

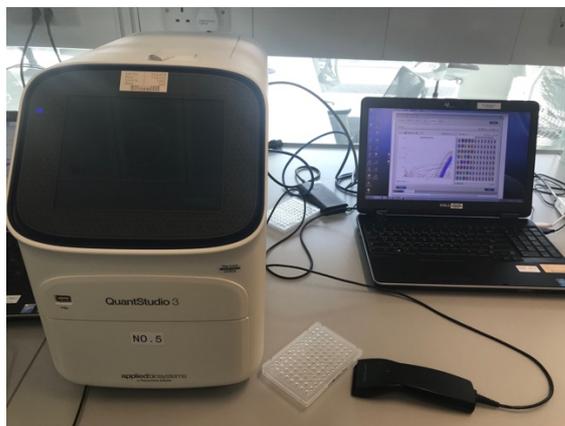
## Supplementary Method 7 RT-PCR for SARS-CoV-2 using the BGI kit

### 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°C	20 min	N
2	1 cycle	95°C	10 min	N
3	40 cycles	95°C	15 sec	N
		60°C	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.