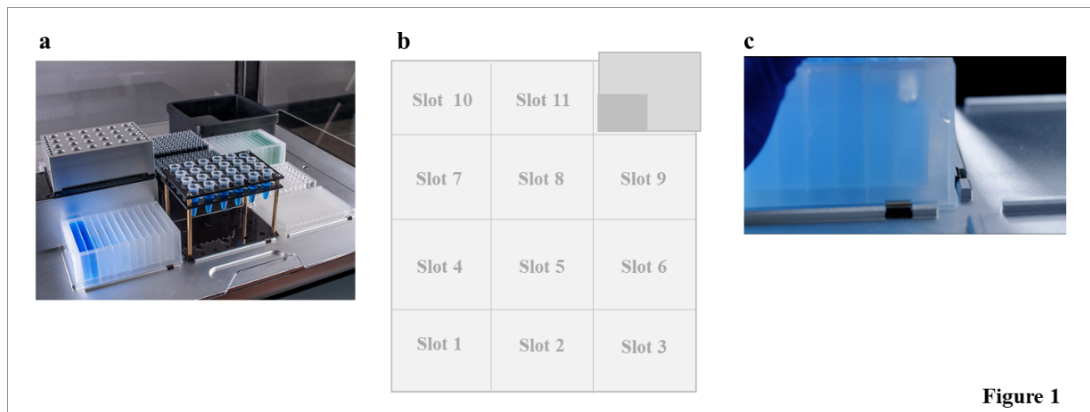


## OPERATING OPENTRONS MACHINES BELONGING TO STATION A

### ATTENTION

- Before starting check that the OpenTrons Machines belonging to Station A 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



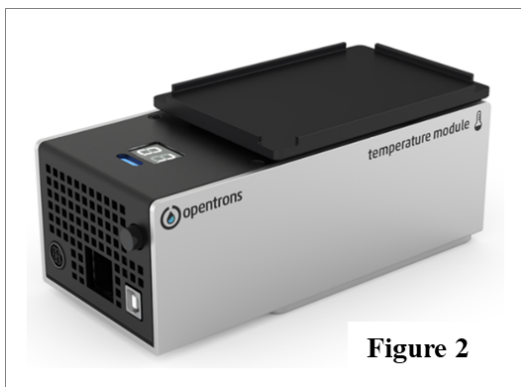
- make sure that the labware is inserted in the right direction, *i.e.* the well numbered as A1 or as 1 is at the top left corner

- **This procedure applies to the OpenTrons Machines belonging to Station A, which are equipped with a p20 multi channel and a p300 single channel pipettes**
- **Materials required for operating each OpenTrons Machine belonging to Station A**

Item	Quantity
Temperature module	1
Rack for 200 µL filter tips	2
200 µL filter tips	192 (96*2)
Rack for 20 µL filter tips	1
20 µL filter tips	96
Sterile 96 2 mL deep well plate	1
24 position tube rack	4
96 position aluminum block	1
200 µL PCR tube strips	2
Pipette for measuring 132 µL	1
Filter tip for measuring 132 µL	1
Internal extraction control RNA	2.112 mL (132 µL*16)
6 position tube rack	1
50 mL sterile Falcon tube	1
Pipette for measuring 1 mL	1
Filter tip for 1 mL	2
Graduated cylinder for measuring 20 mL	1
Incubator	1
Aluminum seal	1
Ethanol for cleaning the external surface of collection tubes	n.a.
Lysis buffer	15 mL
Proteinase K	1 mL

## STEPS

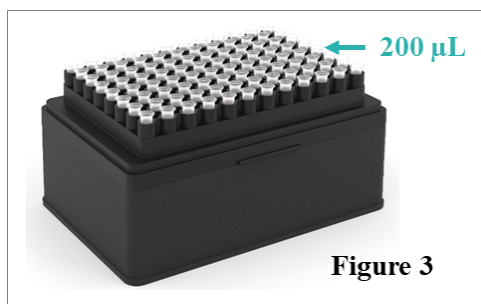
1. Place the temperature module in SLOT 10 of the deck of each OpenTrons machine belonging to Station A (Figure 1)



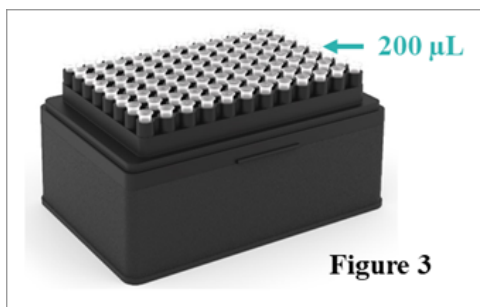
2. Start pre-cooling the temperature module to 4 °C. This is used to actively cool the internal extraction control RNA that will be added to each sample

3. Place

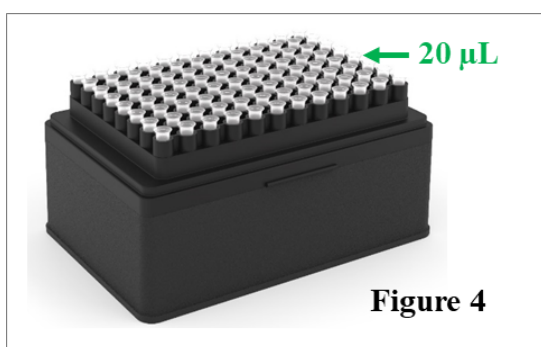
- a sterile rack full of 200  $\mu$ L filter tips in SLOT 9 of the deck of each OpenTrons machine belonging to Station A (Figure 3)



- a sterile rack full of 1000  $\mu$ L filter tips in SLOT 8 of the deck of each OpenTrons machine belonging to Station A (Figure 3)



- a sterile rack full of 20 µL filter tips in **SLOT 7** of the deck of each OpenTrons machine belonging to Station A (**Figure 4**)



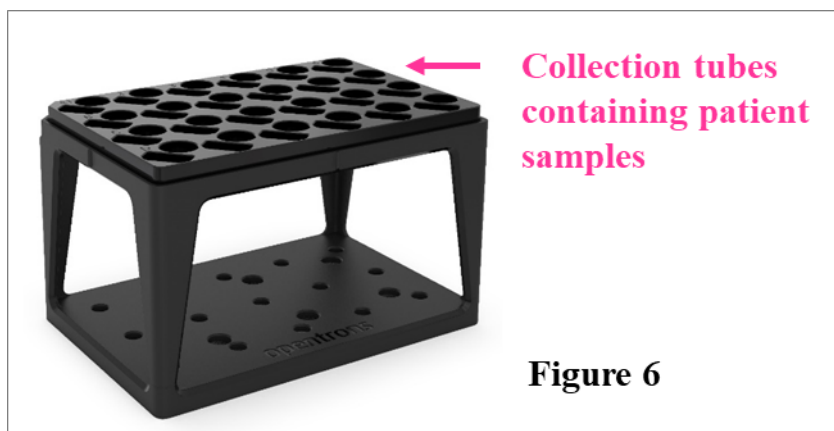
- an empty, sterile 96 2 mL deep well plate in **SLOT 1** of the deck of each OpenTrons machine belonging to Station A (**Figure 5**)



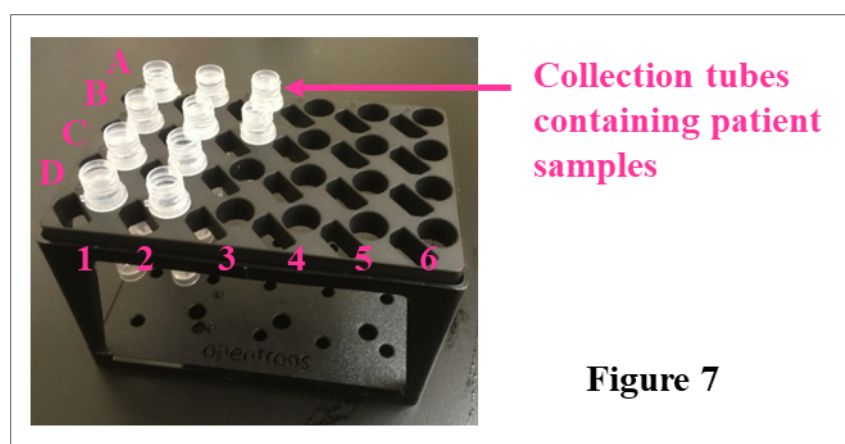
**4. Prepare 4 different 24 position tube racks for patient samples (*i.e.* Rack 1, Rack 2, Rack 3, Rack 4) as follows. Each rack can hold 24 collection tubes**

- Important notes
  - clean with ethanol the external surface of collection tubes before opening
  - observe best practices for safely handling the collection tubes full of patient sample
  - if needed, to give the collection tubes a flick so that all the liquid should sit on the bottom

- always place in position A1 of the Rack 1 a blank collection tube, *i.e.* a collection tube only containing the initial collection medium in which no swabs have been placed. These samples must result negative at the end of the testing process. This way we will have an extra control on the quality of the results from the very beginning of the process
  - always keep position A1 of the Rack 4 empty. This empty position will be used to add the positive control in the Station C
- uncap the collection tubes containing the samples from the patients and insert them in the 24 tube rack (**Figure 6**)



- place the first collection tube in the A1 position, the second one in the B1 position and so on. In other words, the rack has to be filled top to bottom, then left to right. (**Figure 7, Table 1**)



**Table 1a: Outline of Rack 1**

		Column					
		1	2	3	4	5	6
Row	A	BLANK	Sample 4	Sample 8	Sample 12	Sample 16	Sample 20
	B	Sample 1	Sample 5	Sample 9	Sample 13	Sample 17	Sample 21

	<b>C</b>	Sample 2	Sample 6	Sample 10	Sample 14	Sample 18	Sample 22
	<b>D</b>	Sample 3	Sample 7	Sample 11	Sample 15	Sample 19	Sample 23

**Table 1b: Outline of Rack 2 and Rack 3**

		Column					
		1	2	3	4	5	6
<b>Row</b>	<b>A</b>	Sample 1	Sample 5	Sample 9	Sample 13	Sample 17	Sample 21
	<b>B</b>	Sample 2	Sample 6	Sample 10	Sample 14	Sample 18	Sample 22
	<b>C</b>	Sample 3	Sample 7	Sample 11	Sample 15	Sample 19	Sample 23
	<b>D</b>	Sample 4	Sample 8	Sample 12	Sample 16	Sample 20	Sample 24

**Table 1c: Outline of Rack 4**

		Column					
		1	2	3	4	5	6
<b>Row</b>	<b>A</b>	<b>EMPTY</b>	Sample 4	Sample 8	Sample 12	Sample 16	Sample 20
	<b>B</b>	Sample 1	Sample 5	Sample 9	Sample 13	Sample 17	Sample 21
	<b>C</b>	Sample 2	Sample 6	Sample 10	Sample 14	Sample 18	Sample 22
	<b>D</b>	Sample 3	Sample 7	Sample 11	Sample 15	Sample 19	Sample 23

## 5. Place

- Rack 1 in **SLOT 2** of the deck of each OpenTrons machine belonging to Station A
- Rack 2 in **SLOT 3** of the deck of each OpenTrons machine belonging to Station A
- Rack 3 in **SLOT 5** of the deck of each OpenTrons machine belonging to Station A
- Rack 4 in **SLOT 6** of the deck of each OpenTrons machine belonging to Station A

## 6. Ensure the temperature module has reached 4 °C

**7. Prepare the 96 position aluminum block for internal extraction control RNA (Figure 8)**

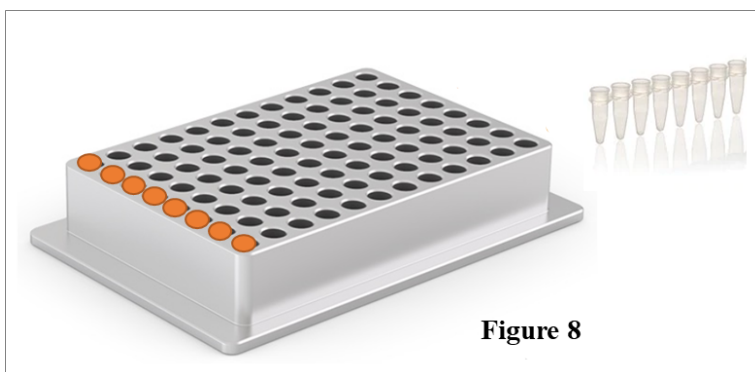


Figure 8

- by placing in column A and column B, a 200  $\mu$ L PCR tube strips, each tube containing 132  $\mu$ L of internal extraction control RNA ( 2 mL and 112  $\mu$ L in total)

**8. Place the 96 position aluminum block containing the 200  $\mu$ L PCR tube strips with the internal extraction control RNA on top of the temperature module already located in SLOT 10 of each OpenTrons machine belonging to Station A**

**9. Prepare the 6 position tube rack for Lysis buffer and Proteinase K (Figure 9)**



Figure 9

- by placing in A1 a 50 mL sterile Falcon tube containing
  - 15 mL of lysis buffer
  - 1.0 mL of proteinase K
- Important note
  - combine Lysis buffer and Proteinase K right before starting the run. Avoid preparing the mixture in advance as it can potentially deactivate the Proteinase K

**10. Place the 6 position tube rack containing the 50 mL Falcon tube with the lysis buffer and Proteinase K in SLOT 4 of each OpenTrons machine belonging to Station A**

- 11. Double-check all the labware to make sure it looks correct. Follow the outline reported below to verify the right positioning of the labware (Figure 10). Notably, it has to be checked 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 slot
  - the tubes are seated flat in their tube racks.
  
- 12. Run the Station A protocol whose file name should be “v1\_station\_a\_S9\_bp\_purebase.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



**Figure 10**



Deck of each OpenTrons machine belonging to Station A

13. **After the addition of the samples, lysis buffer, and proteinase K, the robot will pause**
  - remove the 96 2 mL deep well plate from SLOT 1
  - seal the 96 2 mL deep well plate with an aluminum seal
  - incubate the 96 2 mL deep well plate using the dedicated incubator
    - incubate at 55 - 57° C for 20 minutes
  - once complete, remove the seal and put back the 96 2 mL deep well plate in SLOT 1
  - Hit “Resume” and the robot will resume until the end of the run
14. **Wait for the run to finish**
  - in the meantime, start preparing what is required to run the OpenTrons machine belonging to Station B (see the Standard Operating Procedure for Station B)

- 15. Collect the 96 2 mL deep well plate from SLOT 1, which is be the process output**
- 14. Cleaning / disposal**
  - throw out
    - the used filter tips
    - the 200 µL PCR tube strip
    - the 50 mL sterile Falcon tube
  - remove for cleaning
    - the temperature module
    - the 4 diverse 24 position tube racks
    - the 96 position aluminum block
    - the 6 position tube rack
    - the graduate cylinder for measuring 20 mL
- 15. Remove and store in freezer (- 20 °C) the collection tubes containing the patient samples**
- 16. Clean the deck, the removable parts and the dedicated incubator following the instructions reported in the Standard Operating Procedure for Cleaning**