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nCoV-2019 McGill RT Protocol, Lunascript

Forked from Artic nCoV-2019 McGill modified Lunascript Reverse Transcriptase Nanopore sequencing protocol

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

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ABSTRACT

Artic nCoV-2019 McGill modified Lunascript Reverse Transcriptase sequencing protocol.

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PROTOCOL CITATION

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Cov-19 Nanopore Sequencing, Cov-19 Illumina Sequencing

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MATERIALS

NAME	CATALOG #	VENDOR
Water, nuclease free		
LunaScript RT SuperMix Kit	E3010L	New England Biolabs

cDNA preparation

1 Mix the following components in a 0.2 mL 8-strip tube;

Component	Volume
LunaScript RT SuperMix (5x)	4 µl
Nuclease-free water	5 µl
Template RNA	11 µl
Total	20 µl

Viral RNA input from a clinical sample should be between Ct 18-35. If Ct is between 12-15, then dilute the sample 100-fold in water, if between 15-18 then dilute 10-fold in water. This will reduce the likelihood of PCR-inhibition.

A mastermix should be made up in the **mastermix cabinet.** Tubes should be wiped down when entering and leaving the mastermix cabinet.

2

Gently mix by pipetting and pulse spin the tube to collect liquid at the bottom of the tube.

3

Incubate the reaction as follows:

25 °C for **00:02:00**

55 °C for **00:20:00**

95 °C for **00:01:00**

Place on ice for **00:01:00 or store cDNA at -20C**

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