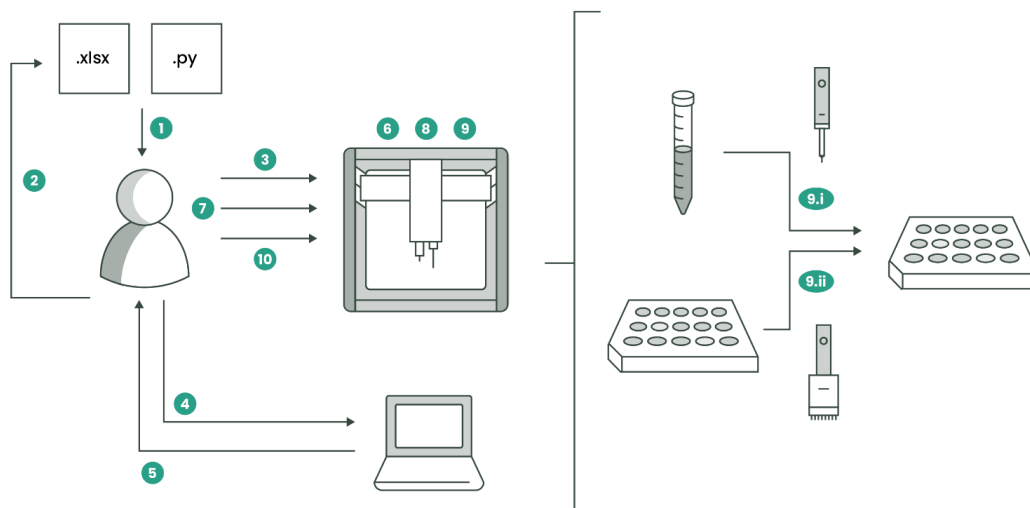


Instructions to use



1. Download the Excel template and Python script.
2. Fill the Excel file with the values of your variable to customise the experiment according to the guidelines at the [end of this document](#).
3. Send the file to the robot that you are going to perform the protocol. If you are using a Unix system, you can send the file using the command `scp` to the folder `/data/user_storage`

The command should look similar to

```
scp -i [password file] VariablesPlateIncubation.xlsx
root@[IP_Robot]:/data/user_storage
```

For more information about sending files to an OpenTrons robot visit <https://support.opentrons.com/s/article/Copying-files-to-and-from-your-OT-2-with-SCP>

4. Import the Python script to the OpenTrons App. A warning will appear on the protocol simulation because the script is not designed to read the Excel variable file from your computer but from the robot system.
5. Start setup in the robot you send the files. The OpenTrons app will simulate the script in the chosen robot, providing you with an error message if something went wrong or with the required labware layout and needed reagents. The colours of the reagents can change due to

them being generated randomly, but the volumes will always be the same ones given the same Excel file.

The volumes of the reagents are exactly what the protocol needs so it is recommended to pour in the falcon tubes between 1-2 mL more of that reagent.

On the other hand, the liquid of the samples in the source plates corresponds to 90% of the max volume that the well can hold, it is only a recommendation, it does not mean that is the volume needed of that sample.

6. (Optional) Turn on the HEPA filter to lower the chances of contamination.
7. Set the different reagents and labware as the App shows. In the setup tab the different labware and reagents will have labels to help you recognise where each one of them should be placed. The names of the labware will include the combination sample-media that will be created.
8. Perform a labware offset, ensuring all the labware is calibrated correctly.
9. Close the door of the Opentrons and press the button 'Start run'. The Opentrons robot will follow the following stages:
 - i) Transfer media to final labware with the single channel pipette
 - ii) Transfer samples to final labware with the multi-channel pipette
10. Wait for the protocol to finish and, if needed, change the tip racks as stated in the App.

Guidelines to fill in the Excel file

General Variables Sheet

Name Source Plate

- **Type of Value:** String
- **Description:** Opentrons API name of the labware with the samples that will be inoculated with different media. The labware must have eight rows so samples can be aspirated by multi-channel pipette.
- **Example:** vwrblueprintdepth105_96_wellplate_390ul

Number of Source Plates

- **Type of Value:** Number (Integer)
- **Description:** The total number of source plates that will contain the samples
- **Example:** 2

Name Final Plate

- **Type of Value:** String
- **Description:** Opentrons API name of the final labware in which the selected samples of the source plates will be mixed with the different media. It needs to have eight rows so samples can be dispensed by the multi-channel pipette.
- **Example:** vwrblueprintdepth105_96_wellplate_390ul

Volume of Sample to Transfer (uL)

- **Type of Value:** Number
- **Description:** Volume, in uL, that each sample will be transferred to the final labware.
Ensure there is enough volume for all the final plates and extra volume so the multi-channel pipette can pick all the required volume.
Make sure the multi-channel pipette can pick this volume as well, in other words, that this value is equal or greater than the min volume of the selected pipette.
- **Example:** 25

Name Medias

- **Type of Value:** String | List
- **Description:** Name of all the types of media separated by commas. Do not put any spaces or special characters in the names and make sure that the names correspond to the ones used in the variable 'Media(s) per plate' of the Sheet 'PerPlateVariables'.
- **Example:** Km, Amp

Volume of Media to Transfer (uL)

- **Type of Value:** Number
- **Description:** Volume, in uL, that will be transferred from each media type to each well in the final labware as set in the variable 'Media(s) per plate'.
Make sure that the single channel pipette can aspirate this volume, i.e., that the volume is equal or greater to the min volume of the selected pipette.
- **Example:** 100

Name 15mL Tuberack

- **Type of Value:** String
- **Description:** Opentrons API Name that will contain the 15mL falcon tubes with the media established in the variable 'Name Medias'.
- **Example:** opentrons_15_tuberack_falcon_15ml_conical

Pipette Variables Sheet

Name Right Pipette (Multichannel)

- **Type of Value:** String
- **Description:** Opentrons API name of the pipette that will be in the right mount of the Opentrons arm.
This pipette must have eight channels.
- **Example:** p300_multi_gen2

API Name Right Pipette TipRack

- **Type of Value:** String
- **Description:** Opentrons API name of the tip rack associated with the right mount pipette. This script does not check that the pipette and the tip rack are compatible.
If the tip rack from the right and left pipette are the same, both pipettes will pick up from the same tip rack, and the initial tip should be the same.
In case of pipette and tip rack incompatibility errors like “Arc out of bounds in the Z-axis” could happen
- **Example:** `opentrons_96_tiprack_300ul`

Initial Tip Right Pipette

- **Type of Value:** String
- **Description:** First tip that will be picked by the right pipette. Make sure that the tip starts with the A, because multi channels pick up entire columns and not just 1 tip.
- **Example:** `A1`

Name Left Pipette (Singlechannel)

- **Type of Value:** String
- **Description:** Opentrons API name of the pipette that will be in the left mount of the Opentrons arm. This pipette must have only one channel.
- **Example:** `p20_single_gen2`

API Name Left Pipette TipRack

- **Type of Value:** String
- **Description:** Opentrons name of the tip rack from which the left pipette will pick up tips from.
This script does not check that the pipette and the tip rack are compatible.
If the tip rack from the right and left pipette are the same, both pipettes will pick up from the same tip rack, and the initial tip should be the same.

In case of pipette and tip rack incompatibility errors like “Arc out of bounds in the Z-axis” could happen.

- **Example:** `opentrons_96_tiprack_20ul`

Initial Tip Left Pipette

- **Type of Value:** String
- **Description:** First tip that will be picked by the left pipette.
- **Example:** `True`

Replace Tiprack

- **Type of Value:** Boolean
- **Description:** Value that will determine that, in case of more than one tip rack needed for the run of the protocol, the tip rack will be replaced and not added to the labware layout.
If the single and multi-channel tip racks are the same, and this variable is set as True, only one slot in the layout will be occupied by tip racks.
This variable only accepts two values, True (tip racks will be replaced) or False (tip racks will not be replaced but added to the layout).
- **Example:** `True`

Per Plate Variables Sheet

Samples per plate

- **Type of Value:** Number (Integer)
- **Description:** Number of samples that the plate contains. Take in account that this number will be considered from the well stated in “First Well With Sample”.
- **Example:** `54`

Media(s) per plate

- **Type of Value:** String | List
- **Description:** List of all media that the source plate samples will be inoculated with.
This variable will determine how many final plates this source plate will create. There is going to be created 1 plate per type of medium. If the

user wants to create 2 different plates with the same medium, they must put the same medium twice in this variable. The different media should be comma separated.

- **Example:** Amp, Amp, Km

First Well With Sample

- **Type of Value:** String
- **Description:** First well from the plate that contains a sample that is going to be transferred to the final media-sample combination plate(s). Take in account that the multi-channel, the pipette that takes care of transferring the samples, takes entire columns and no single wells. For instance, if this value is set as C2, regardless of having or not samples in A2 and B2, all the samples of the column will be transferred to the final plates because tips will be attached, and they will aspirate. Nevertheless, there will be no media in the wells of the rows that according to this variable, does not have a samples. For example, if the first well with sample is C5, in the final plate the wells A1 and B1 will not have any media and the media transferring will start in the C1 well.
- **Example:** A2

* If needed, this sheet can have more columns to the right, this meaning that if the Opentrons have more than 11 available slots, the sheet can be expanded and filled. This column adding does not guarantee that more final plates can be created because that result depends on more variables, such as the tip rack replacement value or the different combinations media-sample.

The columns of the plate can have any name, these names will be included in the final layout that you will be able to see in the OT-App to help you recognize where each source plate must be placed. Nevertheless, if there is an error that is cause by any of the variables of this sheet, the name of the source plate will be given by the index of the column, being Source Plate 1 the one in furthest on the left.