**Supplementary Method 7 RT-PCR for SARS-CoV-2 using the BGI kit**

## Equipment / Consumables

- Barcode Scanner and laptop computer
- QuantStudio Real time PCR System
- Benchtop centrifuge

## RT-PCR set up procedure

1. Take RT-PCR Plate to PCR lab. Ensure that the PCR plate is correctly assigned to the PCR stage of the process in a LIMS system. If using ClarityLims to confirm receipt of plate, click in empty field box in app, then scan the barcode with a handheld barcode scanner to enter PCR plate name. Hit **SUBMIT**.

2. Check seal integrity on PCR plate and spin briefly in centrifuge (1000rpm, 1min)

3. Open the sample drawer of an available Quant 3 PCR machine and place PCR Plate in the machine.


4 Confirm that the barcode is visible and facing the front of the machine, and A1 is on top-left side

5 On the PCR machine-associated laptop, ensure that the correct annotation for the specific PCR plate is downloaded from a LIMS system. If using ClarityLims, click in empty field box in app and scan the barcode on the PCR plate with handheld barcode scanner to enter PCR plate name. Hit **SUBMIT.**

6 Downloading of text file will happen automatically. Save to designated Plate Layout folder on Desktop with the Barcode as filename.

**7 At this point, a second operator must confirm plate orientation.**

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8 Open QuantStudio software.

**9** Click on the button to "Open Existing Experiment" and find the designated **Covid-19 Master Template.edt** on Desktop

10 When prompted, click on '**Edit**' and enter designated password.

**11** Click in field box for **Barcode** and use handheld scanner to scan plate barcode again to populate the field and replace default.

**12** Click in field box for **Name** and scan barcode again to replace default.

**13** Click in field box for **User** and enter your designated user name.

**14** Under 'File' menu click on 'Import Plate Setup' and browse to identify the text file with same barcode on Desktop\Plate Layout. Hit '**Apply**'.

**15** You should be prompted that current info will be lost and replaced. Hit '**yes**'.

**16** Confirm the following settings by clicking on 'Method' on menu near top of window

| Step | Cycle | Temperature | Duration | Fluorescence measure |
|---|---|---|---|---|
| 1 | 1 cycle | 50℃ | 20 min | N |
| 2 | 1 cycle | 95℃ | 10 min | N |
| 3 | 40 cycles | 95℃ | 15 sec | N |
| | | 60℃ | 30 sec | Y |

17 Confirm following settings by clicking on 'Plate'

- **(Advanced Setup Tab) Target1: FAM, Quencher: None for Covid-19**
- **(Advanced Setup Tab) Target2: VIC, Quencher: None for internal control**
- **(Quick Setup Tab) Passive Reference Dye: None**
  in 'Plate' window in Quick Setup Tab that plate is populated with sample barcodes.

18 Click on 'Run' window, and hit 'Start Run' button

19. When prompted, save barcode.eds file in the designated folder on Desktop.

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20. **FINAL CHECKPOINT (stop the run if the following is not true): The PCR run should last 88min 41sec.**

21 After the amplification is complete, remove PCR plates from the thermal cycler and discard plate for autoclave and decontamination in line with laboratory procedures.