 25Jan2020).

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

STEP MATERIALS

SuperScript™ III Platinum™ One-Step qRT-PCR Kit **Life Technologies Catalog #**11732088

Protocol materials

SuperScript™ III Platinum™ One-Step qRT-PCR Kit **Life Technologies Catalog #**11732088

Mix

1 Oligonucleotides

	Oligo Name	Sequence 5'-3'	Location based on NC_045512*
	WuhanORF1ab-F	AATCCACCTGCTCTACAAGATG	5455-5476
	WuhanORF1ab-R	CATCACCTAACTCACCTACTGTC	5566-5544
	WuhanORF1ab-P	6FAM-AGCTTCACCAGCCCTTGCTCT-BHQ1	5505-5485

*GenBank accession NC_045512 Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1

2 Reagents

SuperScript™ III Platinum™ One-Step qRT-PCR Kit **Life Technologies Catalog #**11732088

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:
AAAATAATACGACTCACTATAGGGTGAAGAGAATCCACAAGGAATTGAAAGCTTCACCAGCC
CTTGCTCTACAGTGTTCAGCAGGTCCTGTTGAAAA

Primer control:
AAAATAATACGACTCACTATAGGGAATCCACCTGCTCTACAAGATGATGATCTGGCACGGGA
CCCTCCAAGACAGTAGGTGAGTTAGGTGATGAAAA

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 a 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 (ul) X1	Final reaction concentration
	Nuclease free water	4.37	
	WuhanORF1ab-F (200uM)	0.07	700nM
	WuhanORF1ab-R (200uM)	0.09	900nM
	WuhanORF1ab-P (100uM)	0.03	150nM
	2 X Reaction mix*	10	1X
	Superscript III/Platinum Taq enzyme mix*	0.4	
	ROX reference dye (25uM)*	0.04	50nM
	TOTAL VOLUME	15	

*Superscript®III Platinum® One-Step qRT-PCR kit

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

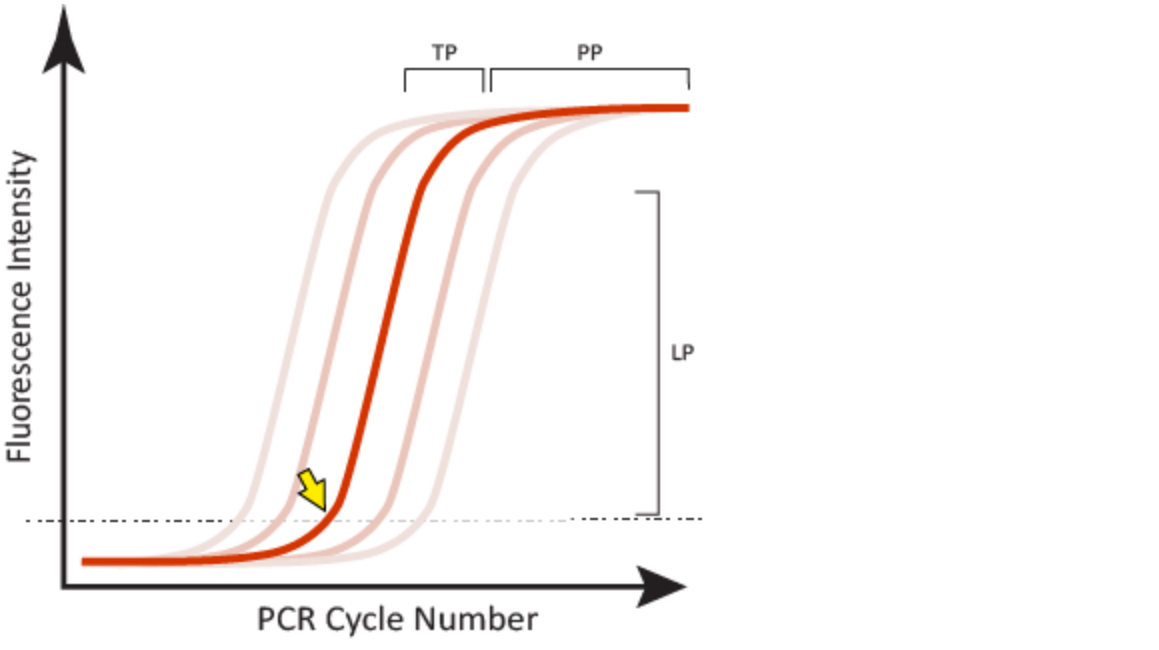

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary)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