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SARS-CoV-2 McGill Artic PCR Protocol, 2.5 ul RT and V3 only + LA1

Forked from a private protocol



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Protocol status: Working

We use this protocol and it's working

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Abstract

V3 only primers for this protocol were designed using **Primal Scheme** 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



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** 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.

Make another primer pool named "Pool LA1 (100 μM)" that contains 5 μL of primer pairs 5, 17, 23, 26, 66, 70, 74, 91, 97, and 10 μL of primer pair 64.

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 recommended that multiple aliquots of each primer pool are made to in case of degradation or contamination.

LA1 primer pool will be diluted to 1 μM primer stock.

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 2.5 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	Pool 1 [10 uM primer]	Pool 2 [10 uM]
Pool LA1 [1 uM]		
Q5 Hot Start High-Fidelity 2X Master Mix	12.5 µL	12.5 µL
12.5 µL		
Primer Pool 1 or 2 (10 µM pool 1+2; 1 µM LA1)	3.7 µL	3.7 µL
3.7 µL		
Nuclease-free water	6.3 µL	
6.3 µL	6.3 µL	
Total	22.5 µL	
22.5 µL	22.5 µL	

Add 2.5 µl RT product as mentioned in 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
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Heat Activation	98 °C	00:00:30	1
Denaturation	98 °C	00:00:15	36
Annealing	65 °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 36.

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. **Keep Pool LA1 separate from the combined Pool 1+2 until after the clean-up!!**

9 Clean-up the amplicons using the following protocol:

Add an equal volume (1:1) of SPRI beads to the sample tube and mix gently by either flicking or 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.

Elute in 30 ul elution buffer.

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.

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

Pool LA1:

1-10 ng/ul for all Ct

- 11 After quantification of Pool 1+2 and Pool LA1, mix them together in following ratio: 89.8% Pool 1+2 and 10.2% Pool LA1. For this, take a new plate and add 135 ng of Pool 1+2 and 15.3 ng of Pool LA1, and add up with nuclease-free water to a total volume of **30 µl (= 150 ng or 5 ng/µl)**.

