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nCoV-2019 McGill RT Protocol, Lunascript

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

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

Materials

MATERIALS

Water, nuclease free

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