Arianna_Tronde_README.docx

Our protocols require user input which cannot be provided through the Opentrons App. For this reason, the protocols have to be run from a Jupyter Notebook. Please follow the subsequent steps to run each of our protocols:

File Name	Purpose
Arianna_Tronde_Protocol_1_Simulation.ipynb	File for simulating protocol 1* on Jupyter Notebook.
Arianna_Tronde_Protocol_1_Calibration.py	Calibration file for running protocol 1*. (To be run on OT-2 application).
Arianna_Tronde_Protocol_1_Executable.ipynb	File for executing protocol 1* on OT-2. (To be run on Jupyter Notebook)
Arianna_Tronde_Protocol_2_Simulation.ipynb	File for simulating protocol 2** on Jupyter Notebook
Arianna_Tronde_Protocol_2_Calibration.py	Calibration file for running protocol 2**. (To be run on OT-2 application)
Arianna_Tronde_Protocol_2_Executable.ipynb	File for executing protocol 2** on OT-2. (To be run on Jupyter Notebook)
plate_layout.xlsx	Microsoft Excel spreadsheet provided for the writing of plate layout. (Executable in the last cell of protocol 2**)

*Protocol 1 is for making (bulk) serial diluted (inducer) solutions.

** Protocol 2 is for sample preparation for promoter characterization.

Step 1: Calibration and deck set-up

Before running the protocol from a Jupyter Notebook, you need to upload its corresponding calibration protocol in the **python format (.py)** in the Opentrons App which consists of all the pipettes and labware used in the corresponding protocol. Once uploaded, the Opentrons App will recognise which pipettes to be attached and can calibrated as described in (Jones, n.d.). The Opentrons App will then display a layout of the deck and the labware (e.g., tips racks, well plates, etc…) which can now be positioned onto the deck.

Calibration should be done before running code on Jupyter Notebook since this is not supported by Opentrons-2 (OT-2) at the moment. Otherwise, the existing calibration from previous calibrations in OT-2 will be used.

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The deck layout for protocol 1 and 2 are shown in Fig. 1 and 2, respectively:

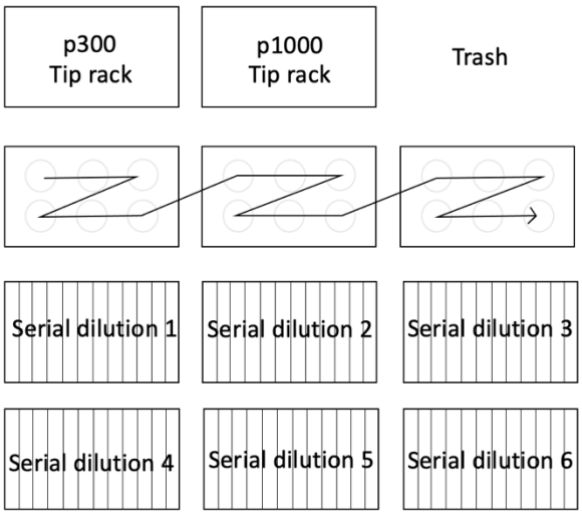

*Figure 1 - The deck layout for protocol 1. The maximum reservoir number and falcon tubes would be loaded according to the number of inducers to be diluted serially in one run. Loading of 5 or less reservoirs should follow the **order** of deck positions **as numbered** above to ensure correct functionality of the protocol. Falcon tubes containing the PBS should be loaded in the racks following the order as indicated by the arrow.*

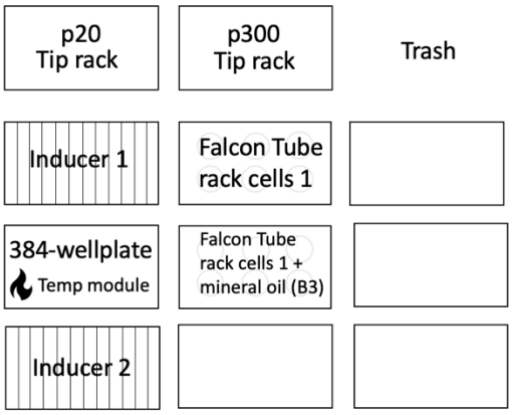

Figure 2 - The deck layout for protocol 2.

Step 2: Additional set-ups

Protocol 1: Each falcon tube should be filled with **40 ml of PBS** for the protocol to run properly. Calculate the total amount of PBS according to what the fold dilution is. The equation for the total amount of PBS needed is given by $\frac{volume\ of\ stock\ solution}{number\ of\ fold\ dilution} * (number\ of\ fold\ dilution - 1) * number\ of\ inducers$. The number of Falcon tubes needed is given by the equation $\frac{Total\ volume\ of\ PBS\ needed\ (mL)}{40\ (mL)}$.

Protocol 2: Each falcon tube should be filled with **30 ml of cell culture** to avoid submersion of the p300 pipette. Since the Greiner 384 well plate does not fit perfectly on the temperature module, this plate should be **pushed to the left upper edge** of the temperature module for pipetting to work as expected.

Step 3: Launching Jupyter Notebook

(Instructions obtained from Copperman, n.d., Jones, n.d. and Jones, n.d.)

Jupyter Notebook is pre-installed in each Opentron robot, which can be accessed through your preferred web browser.

1. Launching the Jupyter Notebook server:

Select '**Open**' under the '**Advanced Settings**' section in Opentrons Application (Fig. 3).

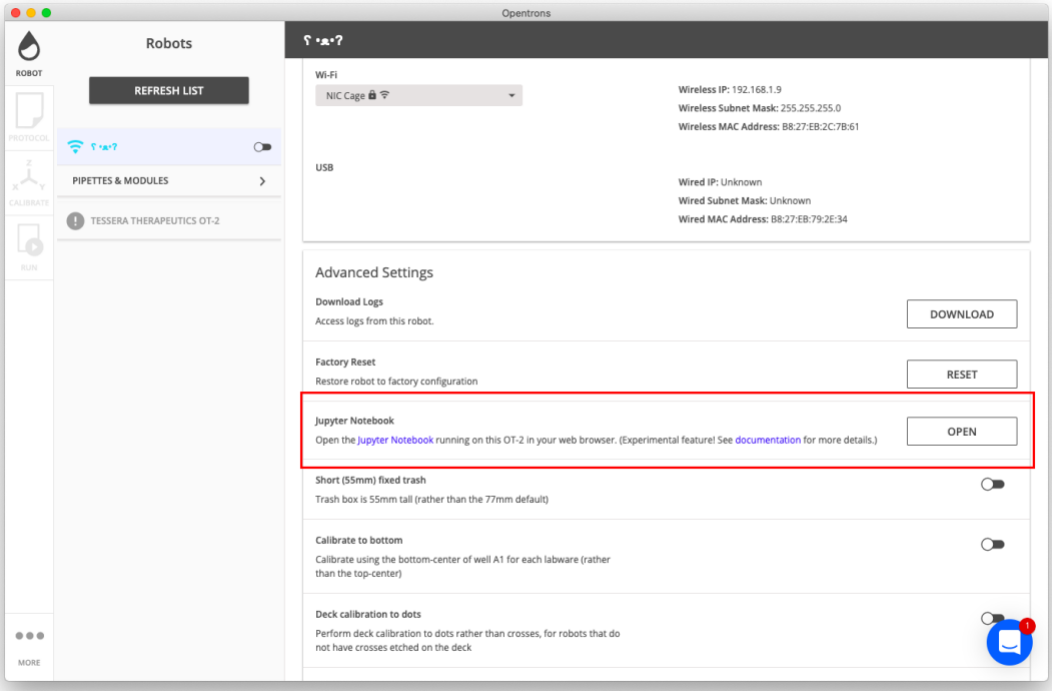

Figure 3 - Launching Jupyter notebook. Screenshot obtained from Opentrons n.d.

2. Upload a file

Select '**Upload'** on the upper right corner of the home page, as shown in Fig. 4.

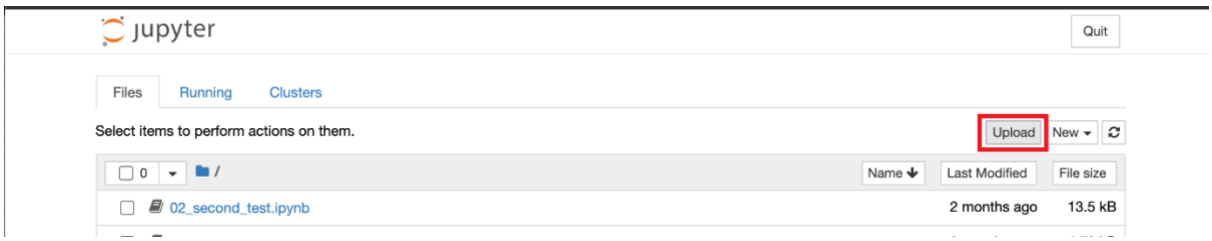

Figure 4 - Select 'Upload' (as highlighted with a red box) to upload a file.

3. Select the protocol

The protocol to be selected should be in **Jupyter Notebook format (file type: .ipynb).** Please take care not to select protocols in Python format (file type: .py) since the code written would not be adapted to run OT-2 via Jupyter Notebook.

The selected protocol file will be uploaded to **/var/lib/jupyter/notebooks** directory.

Please also upload the **Microsoft Excel spreadsheet** for the layout of the plate (plate_layout.xlsx) onto Jupyter Notebook. Please do not change the name of this Excel file as incorrect name may lead to unrecognizability by the code.

4. Running the protocol

Click on '**Run'** to run the cell containing the serial dilution/ main code. User input will be prompted in the output section. Please enter the desired parameters in the textbox underneath the questions and click 'Enter/Return' on your keyboard. It should take a few seconds before OT-2 starts running.

5. Exporting the plate layout in an excel file

After running the protocol, run the cell immediately below the main code to get the Excel file output of the plate layout generated by protocol 2. The Excel file represents the 384 well plate where each cell from the table provides information about the promoter, the inducer and the concentration used per well. For instance, P1_I1_C1 corresponds to promoter number 1, inducer number 1, concentration (of inducer) 1. Please note that the file name of the saved Excel spreadsheet should be precisely plate_layout.xlsx before the cell is being executed. This is crucial for Python 3 to recognise the file and know where to write the output into.

Note: If the cell is interrupted before fully running, the pipette may not remove its tip(s) automatically. To remove the tip(s), simply run the cells below the main code which instructs OT-2 to do so.

References

Copperman, A. J. (n.d.) *Opentrons Help Centre: Uploading files through Jupyter Notebook*. (Available from https://support.opentrons.com/en/articles/5107690-uploading-files-through-jupyter-notebook) [Accessed on 12th December 2021]

Jones, E. (n.d.) *Opentrons Help Centre: Get started: Calibrate the deck*. (Available from https://support.opentrons.com/en/articles/2687620-get-started-calibrate-the-deck) [Accessed on 12th December 2021]

Jones, E. (n.d.) *Opentrons Help Centre: Running the robot using Jupyter Notebook*. (Available from https://support.opentrons.com/en/articles/1795367-running-the-robot-using-jupyter-notebook) [Accessed on 12th December 2021]