

## Supplementary Method 6 Automated transfer of RNA to PCR master mix plate on Biomek FX

### Equipment and Consumables

Consumables	Used/run
Eppendorf 96-well skirted plates LoBind (Eppendorf, Cat.no. 0030129512)	1
MicroAmp™ Fast optical 96-well plate with Barcode (ThermoFisher, Cat no. 4366932)	1
MicroAmp™ Optical Adhesive Film (Thermo Fisher, Cat no. 4311971)	1
MicroAmp™ Adhesive Film Applicator (Thermo Fisher, Cat no. 4333183)	1
Adhesive PCR Plate Seals (Thermo Fisher, Cat no. AB0558)	1
Biomek FX 50 µL tips, Filtered, Sterile (Beckman Coulter, Cat no. A21586)	1
RNAse ZAP wipes	1
Equipment	
Beckman Biomek FX workstation	1
Barcode Reader	1

### Reagents

Extracted viral RNA samples (prepared using **Supplementary Method 4**) in a 96-well barcoded plate.

ABI MicroAmp™ Fast Optical PCR plate containing RT-PCR Master Mix for detecting SARS-CoV-2 (prepared using **Supplementary Methods 5**).

### **Positive control**

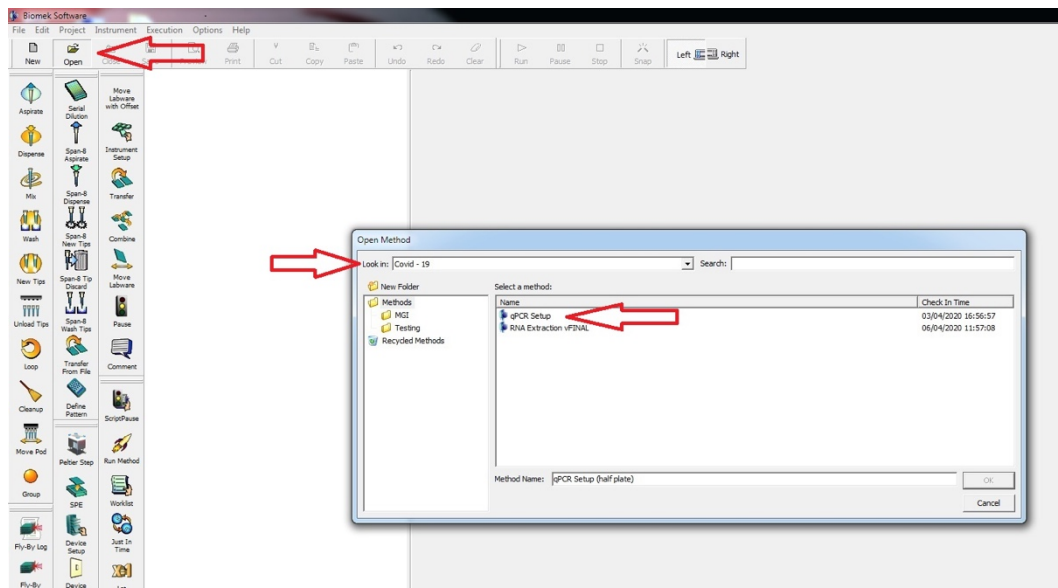
2019-nCoV Positive control - included in the BGI kit

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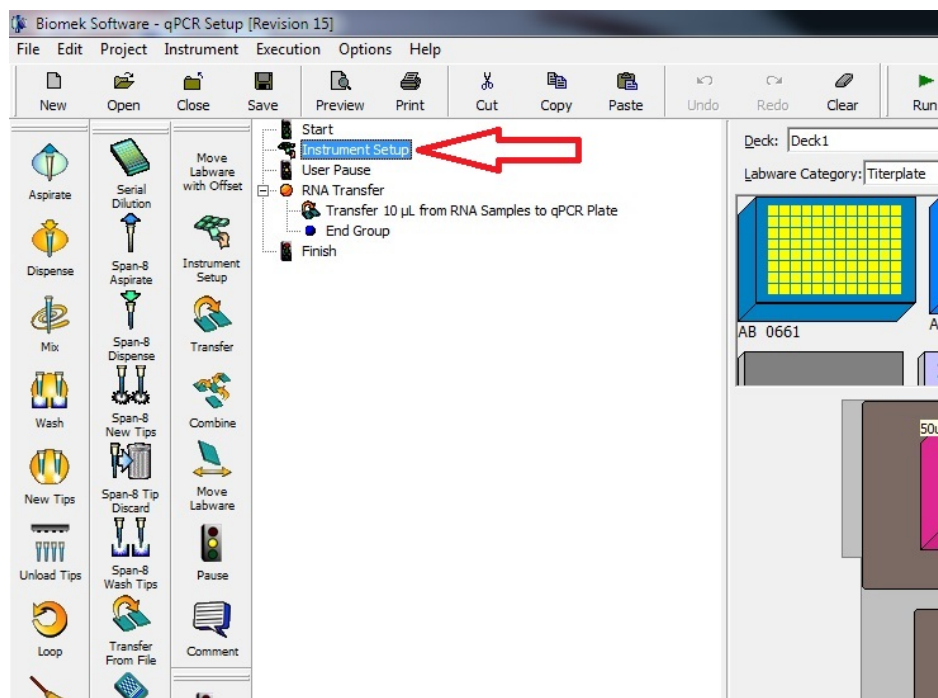
## Procedure

### Biomek FX Setup

1. Open the Biomek software, and select the designated program (see Appendix) for “qPCR Setup” from the relevant location:



2. Click on “Instrument Setup” as shown below to view the deck layout:




## Supplementary Method 6 Automated transfer of RNA to PCR master mix plate on Biomek FX

### FX1 DECK LAYOUT



3. Wipe the deck using an RNase ZAP wipe, followed by an alcohol wipe or 70% ethanol.
4. Place 2 boxes (lid off) of **50  $\mu$ L filter tips** as shown above.
5. Take the **RNA sample plate** (Eppendorf LoBind 96-well skirted plate), remove the seal, and place in position P15 with the SPL barcode on the front side.
6. If the ABI MicroAmp™ Optical plate containing the **qPCR reagents mix** has been stored at 4°C, spin at 1000 rpm for 1 min in the table top centrifuge.
7. Then stack on a black Greiner CellStar plate. Place on the deck in position P16.
8. Make sure you **scan the barcode** of the qPCR plate on the Clarity LIMS. This will link the barcode of the qPCR plate with the RNA samples.
9. Place a black microfuge tube rack in position P18.
10. Place a 2ml screwcap tube (cap off) with TET buffer (or H<sub>2</sub>O) in position A1 and the Positive Control tube (cap off) in position D1 of the above rack.
11. Home the instrument by going to “Instrument” and selecting “Home All Axes”.
12. Make sure to purge the Span-8 syringes until no bubbles are seen in the tubing.
13. Check the level of the Biomek FX Span-8 water container. Re-fill with Milli-Q water if the level is too low. Empty the waste bottles in the sink when they start getting full.

## Supplementary Method 6 Automated transfer of RNA to PCR master mix plate on Biomek FX

14. To START the program, press the Run button (green triangle  ) located in the software.
15. The prompt on the screen will remind you to remove two tips from positions G12 and H12 of the “50 µL Tips 1” tipbox. Click OK to continue.
16. The next prompt will remind you that the MicroAmp™ plate should be stacked on top of a black Greiner plate.
17. The last prompt will remind you of the microfuge rack setup:  
A1: TET buffer (or H2O) in 2ml tube  
D1: Positive Control
18. When the program is complete, seal the RNA sample plate using a Thermo Fisher PCR seal and place at -80°C freezer for temporary storage.
19. Carefully remove the MicroAmp™ plate from the deck.
20. Seal the plate with an Optical Adhesive Film (located on the bench next to the Biomek).
21. Press the seal down to ensure a good seal on each well using the MicroAmp™ Adhesive Film Applicator.
22. Spin the MicroAmp™ plate at 1000rpm for 1 min using the table top plate centrifuge.

### Archiving

23. Once the RT-PCR is completed successfully (see **Supplementary Method 7**), the RNA plate can be moved to an archive freezer.
24. Archiving information must be recorded in the COVID-19 web app.

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### Appendix

The robot carries out the following automated protocol:

1. Load 50  $\mu$ L tips using pod 1.
2. Aspirate 10  $\mu$ L of RNA samples (10  $\mu$ L/sec).
3. Dispense 10  $\mu$ L to the qPCR plate (10  $\mu$ L/sec).
4. Unload tips.
5. Load one 50  $\mu$ L tip using pod 2.
6. Aspirate 10  $\mu$ L of TET buffer from position A1 of the microfuge rack.
7. Dispense 10  $\mu$ L to the qPCR plate position G12 (this constitutes the 'negative control').
8. Unload tip.
9. Load one 50  $\mu$ L tip using pod 2.
10. Aspirate 10  $\mu$ L of Positive Control from position D1 of the microfuge rack (20  $\mu$ L/sec).
11. Dispense 10  $\mu$ L to the qPCR plate position H12 (30  $\mu$ L/sec).
12. Unload tip.