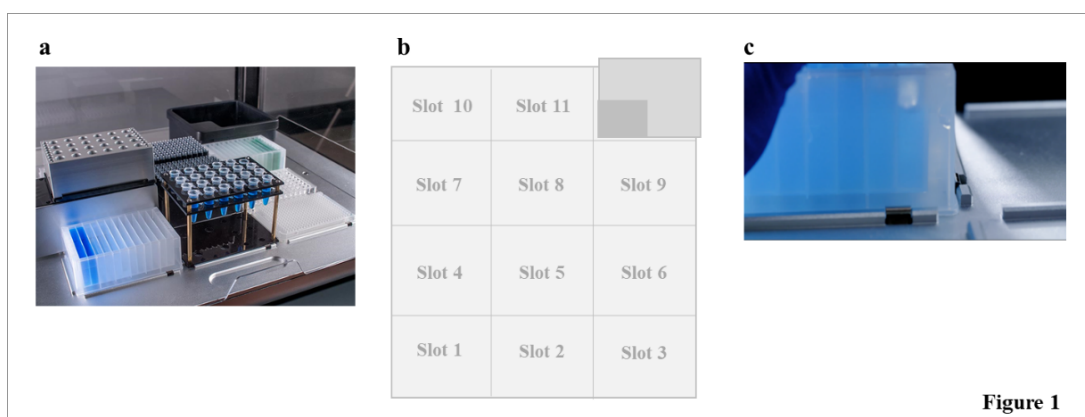


## OPERATING OPENTRONS MACHINES BELONGING TO STATION C

### ATTENTION

- Before starting check that the OpenTrons Machines belonging to Station C and all the removable parts needed to operate the machines have been cleaned, following the instructions reported in the Standard Operating Procedure for Cleaning
- The deck of each OpenTrons machine has 11 different slots for the placement of the labware required for different processes (Figure 1a). A number is virtually assigned to each slot (Figure 1b). When placing any of the labware described below in the dedicate slot
  - check that the labware is properly inserted by pressing the corner into the metal springs (Figure 1c). You should feel a slight click and the labware should sit completely flat



- make sure that the labware is inserted in the right direction, *i.e.* the well numbered as A1 or as 1 is at the top left corner

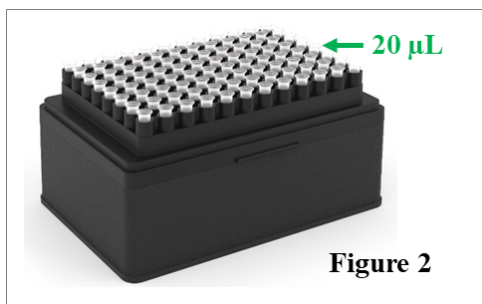
- **This procedure applies to the OpenTrons Machines belonging to Station C. Each of them has to be equipped with which are equipped with a p20 multi channel and a p300 single channel pipettes**
- **Materials required for operating each OpenTrons Machine belonging to Station C**

Item	Quantity
Temperature module	1
96 position aluminum block	3
Sterile 96 well 200 $\mu$ L BIO-RAD PCR plate	1
Rack for 200 $\mu$ L filter tips	1
200 $\mu$ L filter tips	96
Rack for 20 $\mu$ L filter tips	6
20 $\mu$ L filter tips	576 (96*6)
24 position aluminum block	1
1.5 mL sterile tube (screw cap type)	6
Pipette for measuring 8 $\mu$ L	1
Filter tip for measuring 8 $\mu$ L	1
Pipette for measuring 990 $\mu$ L	1
Filter tip for measuring 990 $\mu$ L	1
Pipette for measuring 90 $\mu$ L	1
Filter tip for measuring 90 $\mu$ L	1
Pipette for measuring 10 $\mu$ L	1
Filter tip for measuring 10 $\mu$ L	3
Pipette for measuring 1 mL	1
Filter tip for measuring 1 mL	1
Pipette for measuring 1.2 mL	1
Filter tip for measuring 1.2 mL	1
Vortex	1
200 $\mu$ L PCR tube strips	1
Nuclease free water	2070 $\mu$ L (990 $\mu$ L*2 + 90 $\mu$ L)

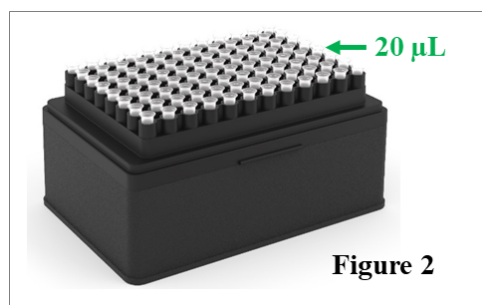
Resuspended PCT	10 $\mu$ L
2x One-Step RT-qPCR Mastermix1	1.2 mL
Primer/Probe mix (nCov and IEC primers)	241 $\mu$ L

## STEPS

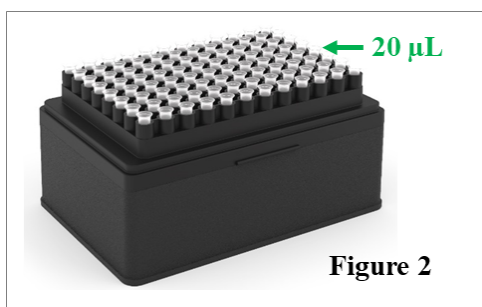
1. Keep the 24 position aluminum block and a 96 position aluminum blocks cold by placing them in freezer at - 20 °C
2. Start pre-cooling the temperature module to 4 °C
3. Place
  - a sterile rack full of 20  $\mu$ L filter tips in **SLOT 11** of the deck of each OpenTrons machine belonging to Station C (**Figure 2**)



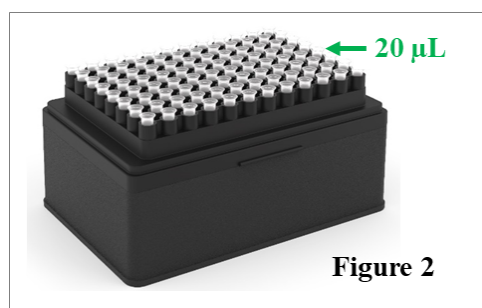
- a sterile rack full of 20  $\mu$ L filter tips in **SLOT 10** of the deck of each OpenTrons machine belonging to Station C (**Figure 2**)



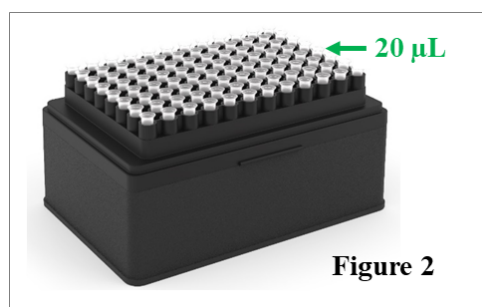
- a sterile rack full of 20  $\mu$ L filter tips in **SLOT 9** of the deck of each OpenTrons machine belonging to Station C (**Figure 2**)



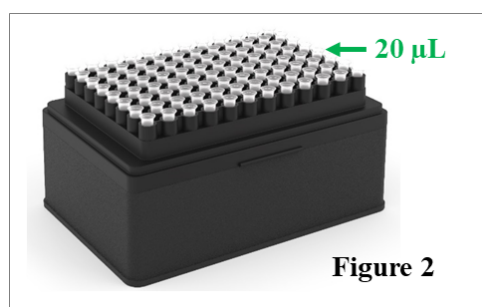
- a sterile rack full of 20  $\mu$ L filter tips in **SLOT 8** of the deck of each OpenTrons machine belonging to Station C (**Figure 2**)



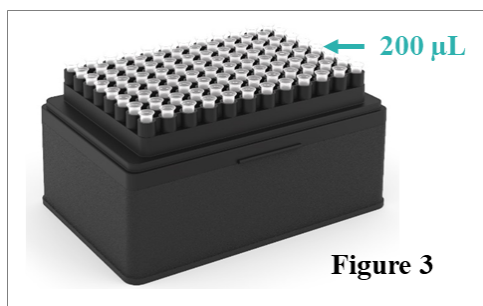
- a sterile rack full of 20  $\mu$ L filter tips in **SLOT 6** of the deck of each OpenTrons machine belonging to Station C (**Figure 2**)



- a sterile rack full of 20  $\mu$ L filter tips in **SLOT 3** of the deck of each OpenTrons machine belonging to Station C (**Figure 2**)



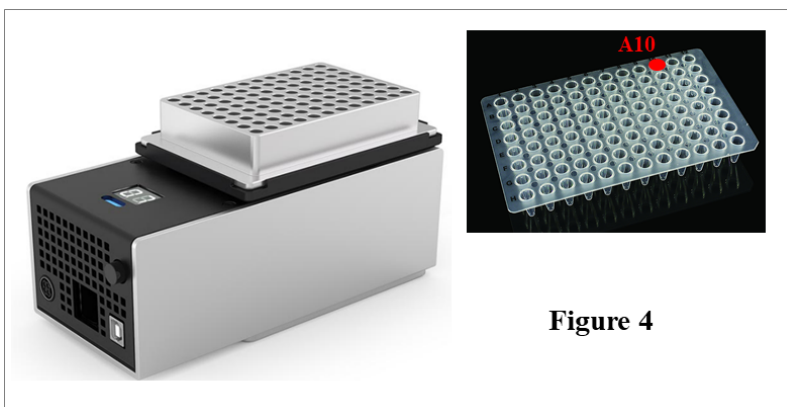
- a sterile rack full of 200  $\mu$ L filter tips in **SLOT 2** of the deck of each OpenTrons machine belonging to Station C (**Figure 3**)



**4. Ensure the temperature module has reached 4 °C**

**5. Prepare the temperature module (Figure 4) by**

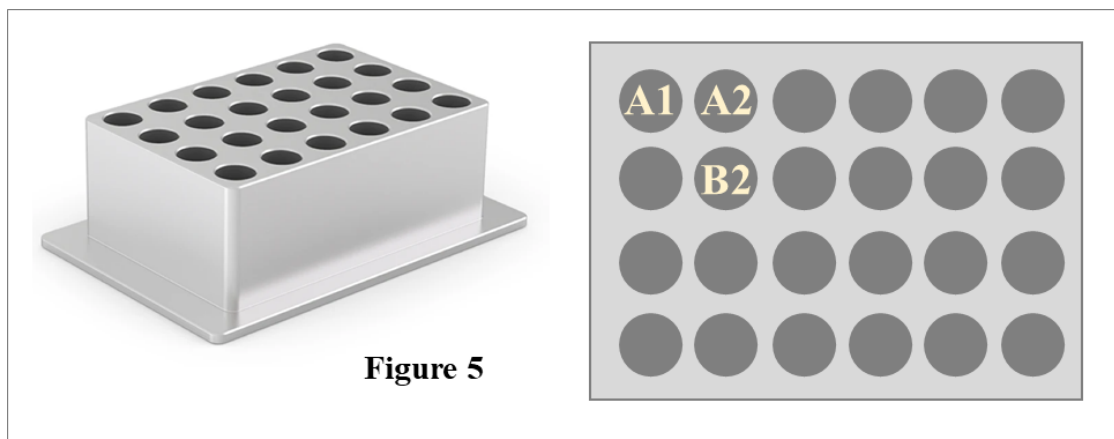
- placing on top of it a 96 position prechilled aluminum block
- placing on top of the 96 position prechilled aluminum block a sterile 96 well 200  $\mu$ L BIO-RAD PCR plate that was prepared as follows



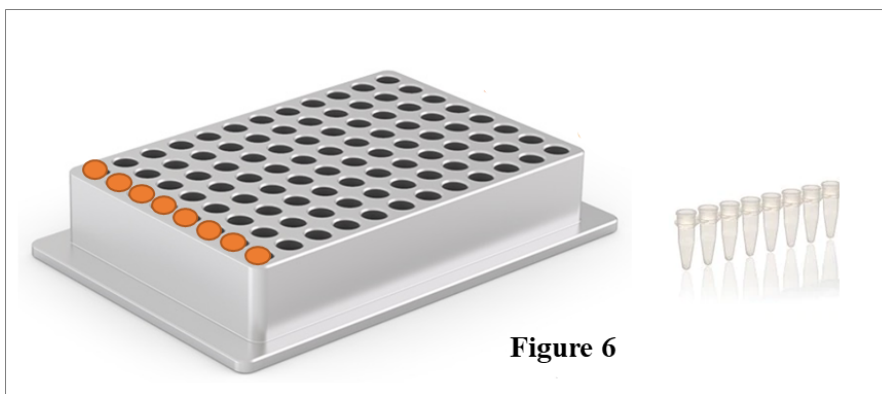
- in A10: pipette 8  $\mu$ L a positive control template diluted down to 1.7 copies/ $\mu$ L. The tube should be prepared as follows:
  - pipette 990  $\mu$ L of nuclease free water into two different 1.5 mL sterile tubes. Label these tubes as 1 and 2
  - pipette 90  $\mu$ L of nuclease free water into one 1.5 mL sterile tube. Label this tube as 3.
  - transfer 10  $\mu$ L of resuspended PCT tube into the tube 1. Vortex 20 times.
  - transfer 10  $\mu$ L of the content of tube 1 to tube 2. Vortex 20 times.
  - transfer 10  $\mu$ L of the content of tube 2 to tube 3. Vortex 20 times. This way tube 3 contains 1.7 copies/ $\mu$ L

6. Place the temperature module equipped with the 96 position aluminum block and the sterile 96 well 200  $\mu$ L BIO-RAD PCR plate containing the positive control in SLOT 7 of the deck of each OpenTrons machine belonging to Station C

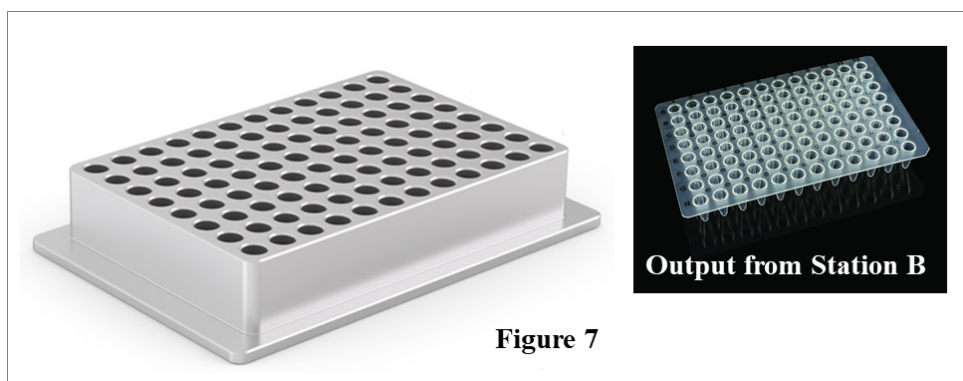
7. Prepare the 24 position prechilled aluminum block for reagents (Figure 5)



- by placing
    - in A1: an empty 1.5 mL sterile tube (screw cap type)
    - in A2: a 1.5 mL sterile tube (screw cap type), containing at least 1.2 mL and of 2x One-Step RT-qPCR Mastermix1
    - in B2: a 1.5 mL sterile tube (screw cap type), containing at least 231  $\mu$ L of Primer/Probe mix (nCov and IEC primers)
8. Place the 24 position prechilled aluminum block containing reagents in SLOT 5 of the deck of each OpenTrons machine belonging to Station C
  9. Prepare a 96 position prechilled aluminum block (Figure 6) by placing into column A a 200  $\mu$ L PCR tube strip



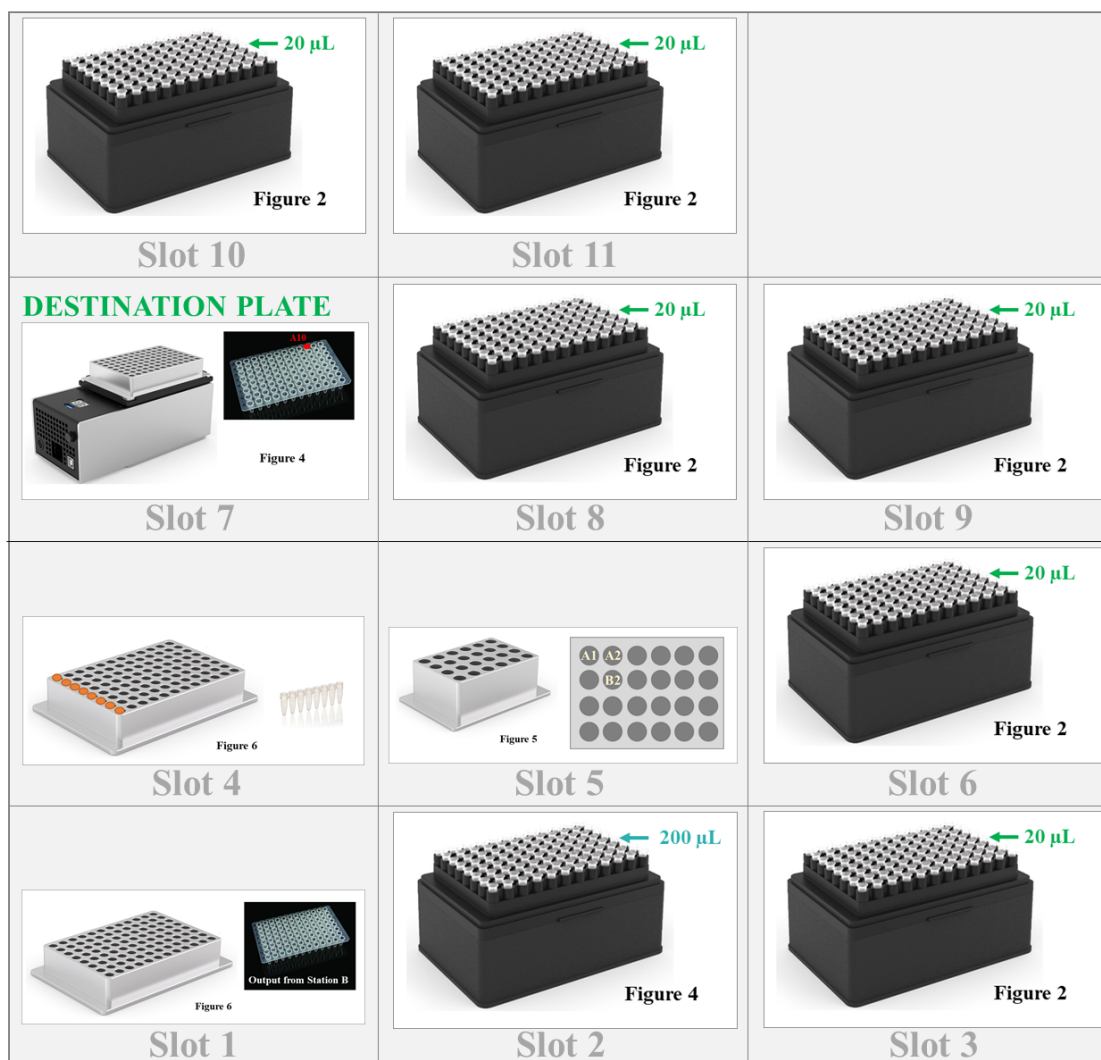
10. Place the 96 position aluminum block containing the 200 µL PCR tube strip in SLOT 4 of the deck of each OpenTrons machine belonging to Station C
11. Remove from the temperature module of Station B the 96 position aluminum block with on top of it the 96 well 200 µL PCR plate that was the output of Station B and place them in SLOT 1 of the deck of each OpenTrons machine belonging to Station C



- 12. Double-check all the labware to make sure it looks correct. Follow the outline reported below to verify the right positioning of the labware before described (Figure 8). Notably, it has to be check that**
- the labware is inserted the right way around (well A1 or 1 at the top-left)
  - the labware is properly clicked into each deck slots
  - the tubes are flat in the aluminum block



**Figure 8**



Deck of each OpenTrons machine belonging to Station C

**13. Run the Station C protocol whose file name should be “v1\_station\_c\_S9\_bp\_primerdesign\_p20multi.py”, by clicking the button “Start run” in the “Run tab”**

- do not click “Start run” more than once
- if you need to cancel the protocol for any reason, use the power switch to turn off the machine. When it turns back on, the pipettes will rise. If the pipettes had tips attached, you will need to manually remove them before starting again

**14. Wait for the run to finish**

- in the meantime, start preparing the RT-qPCR machine
- 15. Collect the sterile 96 well 200  $\mu$ L BIO-RAD PCR plate from SLOT 4 (containing approximately 20  $\mu$ L), which is be the process output**
  - 16. Seal the sterile 96 well 200  $\mu$ L BIO-RAD PCR plate from SLOT 4 before moving it to the PCR room**
  - 17. If the remaining extractions are required to be stored, collect and seal the 96 well 200  $\mu$ L PCR plate with the remaining extractions (containing approximately 22  $\mu$ L) from SLOT 1 and keep it in freezer at - 20  $^{\circ}$ C**
  - 18. Cleaning / disposal**
    - throw out
      - the used filter tips and their racks
      - the sterile 1.5 mL tubes
      - the 2 different 200  $\mu$ L PCR tube strip
    - remove for cleaning / reuse
      - the temperature module
      - the 2 different 96 position aluminum block
      - the 24 position aluminum block
  - 19. Clean the deck and the removable parts following the instructions reported in the Standard Operating Procedure for Cleaning**