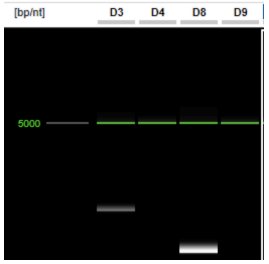
Jun 01, 2020 Version 3

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



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Public Health Virology, F... Coronavirus Method De...

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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

Protocol status: Working
We use this protocol and it's working

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Keywords: COVID-19, SARS, coronavirus, Rd-Rp, RT-PCR, PCR, SARS-CoV-2, severe, coronavirus rt, severe acute respiratory syndrome coronavirus, other coronavirus, specific to sar, sar, pcr, nested rt, rdrp region, rdrp,

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.

Materials

STEP MATERIALS

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

MyFi Mix **Bioline Catalog #**BIO-25049

Protocol materials

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

MyFi Mix **Bioline Catalog #**BIO-25049

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 **Invitrogen - Thermo Fisher Catalog #**12574026

MyFi Mix **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

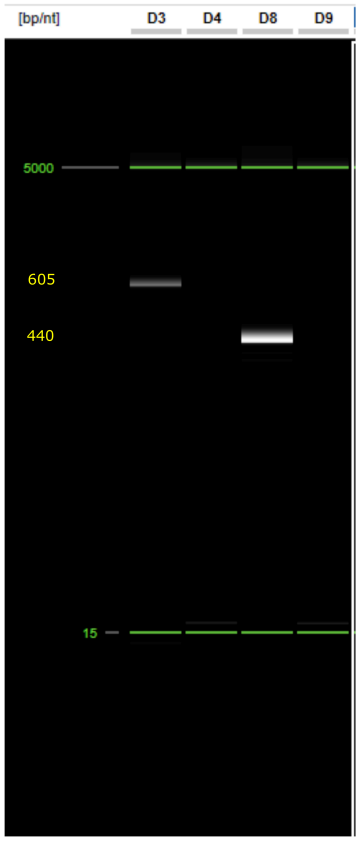
- Dilute each of the round 1 amplicons 1/100 in nuclease-free water (2ul + 198ul is ideal)
- Thaw required number of tubes with round 2 mix.
- Add 5µl of the diluted round 1 amplicon.
- Total reaction volume is 20µl.

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.