Oct 22, 2025 Version 4

OT-2 Counter-Selection V.4

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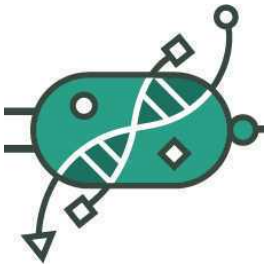

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Protocol status: Working
We use this protocol and it's working

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Protocol Integer ID: 101231

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Abstract

This protocol is meant to select samples from one or more source plate(s) that has 2 different selecting conditions: one condition gives higher values and the other lower values (i.e different fluorescence values or OD_{600nm} values to indicate growth/no growth). From these selected samples this protocol creates final plates with given reactive(s) (i.e media with different antibiotics or inductors) and inoculates samples from a source plate.
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 select variants to keep as glycerol stock and perform subsequent PCRs to assign a genotype. In particular, this protocol is used for selecting bacterial variants grown in two 96-well plates with different antibiotics by measuring OD_{600nm} after overnight growth. Bacterial variants are selected based on growth/no growth and inoculated in final plates.
Final plates consist of 96-well format plates with molecular biology grade water to perform subsequent PCR on selected samples and 2 stock plates containing 30% glycerol to perform bacterial stocks of selected samples.

To run this protocol a python script for an Opentrons OT-2 robot and an excel file with several variables are needed . This protocol is a set of instructions or description of the **LAP repository** entry **LAP-ColonyCounterSelection-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:

- **Change of name variable API *Name Rack 15mL Falcon Reactives*** which is called now *API Name Rack Falcon Reactives*
- **The program now accepts falcon tubes and tube racks of 15ml or 50ml.** It does not accept mix tube racks.
- **Description of robot and protocol setup in a separate protocols.io entry** (Setting and Customizing OT-2 for LAP Entries)
- Sheets that corresponds to the map of values to compare now need to have the **name of the rows and columns of the source labware**

Guidelines

This protocol was developed with python 3.7.1, OT App Software Version 6.3.1 and 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 and numpy (1.15.1)

It has been tested with cultures from *Pseudomonas putida KT2440* as part of a counter-selection step in the High-throughput workflow for the genotypic characterization of transposon library variants.
30% glycerol and water have been successfully dispensed using this protocol.

The maximum number of 96-well plates per run given 1 source plates, 1 pipette and replacement of the tiprack set as True is 8 final plates (using 1 tip rack and 1 tube rack for falcons tubes).

Materials

Software

- Python 3.7.1
- opentrons software version 7.0.2
- python packages: opentrons (7.0.2), pandas (0.25.3), openpyxl (3.1.2), numpy(1.15.1), 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

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

- 15mL Falcon tubes

Equipment

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

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) 300uL	NAME
Opentrons Pipette	TYPE
Opentrons	BRAND
-	SKU
https://shop.opentrons.com/single-channel-electronic-pipette-p20/	LINK

Safety warnings

It is important to use HEPA module to work in sterility

Before start

Note that this protocol needs 2 values to do a selection for each source plate

Files Preparation

1 **Preparing Customized Template**

Preparing the template (a .xlsx) with the specific variables for each experiment.

Here we attach a template of the variable file with several sheets and a PDF file explaining each variable:

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 related to the specifications of each source plate
4. **Sheet(s) for comparable values (OD, fluorescence, etc):** values that will be compared against the threshold established in the PerPlateVariables sheet → *Not in the template but should be present in the final file*

TemplateVariablesCounterSelectio...

CounterSelectionInstructionsv200....

Note

The most updated Excel template can be found in the **LAPrepo page with all the available entries**

1.1 *Fill the template with the corresponding values*

1.2 *Store it with the name VariablesCounterSelection.xlsx*

Note

The file should be spelt **exactly** *VariablesCounterSelection.xlsx* or the Python script won't work correctly

Setting the robot

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

For this protocol to work we need to transfer the *VariablesCounterSelection.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

Biocomputation Lab

Preview

Running 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	REPOSITORY
Opentrons App	Windows >=10, Mac >=10 , Ubuntu >=12.04	Opentrons	https://opentrons.com/ot-app/

3.1 *Load the script in the OT App*

Protocols → Import → Drag Python script

CounterSelectionScript_v200.py

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. The name of the directory should be **LAP-ColonyCounterSelection-OT2** followed by the version.

As well we can find the latest version of the script at **https://www.laprepo.com/repository/** with the same name as in GitHub

Software

NAME	DEVELOPER	REPOSITORY
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 *VariablesCounterSelection.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. A lot of errors are contemplated already and have a specific message that gives a hint of 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 it 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 exactly what is needed, so it is **suggested to pour always more to take into account the error of pipetting**

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 the 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 4 parts:

1. Select colonies that comply with the selected parameters (threshold)
2. Distribute reagents to respective plates (if set in the input file)
3. Distribute to all plates the selected samples
4. Generate identity maps (to be exported in the following steps)

Expected result

Several plates, with different reagents but the same samples in the same order, in addition to a **file with all the map(s) (XLSX file) located in the folder */data/user_storage*** that will give the position in these plates with their identifiers (location in the source plate)

This set of results will be given for each source plate the user has provided

After-Running

5 **Retrieve labware from the OT**

6 **Import map(s) from robot**

There will be 1 final file called [Name Final File Maps].xlsx with as many sheets as source plates. Each sheet will have the name set in the variable 'Final Map Name' for the corresponding source plate

To retrieve it, we can go to step #2 Linked Protocol and reproduce partially the protocol given there it by transferring the files from the robot to the computer.

The map will be in the directory */data/user_storage*

Take in account that files overwrite, so if you have given the same name to the final map in 2 consecutive runs, you will get only the results of the last run

Expected result

The map contains the identity (position in original plate) of the samples selected in the places that they have been placed/distributed being the name of the column as the name of the plate

Example

1h 53m

7 **We want to select the samples that have an OD_{600nm} higher than 0.5 in the Antibiotic Transposition genome file and have an OD_{600nm} lower than 0.5 in the Antibiotic ampicillin plasmid file in one of the source plates and the same selection in the second one but with the value 0.6**

After selecting the samples, we want to transfer those samples to final plates that will have different media

We will use a computer with a Windows 10 system

7.1 Excel template that we can find go to step #1 filled and saved with the name *VariablesCounterSelection.xlsx* 10m

VariablesCounterSelection.xlsx

7.2 Upload custom labware to app and robot system 2m

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/**)

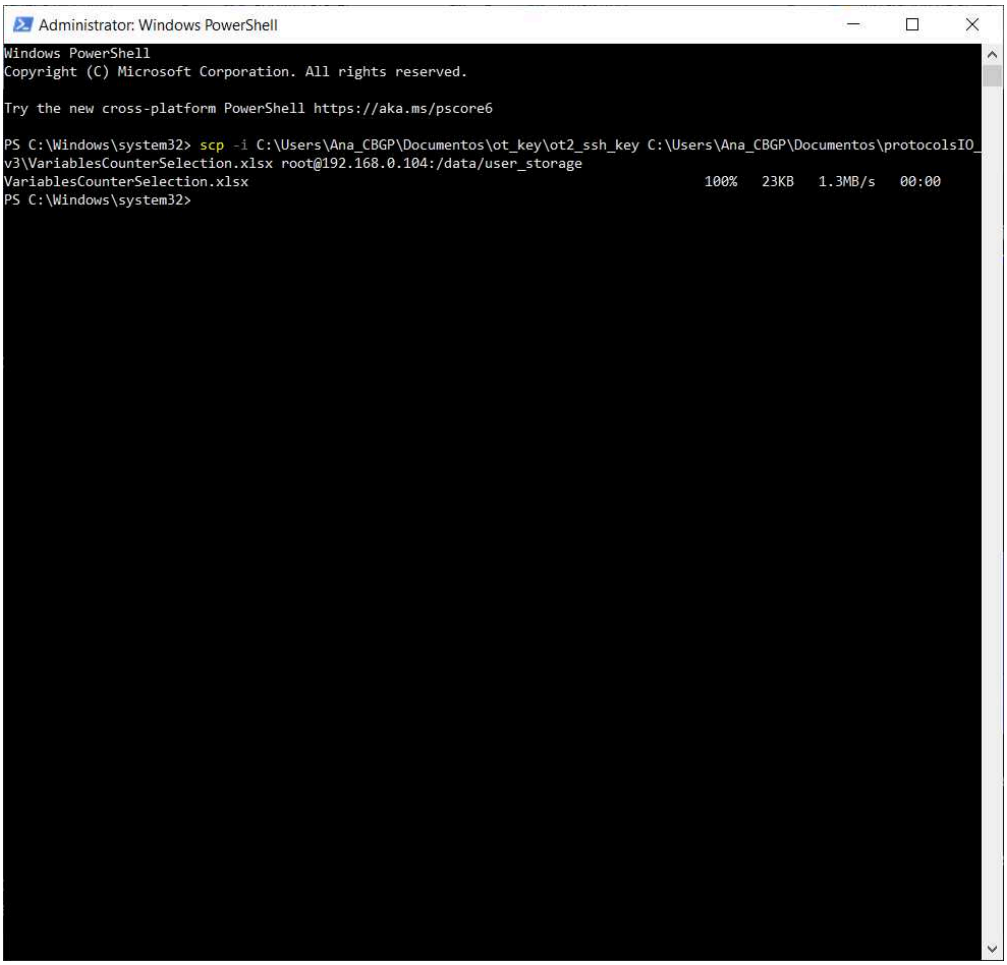
vwrblueprintdepth105_96_wellplate... 11.7KB

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...

7.3 Send the variable file to *the /data/user_storage* folder in the robot 2m



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

7.4 Import the script that we have downloaded from the step go to step #3 (I named it *ColonyScreening_example_v200.py*) to the OT-App 30s

ColonyScreening_example_v200.py

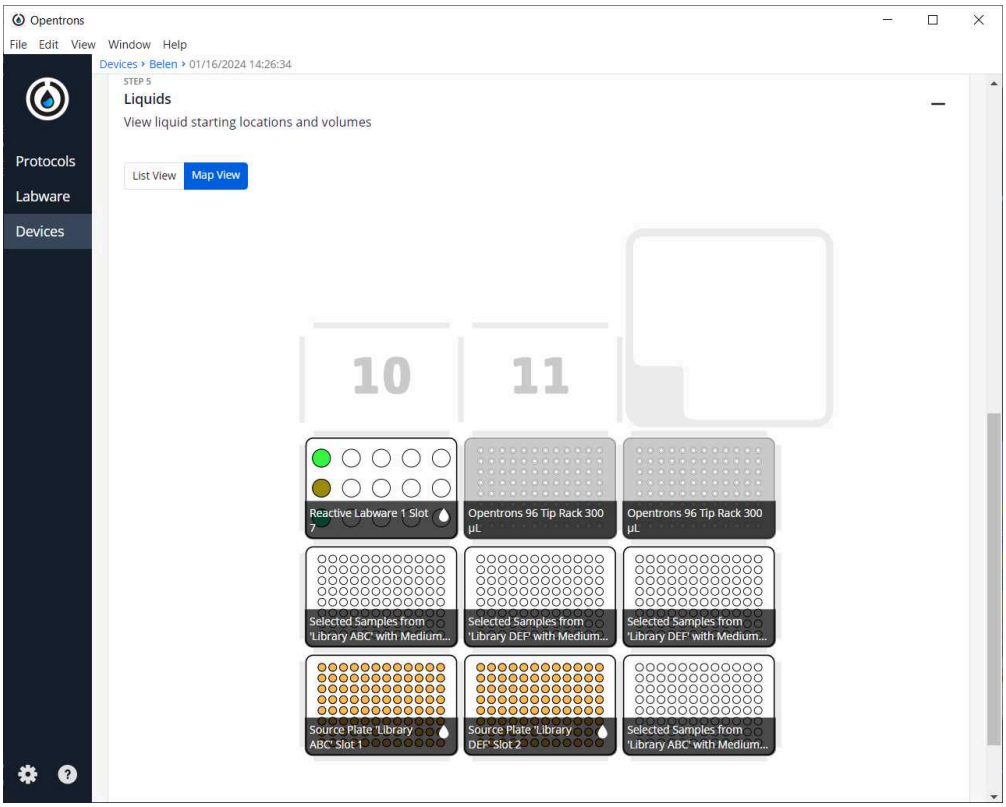
Result of importing the Python script in the OT-App

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

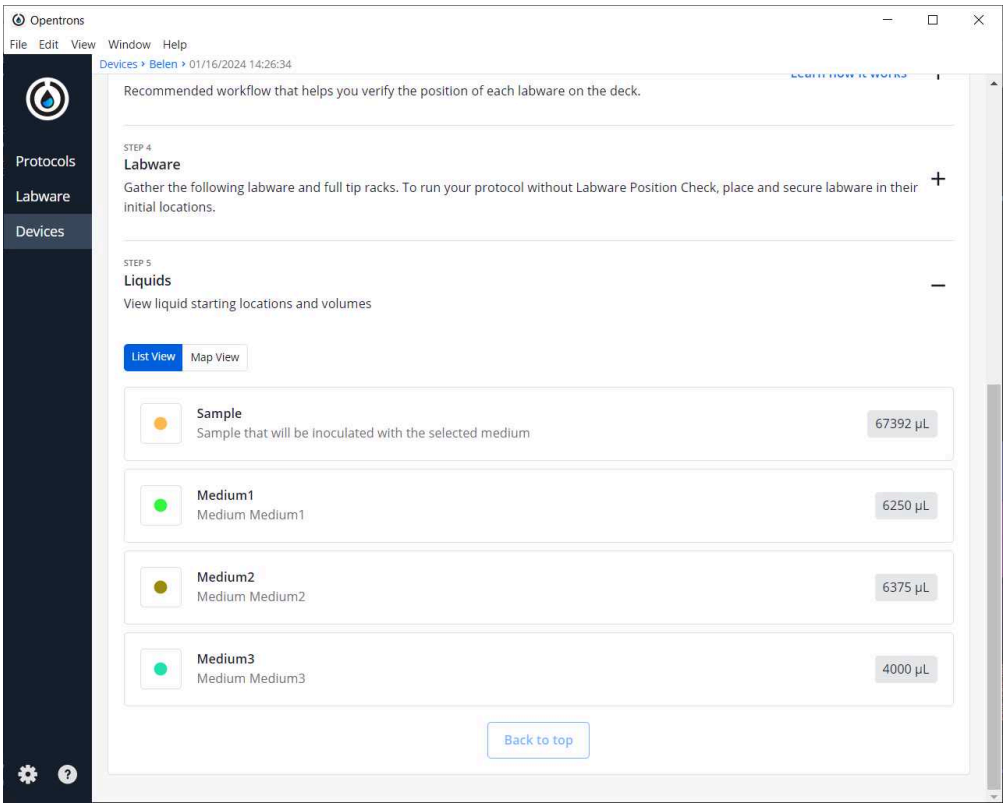
7.5 Run the protocol in the robot that we have transferred the Excel file 1m

ColonyScreening_example_v200.py → Start setup → Select robot in which we are going to run the protocol

If we do not have any errors, the output should look similar to the following pictures



Labware and liquid set-up layout



Volumes of the needed liquids to perform the protocol

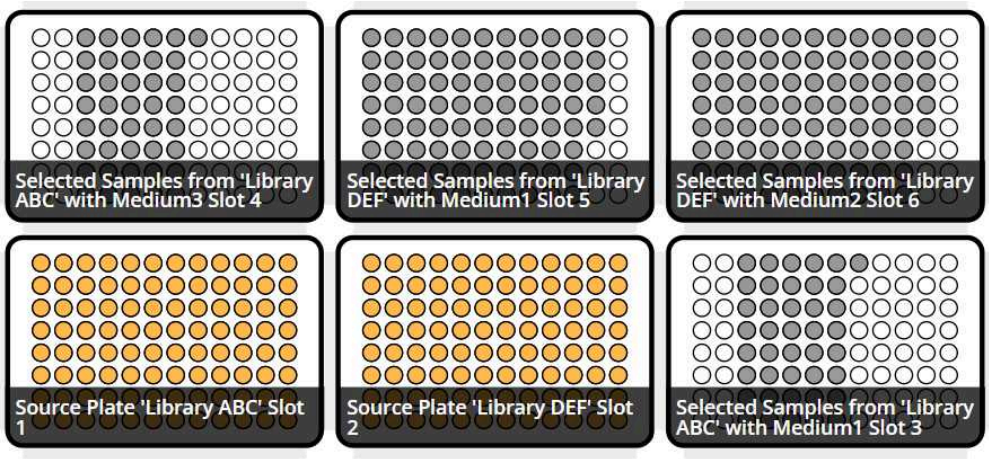
7.6 Turn the HEPA filter module — 30s

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

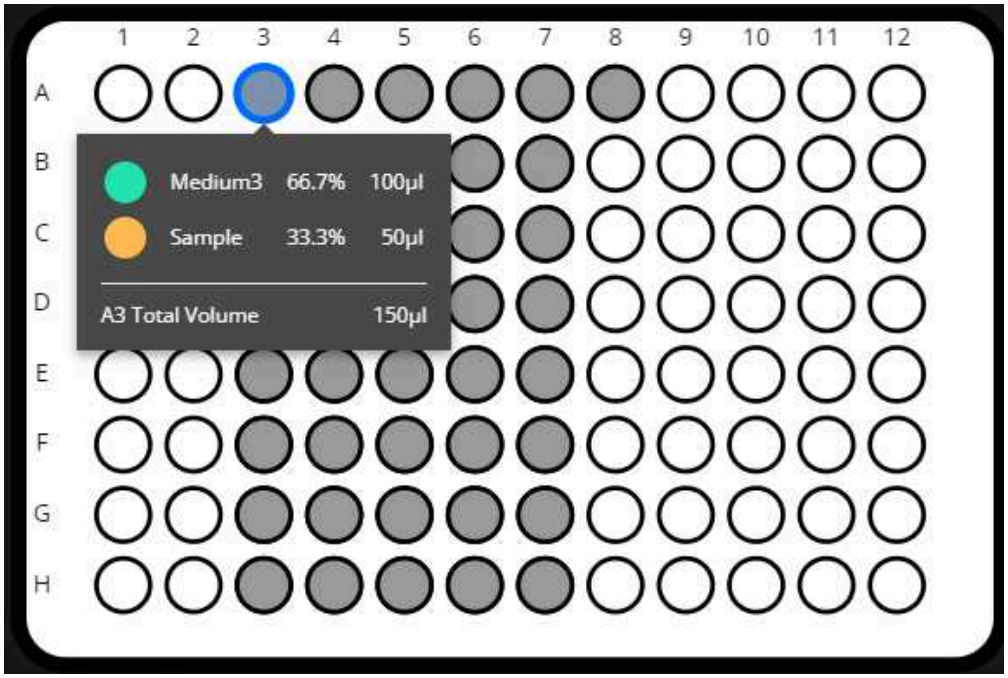
7.8 Prepare all reagents and labware in the places the App is showing and taking into account the notes in step go to step #3.2 Notes — 5m

7.9 Start run — 1h 22m

Expected result



Final layout of source and final plates in the run

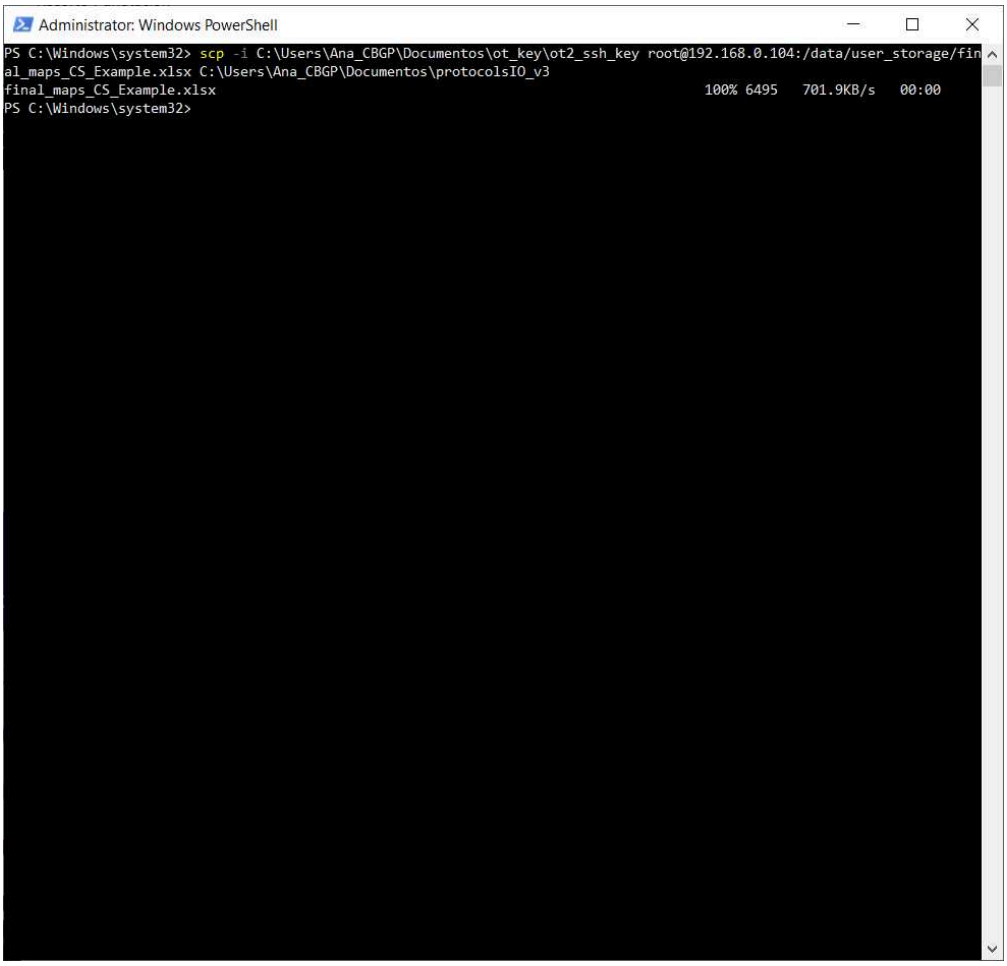


Example of the content of A3 in the labware *Selected Samples from 'Library ABC' with Medium3 Slot 4*

Here, we will obtain the mix between the volume of media and the samples set in the variable file in the final plates. These positions are seen in the image by the grey wells, and we can see the info of the plate and the media in the name of the labware.

7.10 Retrieve labwares from the OT 5m

7.11 Retrieve the final maps, in this case, they will be in a file called *final_maps_CS_Example.xlsx,* of the IDs of the samples that fulfilled the requisites that have the names set in the variable file by us. 2m
This file has 2 sheets, one per source plate, called *mapSelectedColoniesABC* and *mapSelectedColoniesDEF* for the columns *Library ABC* and *Library DEF*, respectively.



command line windows with the transfer command of the samples map from the OT to our computer

final_maps_CS_Example.xlsx

For more information about how to retrieve files from the robot

go to step #2 Linked Protocol

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