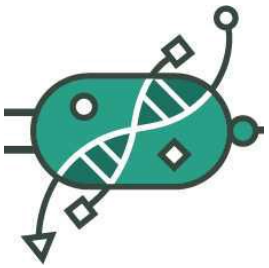
Sep 11, 2024 Version 3

OT-2 Media dispensing and culture inoculation protocol V.3



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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is meant to distribute reactive(s) (i.e bacterial culture media) into final 96-well plates with a single channel pipette and then transfer culture samples from source plate(s) to these plates with a multi-channel pipette.
In our laboratory, this protocol has been used as part of the "High-throughput workflow for the genotypic characterization of transposon insertion library variants" also available in protocols.io to prepare cultures plates to perform a subsequent counter-selection.

To run this protocol a python script in LAP format for an Opentrons OT-2 robot and an excel file with variables such as culture volume, transfer volume etc... This protocol provides a set of instructions or description of the **LAP repository** entry **LAP-CellMediaInoculation-OT2-2.0.0.** You can find the script and complementary information for this specific version of the protocol in this **LAP entry link** and **GitHub link to LAP entry documents**

The major changes from previous version are:

- **New variables are added to GeneralVariables sheet**: *Change Tip In Media Distribution*, *Change Tip In Sample Transfer*, *Position Transfer Sample*, *Touch Tip After Transferring Sample*, *Touch Tip In Distribution Media*, *Mixing Volume Before Sample Transfer (uL)*, *Number Times of Mixing Volume* and *Flow Rate Mixing*.
- **Change of name variable *Name 15mL Tuberack*** which is called now *Name Tuberack*
- **New variables are added to PerPlateVariables sheet**: *Number of Replicas*, *Only Media(s) Plate Creation* and *Only Sample(s) Plate Creation*
- **The program now accepts falcon tubes of 15ml and 50ml**
- **Description of robot and protocol setup in a separate protocols.io entry** (Setting and Customizing OT-2 for LAP Entries)

For all the new variables you can find more information in the PDF attached in the first step of this protocol or in the LAP entry of this program in the LAP entry link given before

Guidelines

This protocol was run in a python 3.7.1, OT App Software Version 7.0.2 and opentrons API level version 2.14 in a Linux 4.14.74 system (these are the OT-2 specifications). In the script several packages are used: pandas (0.25.3), openpyxl (3.1.2), math, random

It has been tested with cultures from *Pseudomonas putida KT2440* as part of the High-throughput workflow for the genotypic characterization of transposon library variants. LB and M9-citrate media with either kanamycin, gentamicin, streptomycin or ampicillin haven been successfully run.

The maximum number of 96-well final plates per run given **1 source plate**, 2 different types of tip racks (with the replacement of the tip rack set as True) is **7 final plates** (use 1 falcon tube rack and 2 tip racks)

Materials

Software

- Python 3.7.1
- opentrons software version 7.0.2
- python packages: pandas (0.25.3), openpyxl (3.1.2), math, random
- OT App
- Excel

OT-2 Labware

- Opentrons Tip racks

Equipment	
Opentrons 96 Tip Rack 300 µL	NAME
Tip rack	TYPE
Opentrons	BRAND
-	SKU
https://labware.opentrons.com/opentrons_96_tiprack_300ul?category=tipRack	LINK

Equipment

Opentrons 96 Tip Rack 20 µL	NAME
Tip rack	TYPE
Opentrons	BRAND
-	SKU
https://labware.opentrons.com/opentrons_96_tiprack_20ul?category=tipRack	LINK

- 96-well plates

Equipment

96-well plates, flat bottom, non treated	NAME
Cell culture plates	TYPE
VWR	BRAND
734-2781	SKU
https://es.vwr.com/store/catalog/product.jsp?catalog_number=734-2781	LINK

- Opentrons Falcon Tube Rack

Equipment

Opentrons 15 Tube Rack with Falcon 15 mL Conical	NAME
OT Tube Rack	TYPE
Opentrons	BRAND
-	SKU
https://labware.opentrons.com/opentrons_15_tuberack_falcon_15ml_conical/	LINK

- 15mL Falcon tubes

Equipment

Falcon® Conical Centrifuge Tubes 15mL	NAME
Flaocn Tube	TYPE
Falcon	BRAND
352096	SKU
https://ecatalog.corning.com/life-sciences/b2c/US/en/Liquid-Handling/Tubes,-Liquid-Handling/Centrifuge-Tubes/Falcon%C2%AE-Conical-Centrifuge-Tubes/p/falconConicalTubes	LINK

Equipment:

Equipment

OT-2	NAME
Liquid handler	TYPE
Opentrons	BRAND
OT-2	SKU

Equipment

HEPA Module	NAME
Opentrons	BRAND
OT-2-HEPA	SKU
https://opentrons.com/modules/hepa-module/	LINK

Equipment

Single Channel Electronic Pipette (GEN2) 1000uL	NAME
Opentrons Single Channel Pipette	TYPE
Opentrons	BRAND
-	SKU
https://shop.opentrons.com/single-channel-electronic-pipette-p20/	LINK

Equipment

8 Channel Electronic Pipette (GEN2) 20uL	NAME
Multi channel pipette	TYPE
Opentrons	BRAND
-	SKU
https://shop.opentrons.com/8-channel-electronic-pipette/	LINK

Safety warnings

It is important to use HEPA module to work in sterility

Before start

Note that the source and final 96-well plates will follow the same order (If the wells of a column of the source plates has empty wells the multichannel will aspirate in that well) but media will not be dispensed in those corrresponding final wells

Files Preparation

1 **Preparing Customized Template**

Preparing the template (a .xlsx) with the specific variables for each experiment and a .pdf that contains the instructions on how to fill the template

Here we attach one Excel with several sheets:

1. **GeneralVariables:** variables related mainly to the labware that is going to be used
2. **PipetteVariables:** variables related to the pipettes that are going to be used
3. **PerPlateVariables:** variables associated with the specifications of each source plate

CellAndMediaInoculationInstructions…

TemplateVariablesCellInoculation.xlsx

> Note
>
> The most updated Excel template can be found in the **LAPrepo Repository Page**

1.1 *Fill the template with the corresponding values*

1.2 *Store it as VariablesPlateIncubation.xlsx*

> Note
>
> The file should be spelt **precisely** as *VariablesPlateIncubation.xlsx* or the Python script won't read it

Setting the robot

2 **Prepare the system of the robot to run the protocol**

For this protocol to work we need to transfer the *VariablesPlateIncubation.xlsx* to the directory */data/user_storage* of the OT system that we will use to perform the protocol

As well, if we are using custom labware we need to upload it to the OT App and send it to the directory */data/labware/v2/custom_definitions/custom_beta* if the labware is not there yet.

Finally, we need to make sure the package *openpyxl* is installed in the robot system

We can do this entire step by following the protocol *Setting and Customizing OT-2 for LAP Entries* with the specifications given in the text above

Protocol

NAME

Setting and Customizing OT-2 for LAP Entries

CREATED BY

biocomp.cbgp Biocomputation Lab

PREVIEW

Run Protocol

3 **Load script in OT-App**

Now that we have transferred the variable files to the robot, we can load the script and run it in the selected robot

Note

This whole step has been developed and tested with version 7.0.2 of the OT-App

Indications may vary from version to version of the opentrons App and the version of the script.

Software

NAME	OS	DEVELOPER	SOURCE LINK
Opentrons App	Windows >=10, Mac >=10 , Ubuntu >=12.04	Opentrons	https://opentrons.com/ot-app/

3.1 *Load the script in the App*

Protocols -> Import -> Drag Python script

This version of the protocol was developed when the last version available of **LAP-CellMediaInoculation-OT2** was the 2.0.0 which script you can find attached

ScriptPlateGenerationAndIncubation…

The name of the python file is user's choice, it will work with any name in the app.

Note

The last script version can be found at https://github.com/BiocomputationLab/LAPrepository/tree/main/LAPEntries in the directories with the name **LAP-CellMediaInoculation-OT2** followed by the version.

As well we can find the latest version of the script at **LAPrepo Repository Page** with the same name as in GitHub

Software

NAME	DEVELOPER	SOURCE LINK
LAP Repository	https://biocomputationlab.com/	www.laprepo.com

Note

The App with version 7.0.2 analyzes your protocol before setting a robot to run, so the labware will not be shown before assigning the protocol to a specific robot when you import it into the App.

3.2 *Select Robot to Perform Script*

Click in the protocol -> Start setup -> Choose the OT where the file *VariablesPlateIncubation.xlsx* is -> Proceed To Setup

After clicking on Proceed to Setup, you should obtain the labware positions in the *Labware* tab and the reagents, with their corresponding volume, in the *Liquids* tab.

In case the protocol with the set variables cannot run, an error will occur during the run of that simulation. Many errors are contemplated already and have a specific message that hints the user what could have gone wrong.

Note

The volume of the initial samples is established to be 90% of the max volume of the well, but this is only a recommendation. **Just make sure that there is enough volume to transfer to all the final plates.**

On the other hand, the volume of the reagents is precisely what is needed, so it is **suggested always to add more to consider the pipetting error**.

Note

It is recommended that you perform a labware position check.

You can do it with test plates after loading the script but before cleaning the surface. That way, you reduce the probability of contamination (using test plates and labware) and pipetting errors (position check).

4 Run Protocol in OT

4.1 *Make sure the needed calibrations are done*

Pipettes, tip racks and tip length calibrations need to be done for the items used in this run.

4.2 *Labware position check is performed (if needed)*

4.3 *Clean the surface of the robot with 70% ethanol to clean and disinfect the surfaces*

Note

Check the Opentrons page https://support.opentrons.com/s/article/Cleaning-your-OT-2? for more information about cleaning the OT-2 robot with the proper materials.

4.4 *Set the labware and reagents as shown in the OT-App*

4.5 *Start Run*

The procedure that the robot is going to do is mainly divided into 2 parts:

1. Distribute each reactive to the final plate(s) with the single-channel pipette
2. Distribute samples to the final plate(s) with the multi-channel pipette

Expected result

One or more plates with different reagents and same samples as set in the input variable file

This set of plates will be given for each source plate the user has provided with the reagents the user has provided, as well, the number of them is dependent of how many replicas the user has set

After-Running

5 **Retrieve labware from the OT**

Example

1h

6 **We have 2 source plates, only with 96 samples and the other with 50.**

With the first source plate we want to create 1 plate with only samples from the source plate. With the second source we want to create 2 final plates, 1 with Ampicilin and another with Kanamycin inoculated with these samples starting to transfer samples from the 3rd column of the source plate. As well, we want to create 1 replica of those 2 plates.

Finally, we want to create 1 additional plate without only Ampicilin in 73 wells starting from the well C2

We need to change the tip during the sample transferring to be every time the pipette aspirates from the source plate but we just need the tip during the distribution of the media to change everytime it changes of reagent.

We will use a computer with a Windows 10 system.

6.1 Fill the excel template that we can find go to step #1 filled with the name *VariablesPlateIncubation.xlsx*

10m

VariablesPlateIncubation.xlsx

6.2 Upload custom labware to app and robot system

1m

We are using a custom labware called *vwrblueprintdepth105_96_wellplate_390ul* that has been created with the labware creator that opentrons offers (**https://labware.opentrons.com/create/**)

vwrblueprintdepth105_96_wellplate_… 11KB

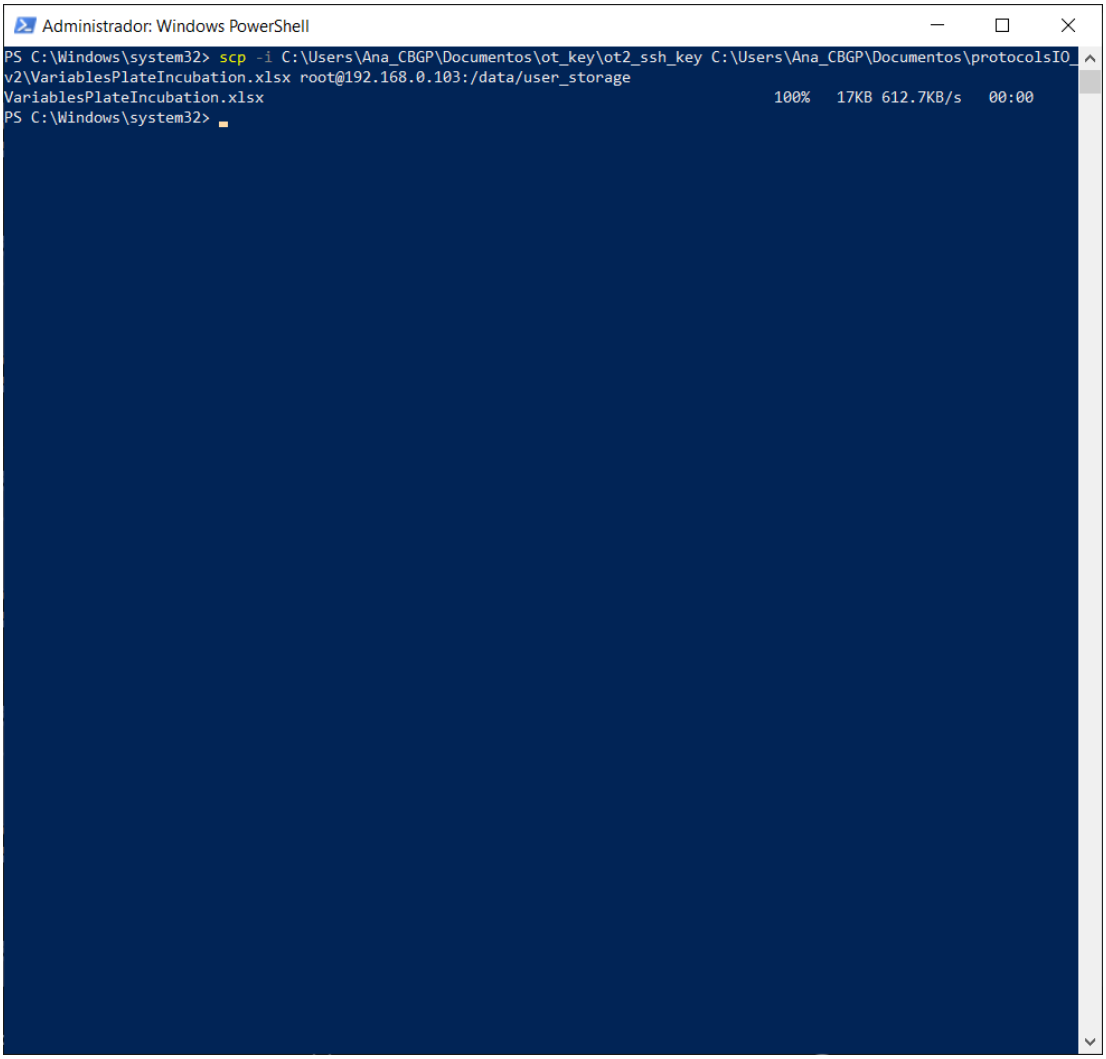
We upload it to the opentrons app (make sure that is in the robot app) and the robot system as stated in the protocol in step Setting and Customizing OT-2 for LAP Entries

vwrblueprintdepth105_96_wellplate_…

6.3 Export the variable file to *the /data/user_storage* folder in the robot. 2m

For more information about sending files to the OT-2

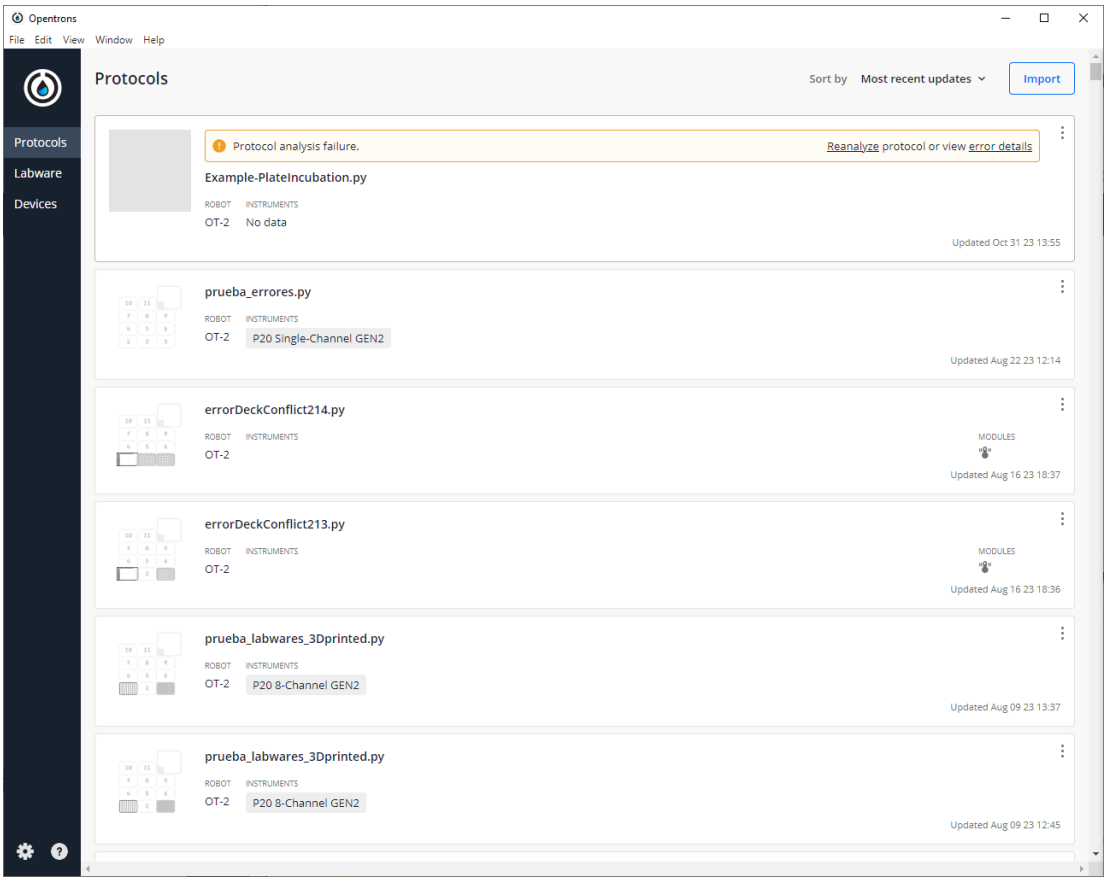
go to step #2 Setting and Customizing OT-2 for LAP Entries



command line window with scp commands to transfer the variables .xlsx from our computer to the OT-2

6.4 Import the python script downloaded from go to step #3 (I named it *Example-PlateIncubation.py*) to the OT-App 30s

Example-PlateIncubation.py



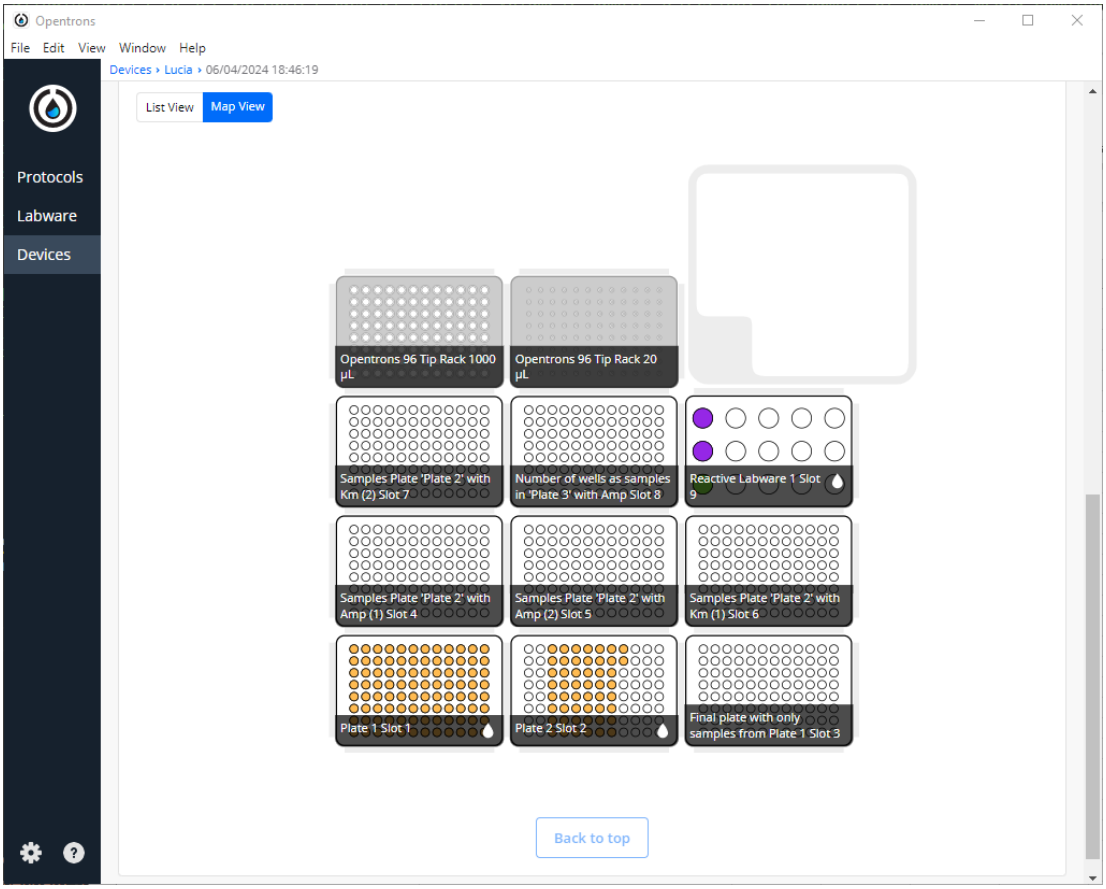
Result of importing the Python script in the OT-App

As we can see, we have an error, but that is programmed because the script is meant to work in the robot but not in your computer

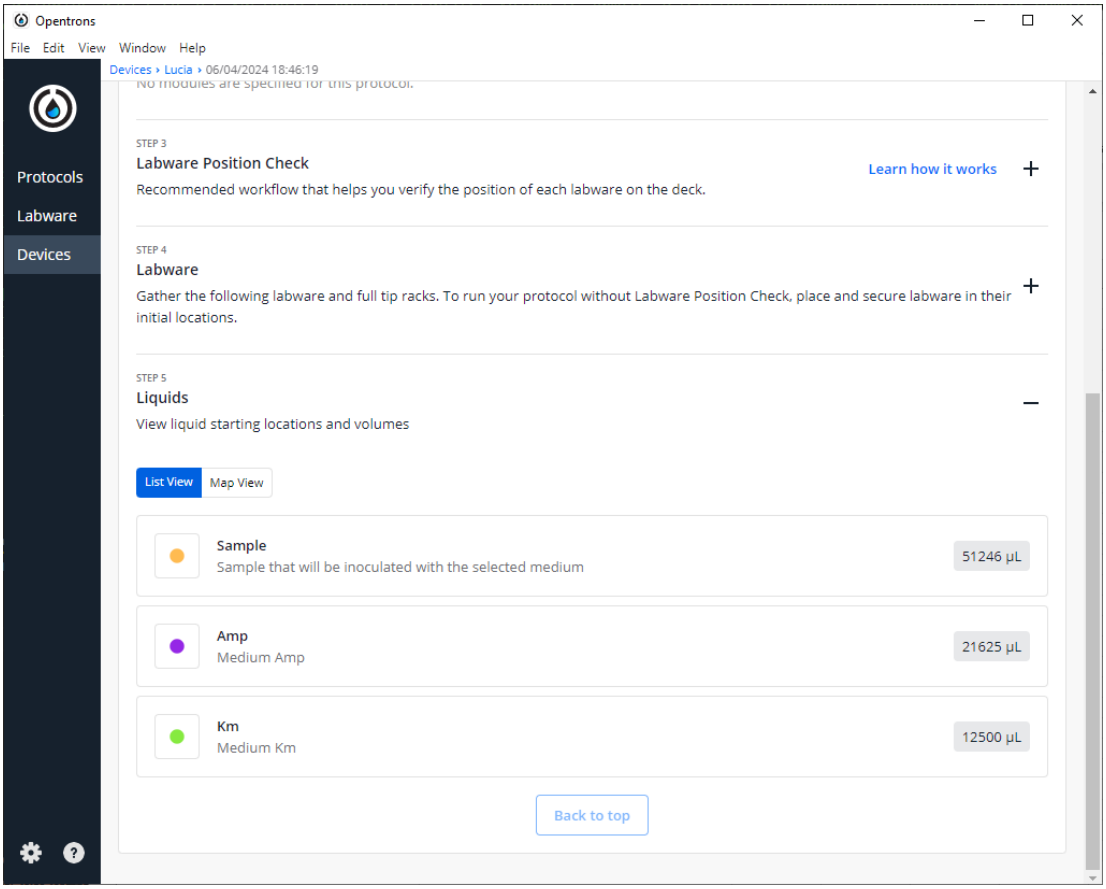
6.5 Run the protocol in the robot that we have transferred the Excel file 1m

***Example-PlateIncubation.py* -> Start setup -> Select** the **robot** in which we are going to run the protocol

If we do not have any errors, the output should look like the following pictures.



Labware and liquid set-up layout



Volumes of the antibiotics needed to perform the protocol

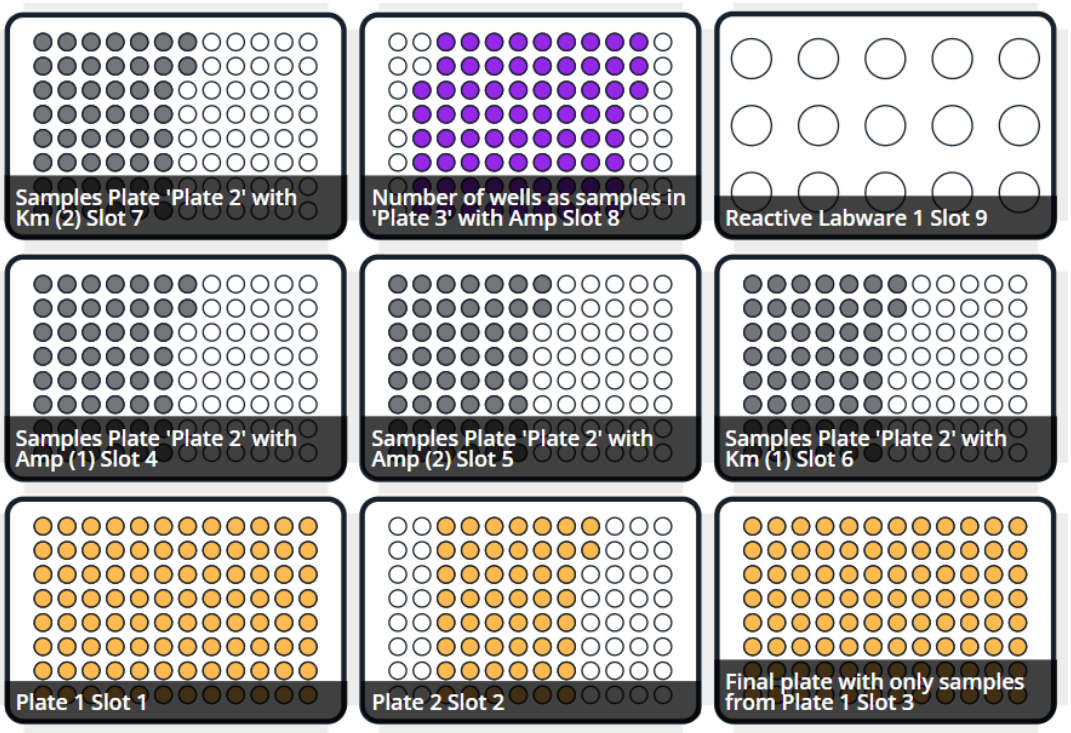
6.6 Turn the HEPA filter module 30s

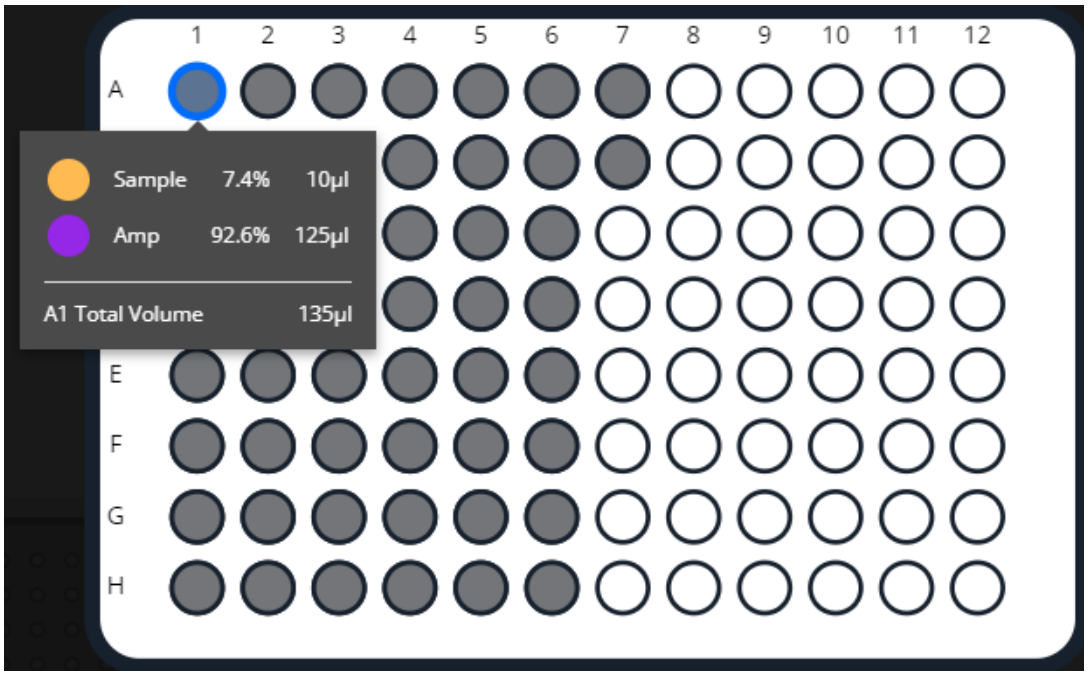
6.7 Clean platform of the robot that we are going to perform the protocol 2m

6.8 Prepare all reagents and labware in the places the App is showing and take into account the notes in step go to step #3.2 Notes 5m

6.9 Start Run 32m

Expected result





Example of the content of A1 in the labware *Samples Plate 'Plate 2' with Amp (1) Slot 4*

Here, we will obtain the mix between the volume of media and the samples set in the variable file in the final plates as well as the finla plates with only samples of media as we state in the excel. These positions are seen in the first image by the grey wells, and we can see the info on the plate and the media in the name of the labware and an example of the composition of 1 well from the plate called "Samples Plate 'Plate 2' with Amp (1) Slot 4" in the second picture.

6.10 Retrieve labwares from the OT 5m

Protocol references

pBLAM1-x: standardized transposon tools for high-throughput screening *(Synthetic Biology)* https://doi.org/10.1093/synbio/ysad012

The Laboratory Automation Protocol (LAP) Format and Repository: A Platform for Enhancing Workflow Efficiency in Synthetic Biology (*ACS Synth. Biol.*) https://doi.org/10.1021/acssynbio.3c00397