VERSION 1

DEC 21, 2020

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.bj9hkr36

Protocol Citation: Sarah J Reiling, Anne-Marie Roy, Shu-Huang Chen, Ioannis Ragoussis 2020. nCoV-2019 McGill Artic PCR Protocol, 5 ul RT and V3 only at 63C.

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<https://dx.doi.org/10.17504/protocols.io.bj9hkr36>

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

Created: Aug 24, 2020

Last Modified: Dec 21, 2020

PROTOCOL integer ID:
40969

🌐 nCoV-2019 McGill Artic PCR Protocol, 5 ul RT and V3 only at 63C V.1

🔗 Forked from [nCoV-2019 McGill Artic PCR Protocol, 5 ul RT and V3 only + LA1 at 63C](#)

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ABSTRACT

V3 only primers can be found here:

https://github.com/sarahreiling/artic-ncov2019/blob/master/primer_schemes/nCoV-2019/V3/nCoV-2019_V3only.scheme.bed

MATERIALS

MATERIALS

Q5 High-Fidelity 2X Master Mix - 500 rxns **New England Biolabs Catalog**
#M0492L

nuclease-free water **Contributed by users**

Fresh 80% Ethanol **Contributed by users**

Quant-iT™ PicoGreen™ dsDNA Assay Kit **Invitrogen - Thermo Fisher Catalog**
#P11496

AmpureXP beads **Beckman Coulter Catalog** **#A63880**

Primer pool preparation

1 PRIMER POOL PREPARATION

If required resuspend lyophilised primers at a concentration of 100 μM each

Note

V3 only primers for this protocol were designed using [Primal Scheme](https://github.com/sarahreiling/artic-ncov2019/blob/master/primer_schemes/nCoV-2019/V3/nCoV-2019_V3only.scheme.bed) and generate overlapping 400 nt amplicons. Primer names and dilutions are listed in the table below.
https://github.com/sarahreiling/artic-ncov2019/blob/master/primer_schemes/nCoV-2019/V3/nCoV-2019_V3only.scheme.bed

- 2 Generate primer pool stocks by adding 5 μL of each primer pair to a 1.5 mL Eppendorf labelled either "Pool 1 (100 μM)" or "Pool 2 (100 μM)". Total volume should be 490 μL for Pool 1 (100 μM) and 490 μL for Pool 2 (100 μM). These are your 100 μM stocks of each primer pool.

Note

Primers should be diluted and pooled in the **mastermix cabinet** which should be cleaned with decontamination wipes and UV sterilised before and after use.

- 3 Dilute this primer pool 1:10 in molecular grade water, to generate 10 μM primer stocks. It is recommend that multiple aliquots of each primer pool are made to in case of degradation or contamination.

Note

Primers need to be used at a final concentration of 0.015 μM per primer. In this case both pools have 98 primers in so the requirement is 3.65 μL primer pools (10 μM) per 25 μL reaction. For other schemes, adjust the volume added appropriately.

Multiplex PCR

4 MULTIPLEX PCR

In the **extraction and sample addition cabinet** add 5 µL RT product to each tube and mix well by pipetting.

Note

The **extraction and sample addition cabinet** should be cleaned with decontamination wipes and UV sterilised before and after use.

- 5 In the mastermix hood set up the multiplex PCR reactions as follows in 0.2mL 8-strip PCR tubes:

Component µM]	Pool 1 [10 uM primer]	Pool 2 [10
Q5 Hot Start High-Fidelity 2X Master Mix	12.5 µL	12.5 µL
Primer Pool 1 or 2 (10µM pool 1+2)	3.7 µL	3.7 µL
Nuclease-free water	3.8 µL	3.8 µL
Total	20 µL	20 µL

Add 20ul of PCR mastermix to the **5 ul RT product** of step 10.

Note

A PCR mastermix for each pool should be made up in the **mastermix cabinet** and aliquoted into PCR strip tubes. Tubes should be wiped down when entering and leaving the mastermix cabinet.

- 6 Pulse centrifuge the tubes to collect the contents at the bottom of the tube.

- 7 Set-up the following program on the thermal cycler:

Step	Temperature	Time	Cycles
Heat Activation	98 °C	00:00:30	1
Denaturation	98 °C	00:00:15	36
Annealing	63 °C	00:05:00	36
Hold	4 °C	Indefinite	1

Note

Cycle number should be 25 for Ct 18-21 up to a maximum of 36 cycles for Ct 35

PCR clean-up

8 PCR CLEANUP

Combine the entire contents of "Pool 1" and "Pool 2" PCR reactions for each biological sample into to a single 1.5 mL Eppendorf tube.

9 Clean-up the amplicons using the following protocol:

Add an equal volume (1:1) of AmpureXP beads to the sample tube and mix by pipetting.

Incubate for 5 min at room temperature.

Pellet on magnet for 5 min. Remove supernatant.

Add 200 ul of 80% ethanol to the pellet and wash twice.

Let the beads dry for 3 min.

Add 30 ul elution buffer and resuspend the beads. Incubate for 3 minutes.

Pellet on magnet for 5 min. Remove and keep eluate (30 ul).

Note

Amplicon clean-up should be performed in the **post-PCR cabinet which** should be cleaned with decontamination wipes and UV sterilised before and after use.

Amplicon Quantification and normalisation

10 AMPLICON QUANTIFICATION AND NORMALIZATION

Quantify the amplicon pools using a fluorimetric dsDNA assay. (e.g: PicoGreen with a standard curve 0-200ng)

We expect following concentrations:

Pool 1+2 combined:

100-150 ng/ul for Ct 14-24

30-80 ng/ul for Ct 25-29

10-30 ng/ul for Ct 30-36

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Nextera Flex Library Prep:

After quantification of Pool 1+2, take a new plate and add 150 ng of Pool 1+2 and add up with nuclease-free water to a total volume of **30 ul (= 5 ng/ul)**.

Nanopore Library Prep:

After quantification of Pool 1+2, take a new plate and add 200 ng of Pool 1+2 and add up with nuclease-free water to a total volume of **20 ul (= 10 ng/ul)**.