OPERATING OPENTRONS MACHINES BELONGING TO STATION B

ATTENTION

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

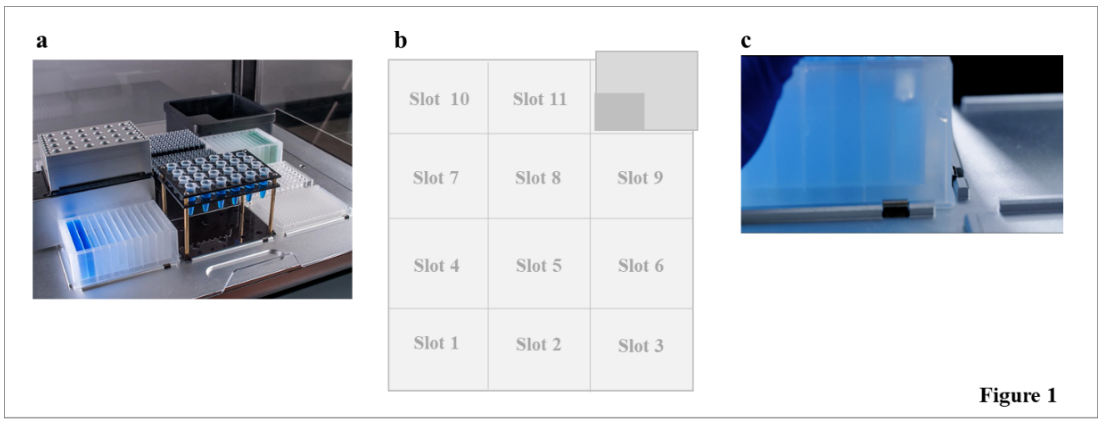

Figure 1

    - 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 B. Each of them has to be equipped with a p300 8 channel pipette**

- **Materials required for operating each OpenTrons Machine belonging to Station B**

Item	**Quantity**
NEST 1-well reservoir	2
Temperature module	1
Sterile NEST 100 µL PCR plate	1
Rack for 200 µL filter tips	4
96 200 µL filter tips	384 (96*4)
Magnetic module	1
5 mL Falcon tubes	2
NEST 12-well reservoir	1
Pipette for measuring 8 mL	1
Tip for 8 mL	1
Pipette for measuring 500 µL	1
Tip for 500 µL	1
Vortex	1
Pipette for measuring 10 mL	1
Tip for 10 mL	1
Pipette for measuring 3 mL	1
Tip for 3 mL	1
Pipette for measuring 8.5 mL	1
Tip for 8.5 mL	1
Graduated cylinder for measring 96 mL	1
Isopropyl alcohol	16 mL (8 mL*2)
100% Ethanol	70 mL
Distilled water	30 mL
Nuclease free water	3 mL
Magnetic beads	1000 µL (500 µL*2)

Wash buffer 1	50 mL (12.5*4)
Wash buffer 2	50 mL (12.5 mL*4)

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STEPS

1. **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**

2. **Place**
   - a first sterile 195 mL NEST 1-well reservoir (**Figura 2**) for collecting organic liquid waste in **SLOT 11** of the deck of each OpenTrons machine belonging to Station B

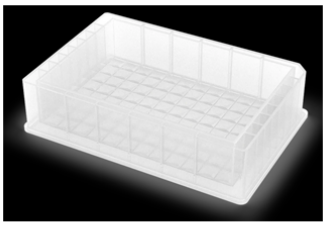


Figure 2

   - a sterile racks full of 200 µL filter tips in **SLOT 10** of the deck of each OpenTrons machine belonging to Station B (**Figura 3**)

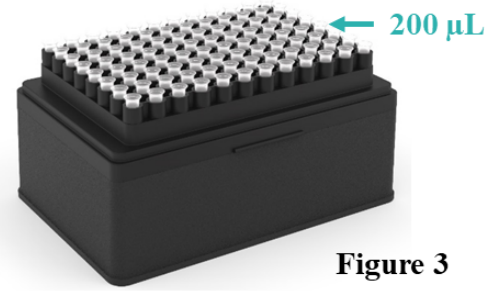
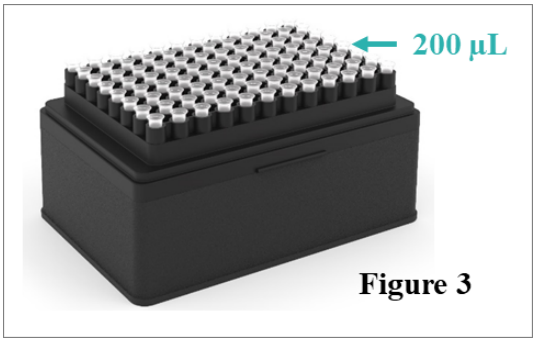
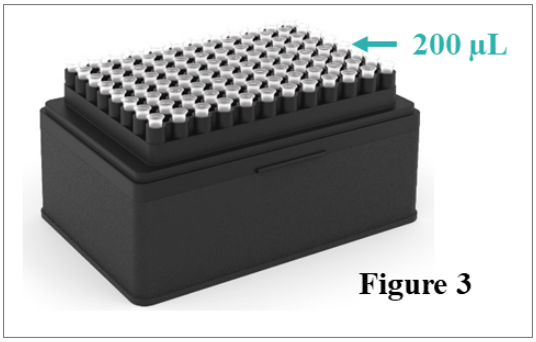


Figure 3

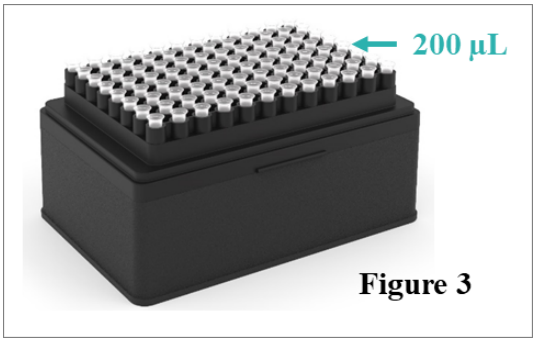
- a sterile rack full of 200 µL filter tips in **SLOT 9** of the deck of each OpenTrons machine belonging to Station B (**Figura 3**)



- a sterile rack full of 200 µL filter tips in **SLOT 6** of the deck of each OpenTrons machine belonging to Station B (**Figura 3**)



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



- the temperature module equipped with the 96 position aluminum block and an empty, sterile NEST 100 µL PCR plate in **SLOT 1** of the deck of each OpenTrons machine belonging to Station B **(Figure 4**)

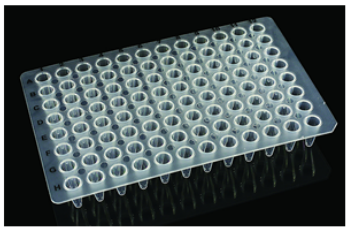


Figure 4

- a magnetic module with nothing on top of it in **SLOT 4** of the deck of each Open-Trons machine belonging to Station B (**Figura 5**)

Figure 5

3. **Prepare 2 sterile Falcon tubes of 15 mL as below described. At the end of the preparation each 15 mL Falcon tube (*i.e.* Falcon 1, 2) will contain 8 mL of isopropyl alcohol and 500 µL of magnetic beads**
    - in a sterile 15 mL Falcon tube, pipette 8 mL isopropyl alcohol
    - vortex (20 seconds at high speed) BP Genomics magnetic beads in their container
    - pipette 500 µL vortexed magnetic beads to the Falcon tube

4. **Use a second sterile 195 mL NEST 1 well reservoir to prepare the Reagent Trough 1 as below described**
    - measure in a 100 mL gradutae cylinder 70 mL of 100% ethanol and add 30 mL of distilled water
    - fill the reservoir with 96 mL of freshly diluted 70% ethanol **(Figure 6)**

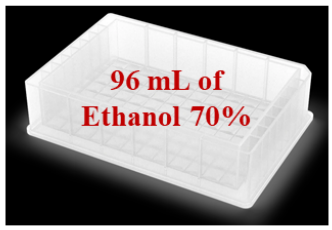


Figure 6

5. Place the Reagent Trough 1 in SLOT 2 of the deck of each OpenTrons machine belonging to Station B

6. Use a sterile NEST 12 well reservoir to prepare the Reagent Trough 2 as below described (Figure 7)

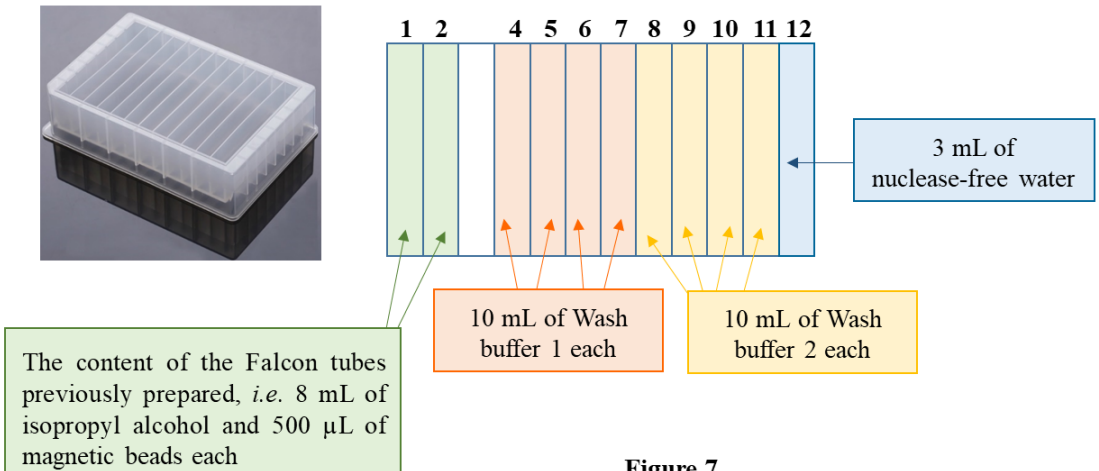


Figure 7

- pipette 3 mL of nuclease-free water to well 12
- pipette 12.5 mL of Wash buffer 2 to each of the wells 8, 9, 10 and 11 (for a total of 50 mL)
- pipette 12.5 mL of Wash buffer 1 to each of the wells 4, 5, 6 and 7 (for a total of 50 mL)
- vortex the 2 sterile Falcon tubes of 15 mL containing 8 mL of isopropyl alcohol and 500 µL of magnetic beads
- for each of the wells 1-2, pipette an entire Falcon tube into the well

- consequently, channel 1 will be filled with the entire content of the Falcon 1, channel 2 will be filled with the entire content of the Falcon 2.
    - please note that this step is time-sensitive. As soon as you pipette the beads into well 1, they will start to settle. Continue quickly through the next steps.

7. Place the Reagent Trough 2 in SLOT 5 of the deck of each OpenTrons machine belonging to Station B

8. Place the NEST 2 mL 96 deep well plate that was output from Station A onto the magnetic module already placed in SLOT 4 of the deck of each OpenTrons machine belonging to Station B (Figura 8)

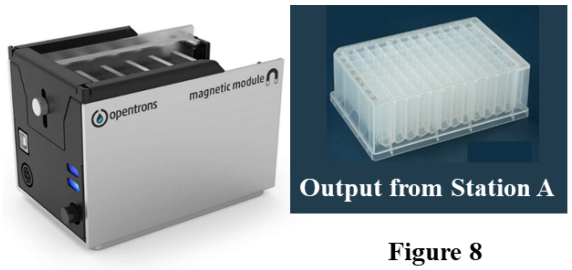


Figure 8

9. Check that the temperature module has reached 4 °C

10. 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 9). 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

Figure 9

Deck of each OpenTrons machine belonging to Station B

11. **Run the Station B 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

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

13. **When you're ready to move on to station C, collect the NEST 100 µL PCR plate sitting on the 96 position aluminum block on top of the temperature module in SLOT 1. This is the process output**
    - the NEST 100 µL PCR plate can be maintained about 1 h on Station B before moving to station C

14. **Cleaning/Disposal of the labware**
    - Throw out
        - the used filter tips
        - the NEST 1-well reservoir for collecting organic liquid waste
        - the NEST 2 mL 96 deep well plate
        - the Falcon tubes
        - the Reagent Trough 1 and the Reagent Trough 2
    - Remove for cleaning
        - the filter that were not used
        - the 4 racks for 200 µL filter tips
        - the temperature module
        - the 96 position aluminum block
        - the magnetic module