Version 2

Jun 23, 2022

nCoV-2019 McGill Artic PCR Protocol, V4.1 at 63C V.2

Version 1 is forked from nCoV-2019 McGill Artic PCR Protocol, 5 ul RT and V3 only + LA1 at 63C

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ABSTRACT

This is the updated SARS-Cov-2 PCR Protocol, with the ARTIC V4.1 primers, that is currently being used at the McGill Genome Center.

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PROTOCOL CITATION

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FORK NOTE

FORK FROM

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MATERIALS TEXT

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

V4.1 only primers for this protocol were designed using Primal Scheme and generate overlapping 400 nt amplicons. V4.1 was added to the V4 primer set for optimization. Primer names and dilutions are listed below.
primer-schemes/nCoV-2019/V4.1 at master · artic-network/primer-schemes · GitHub

For information on V4 primers visit Optimization of the SARS-CoV-2 ARTIC Network V4 Primers and Whole Genome Sequencing Protocol - PMC (nih.gov)

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2 The V4.1 pre-pooled primers in **1.5 mL** Eppendorf labelled tubes are labelled “Pool 1 (100µM)” or “Pool 2 (100µM)". The primers do not require additional preparation.

If the V4.1 primers are not pre-pooled, follow the below pipetting scheme to make the master mix by adding the following primers to the V4 primer pools.

Added to pool 1:
SARS-CoV-2_23_RIGHT_alt1
SARS-CoV-2_27_RIGHT_alt1
SARS-CoV-2_79_RIGHT_alt1
SARS-CoV-2_89_LEFT_alt1
SARS-CoV-2_89_RIGHT_alt1

Added to pool 2:
SARS-CoV-2_10_LEFT_alt1
SARS-CoV-2_10_RIGHT_alt1
SARS-CoV-2_76_LEFT_alt1
SARS-CoV-2_76_RIGHT_alt1
SARS-CoV-2_88_LEFT_alt1
SARS-CoV-2_90_RIGHT_alt1

The guide to pooling volumes are as follows;

2x volume:
SARS-CoV-2_1_LEFT & SARS-CoV-2_1_RIGHT
SARS-CoV-2_7_LEFT & SARS-CoV-2_7_RIGHT
SARS-CoV-2_13_LEFT & SARS-CoV-2_13_RIGHT
SARS-CoV-2_17_LEFT & SARS-CoV-2_17_RIGHT
SARS-CoV-2_27_LEFT & SARS-CoV-2_27_RIGHT
SARS-CoV-2_45_LEFT & SARS-CoV-2_45_RIGHT
SARS-CoV-2_59_LEFT & SARS-CoV-2_59_RIGHT
SARS-CoV-2_60_LEFT & SARS-CoV-2_60_RIGHT
SARS-CoV-2_61_LEFT & SARS-CoV-2_61_RIGHT
SARS-CoV-2_64_LEFT & SARS-CoV-2_64_RIGHT
SARS-CoV-2_79_LEFT & SARS-CoV-2_79_RIGHT
SARS-CoV-2_90_LEFT & SARS-CoV-2_90_RIGHT
SARS-CoV-2_91_LEFT & SARS-CoV-2_91_RIGHT

1x volume: All other primers including alts (from V4.1).

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

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Multiplex PCR

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MULTIPLEX PCR

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

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

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

Component [10 uM]	**Pool 1 [10 uM primer]**	**Pool 2**
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.

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.

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

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

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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

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

PCR clean-up

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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.

8 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).

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

Amplicon Quantification and normalisation

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AMPLICON QUANTIFICATION AND NORMALIZATION

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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).**

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