OPERATING OPENTRONS MACHINES BELONGING TO STATION C

ATTENTION

- **Before starting, clean each OpenTrons machine belonging to Station C, 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

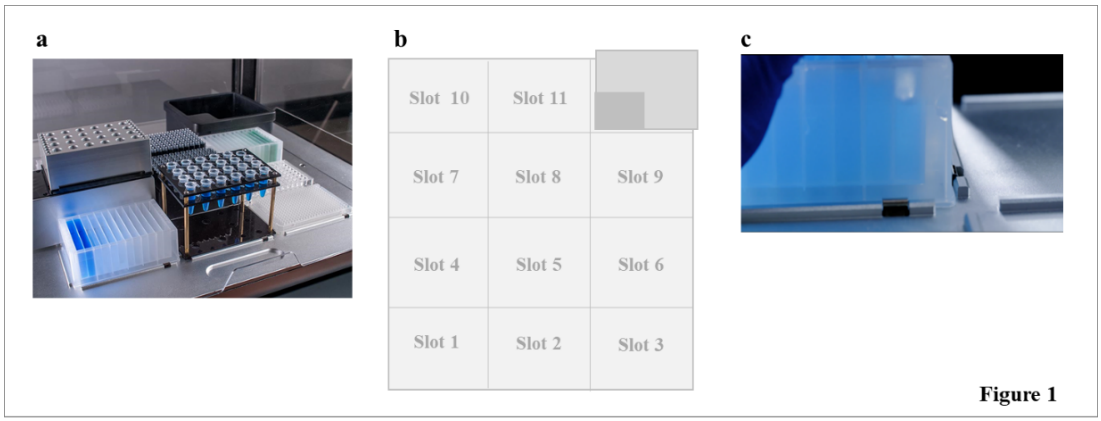

Figure 1

    - 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 NEST 100 µL PCR plate	1
Rack for 200 µL filter tips	1
200 µL filter tips	96
Rack for 20 µL filter tips	6
20 µL filter tips	576 (96*6)
24 position aluminum block	1
1.5 mL sterile tube	3
Pipette for measuring 990 µL	1
Tip for measuring 990 µL	1
Pipette for measuring 90 µL	1
Tip for measuring 90 µL	1
Pipette for measuring 10 µL	1
Tip for measuring 10 µL	3
Pipette for measuring 15 µL	1
Tip for measuring 15 µL	1
Pipette for measuring 1 mL	1
Tip for measuring 1 mL	1
Pipette for measuring 200µL	1
Tip for measuring 200 µL	1
Vortex	1
200 µL PCR strips tubes	2
Nuclease free water	2070 µL (990 µL*2 + 90 µL)

Resuspended PCT	10 µL
2x One-Step RT-qPCR Mastermix	1 mL
Primer/Probe mix (nCov and IEC primers)	1.2 mL

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STEPS

1. **Keep the 24 position aluminum block and the two different 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 µL filter tips in **SLOT 11** of the deck of each OpenTrons machine belonging to Station C **(Figure 2)**

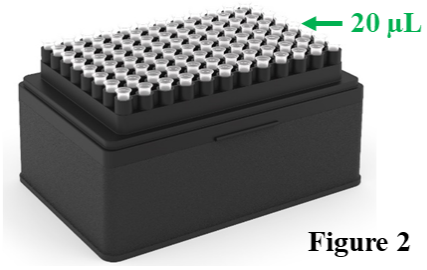


Figure 2

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

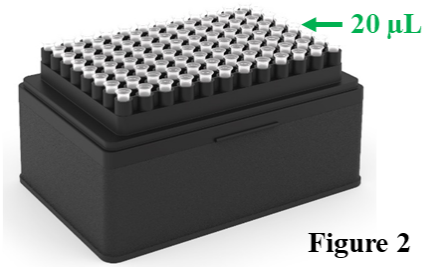


Figure 2

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

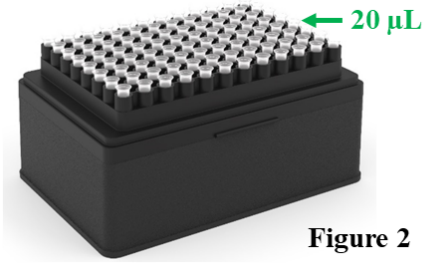


Figure 2

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

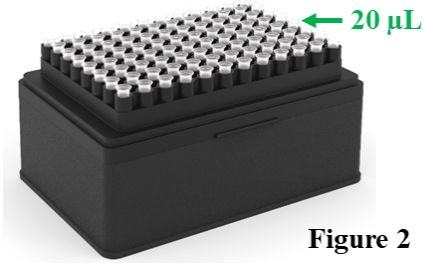


Figure 2

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

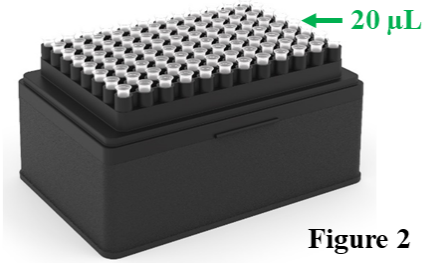


Figure 2

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

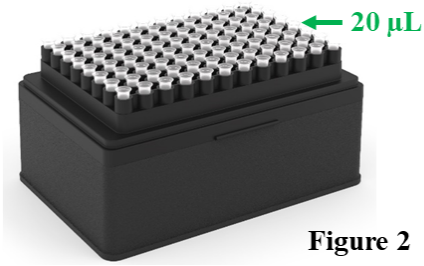


Figure 2

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

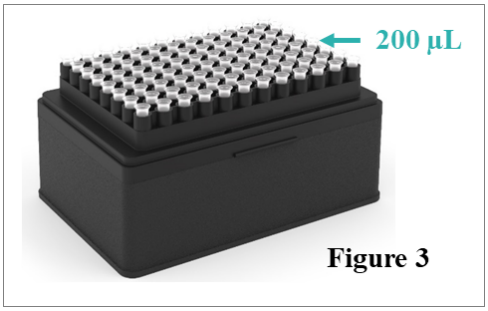


Figure 3

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

5. **Prepare the temperature module (Figure 4) by**
   - placing on top of it a 96 well prechilled aluminum block
   - placing on top of the 96 well prechilled aluminum block a sterile NEST 100 µL PCR plate that was prepared as follows

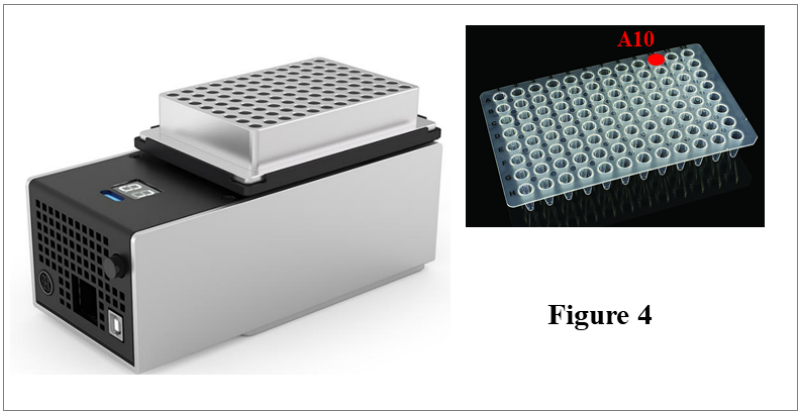


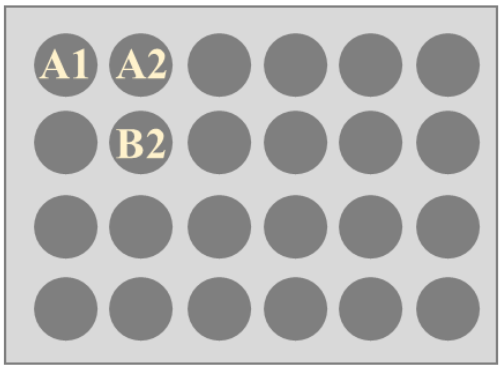
Figure 4

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

6. **Place the temperature module equipped with the 96 well aluminum block and the NEST 100 µL PCR plate containing the positive control in SLOT 4 of the deck of each Open-Trons machine belonging to Station C (Figure 3)**

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

Figure 5



- by placing
  - in A1: an empty 1.5 mL sterile tube
  - in A2: a 1.5 mL sterile tube, containing at least 1 mL of 2x One-Step RT-qPCR Mastermix1
  - in B2: a 1.5 mL sterile tube, containing at least 1.2 mL 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 first 96 position prechilled aluminum block (Figure 6) by placing into column A and B 2 PCR 200 µL PCR strips tubes**

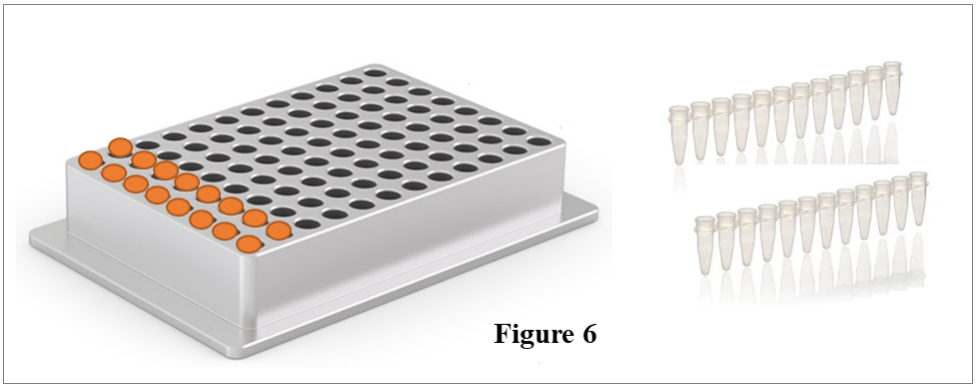


Figure 6

10. **Place the 96 position aluminum block containing the 2 PCR µL PCR strips tubes in SLOT 7 of the deck of each OpenTrons machine belonging to Station C**

11. **Prepare a second 96 position prechilled aluminum block (Figure 7) by placing on top of it the 100 µL PCR plate that was the output from Station B**

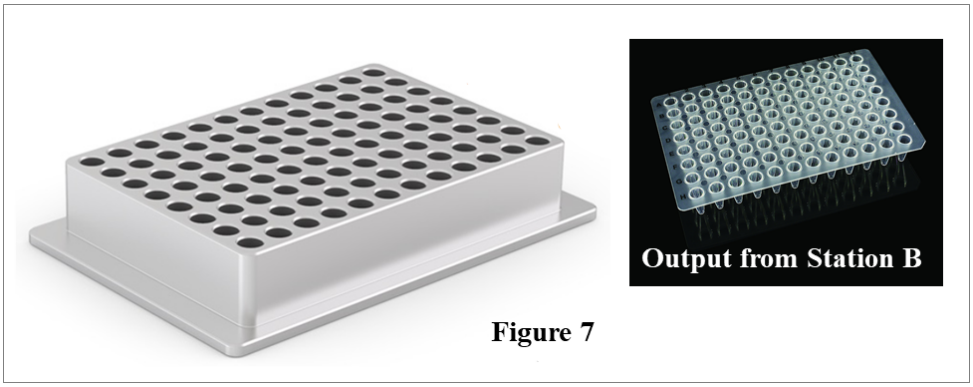


Figure 7

12. **Place the 96 position prechilled aluminum block with on top of it the 100 µL PCR plate that was the output from Station B in SLOT 1 of the deck of each OpenTrons machine belonging to Station C**

13. **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

14. **Run the Station C protocol whose file name should be "__.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

15. **Wait for the run to finish**
    - in the meantime, start preparing the RT-qPCR machine

16. **Collect the sterile NEST 100 µL PCR plate from SLOT 4 (containing 20 µL), which is be the process output**

17. Seal the sterile NEST 100 µL PCR plate from SLOT 4 before moving it to the PCR room

18. If the remaining extractions are required to be stored, collect and seal the 100 µL PCR plate with the remaining extractions (containing 22 µL) from SLOT 1 and keep it in freezer at - 20 °C

14. Cleaning/Disposal of the labware

- throw out
  - the used filter tips
  - the sterile 1.5 mL tubes
  - the NEST 100 µL PCR plate that was output from Station B
  - the 2 200 µL PCR strip tubes
- remove for cleaning
  - the 7 racks for 20 µL filter tips
  - the rack for 200 µL filter tips
  - the temperature module
  - the 2 different 96 position aluminum block
  - the 24 position aluminum block