AUG 24, 2020

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bjgekjte

Protocol Citation: Shu-Huang Chen, Sarah J Reiling, Josh Quick, Ioannis Ragoussis 2020. nCoV-2019 McGill RT Protocol, Lunascript, protocols.io
<https://dx.doi.org/10.17504/protocols.io.bjgekjte>

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

Created: Aug 07, 2020

Last Modified: Aug 24, 2020

PROTOCOL integer ID:
 40166

Keywords: Cov-19 Nanopore Sequencing, Cov-19 Illumina Sequencing

🌐 nCoV-2019 McGill RT Protocol, Lunascript

Forked from a private protocol

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ABSTRACT

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

MATERIALS

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⊗ Water, nuclease free **Contributed by users**

⊗ LunaScript RT SuperMix Kit **New England Biolabs Catalog # E3010L**

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

Note

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.

Note

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