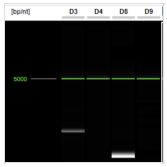


Version 3

Jun 01, 2020

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RdRp nested RT-PCR V.3

Judy Northill[1], Ian Mackay[1]

[1]Public Health Virology, Forensic and Scientific Services

1 Works for me dx.doi.org/10.17504/protocols.io.bgz2jx8e

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

Judy Northill
Public Health Virology, Forensic and Scientific Services

ABSTRACT

A nested RT-PCR targeting the RdRp region of the sub-genus *Sarbecovirus*. The primers are modified from the pan-coronavirus RT-PCR published by Hu *et al.* 2017 to be more specific to SARS-CoV-2, though other coronaviruses may still be detected.

Assay may be used in resource poor settings where real-time cyclers are not available.
Sanger sequencing should be used to confirm SARS-CoV-2 where WGS is not available or where WGS fails due to poor quality sample.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Hu D, Zhu C, Wang Y, Ai L, Yang L, Ye F, et al. Virome analysis for identification of novel mammalian viruses in bats from Southeast China. Scientific Reports. 2017;7(1):10917 .
https://www.nature.com/articles/s41598-017-11384-w

STEPS MATERIALS

NAME	CATALOG #	VENDOR
SuperScript III One-Step RT-PCR System with Platinum Taq	12574026	Invitrogen - Thermo Fisher
MyFi Mix	BIO-25049	Bioline

Oligonucleotides

1

Name	Purpose	5'-3'	Location*
SARS-CoV-2-15283-F	Round 1 forward primer	ATGGGTTGGGATTATCCTAAATG	15283-15305
SARS-CoV-2-15887-R	Round 1 reverse primer	TGTTGAGAGCAAAATTCATG	15887-15868
SARS-CoV-2-15286-F	Round 2 forward primer	GGTTGGGATTATCCTAAATGTGA	15286-15308
SARS-CoV-2-15725-R	Round 2 reverse primer	GCATCGTCAGAGAGTATCATCAT	15725-15703

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

Round 1 amplified products will produce a band of 605bp.
Round 2 amplified products will produce a band of 440bp.

Mix

2

SuperScript III One-Step RT-PCR System with Platinum Taq
by Invitrogen - Thermo Fisher
Catalog #: 12574026

Citation: Judy Northill, Ian Mackay (06/01/2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RdRp nested RT-PCR. https://dx.doi.org/10.17504/protocols.io.bgz2jx8e

MyFi Mix
by Bioline
Catalog #: BIO-25049

Round 1 mix

Reagent	Volume x1 (µl)	Final Concentration
Nuclease-free water	3.2	
2 x Reaction mix*	10	1 X
Wuhan15283-F (20pmol/µl)	0.5	500nM
Wuhan15887-R (20pmol/µl)	0.5	500nM
Superscript/Platinum Taq mix*	0.8	
TOTAL	**15**	

*SuperScript III One-Step RT-PCR System with Platinum Taq #12574026

Dispense mix in 15µl amounts in 0.2ml PCR tubes suitable for thermocycler.
Mix maybe stored frozen if necessary.

Round 2 mix

Reagent	Volume x1 (µl)	Final Concentration
Nuclease-free water	4	
MyFi 2X mix*	10	1X
Wuhan15286-F (20pmol/µl)	0.5	500nM
Wuhan15725-R (20pmol/µl)	0.5	500nM
TOTAL	**15**	

*MyFi™ Mix Bioline #BIO-25049

Dispense mix in 15µl amounts in 0.2ml PCR tubes suitable for thermocycler.
Mix maybe stored frozen if necessary.

AMPLIFICATION ROUND 1

3

- Thaw required number of tubes with round 1 mix.
- Add 5µl of extract, control, or NTC (nuclease-free water) to round 1 mix above for a final reaction volume of 20µl.
- Label the tubes and record details.

The assay has been used with Eppendorf thermocyclers.

PCR cycling times

1 cycle	40 cycles	1 cycle	Hold
50°C 30 minutes	94°C 30 seconds	68°C 5 minute	15°C
94°C 2 minutes	55°C 30 seconds		
	68°C 1 minute		

AMPLIFICATION ROUND 2

4

- Dilute each of the round 1 amplicons 1/100 in nuclease-free water (2ul + 198ul is ideal)
- Thaw required number of tubes with round 2 mix.
- Add 5µl of the diluted round 1 amplicon.

Citation: Judy Northill, Ian Mackay (06/01/2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RdRp nested RT-PCR. https://dx.doi.org/10.17504/protocols.io.bgz2jx8e

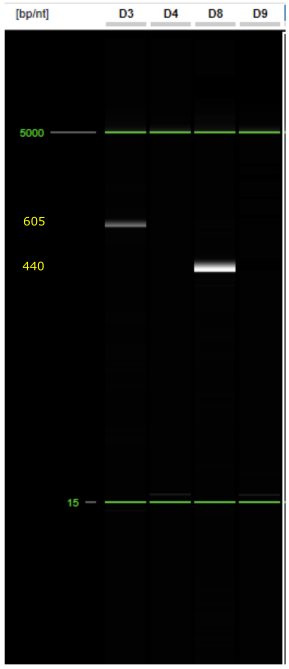
- Total reaction volume is 20µl.

PCR cycling times

1 cycle	40 cycles	1 cycle	Hold
95°C 3 minutes	95°C 15 seconds	72°C 1 minute	15°C
	55°C 15 seconds		
	72°C 15 seconds		

GEL ELECTROPHORESIS

5 Amplified products are analysed by gel electrophoresis or equipment such as a QIAxcel or equivalent.
Round 1 amplified products will produce a band of 605bp.
Round 2 amplified products will produce a band of 440bp.
No template controls (NTC) should be not detected.



Example of result from a QIAxcel.
D3-Round 1 band, D8-round 2 band, D4 and D9 are NTC in round 1 and 2 respectively.

Citation: Judy Northill, Ian Mackay (06/01/2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RdRp nested RT-PCR. https://dx.doi.org/10.17504/protocols.io.bgz2jx8e