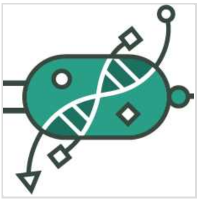

Oct 27, 2022

OT-2 Counter-Selection

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1 *Works for me* Share

dx.doi.org/10.17504/protocols.io.5qpvor5xdv4o/v1

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ABSTRACT

This protocol is meant to select the samples from 1 source plate that has been growing in different conditions and has different values for these different conditions, one condition gives higher values and the other lower values. From this selected samples this protocol create final plates with 2 types of reactives

The instructions for running this protocol are a python script for an Opentrons 2 robot and need a csv file with several variables setted making the protocol modular to reactives, volumes of transfer, type of plates, etc.

In our lab we are selecting samples by their after night OD in 2 different antibiotics and create PCR plates (with water) and stock plates (with glycerol), but it can be used with other selection characteristics as expression of GFP and with other types of reactives.

DOI

dx.doi.org/10.17504/protocols.io.5qpvor5xdv4o/v1

PROTOCOL CITATION

Ana Mariya Anhel, Lorea Alejaldre, Ángel Goñi-Moreno 2022. OT-2 Counter-Selection. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5qpvor5xdv4o/v1

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LICENSE

CREATED

Sep 20, 2022

LAST MODIFIED

Oct 27, 2022

OWNERSHIP HISTORY

Sep 20, 2022	Lorea Alejaldre
Oct 27, 2022	biocomp.cbgp

PROTOCOL INTEGER ID

70288

PARENT PROTOCOLS

In steps of

High-throughput workflow for the genotypic characterization of transposon library variants

GUIDELINES

This protocol was developed with python 3.9.7, OT App Software Version 6.1.0 and API level version 2.13. It has been tested with cultures from *Pseudomonas putida* KT2440 as part of 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 is 1 source plates to 7 final plates (using 2 tip racks per pipette and 1 tube rack for falcons tubes).

MATERIALS TEXT

Software:

- python 3.9.6
- python packages: pandas, logging, math, copy, opentrons
- OT-App
- Excel

OT-2 Labware

- Opentrons Tipracks

Citation: Ana Mariya Anhel, Lorea Alejaldre, ÃÂngel GoÃÂ±i-Moreno OT-2 Counter-Selection https://dx.doi.org/10.17504/protocols.io.5qpvor5xdv4o/v1

Opentrons 96 Tip Rack 300 µL
Tip rack
Opentrons -

Opentrons 96 Filter Tip Rack 20 µL
Tip rack
Opentrons -

- 96- well plates

96-well plates, flat bottom, non treated
Cell culture plates
VWR **734-2781**

- Opentrons Falcon tuberack

Opentrons 15 Tube Rack with Falcon 15 mL Conical
OT Tube Rack
Opentrons -

- 15mL Falcon tubes

Falcon® Conical Centrifuge Tubes 15mL
Flaocn Tube
Falcon 352096

Equipment:

OT-2
Liquid handler
Opentrons OT-2

HEPA Module
Opentrons OT-2-HEPA

Single Channel Electronic Pipette (GEN2) 300uL
Opentrons Pipette
Opentrons -

SAFETY WARNINGS

It is important to use HEPA module to work in sterility

BEFORE STARTING

Note that this protocol can be done for one 96-well source plate at a time. In addition, it can only be performed with two selection variables (2 files with 1 condition per file in which one should be higher than a value and the other lower than this same value).

Files Preparation

1 Preparing Customized Template

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

Here we attach one excel with several sheets:

1. Template to use in protocol
2. Explanation of each variable
3. Example

Template_ColonieScreening.xlsx

1.1 Fill the template with the corresponding values

1.2 Store it as a comma-separated values file (csv) with the name *Variables-ColonieScreening-OT.csv*

⚠ The file should be spelled exactly *Variables-ColonieScreening-OT.csv* or the python script won't work.
Make sure that the final csv is separated by commas, not by semicolons and that the variables have 2 quotation marks and not 6, in case they need quotation marks (these are added when going from excel to csv)

In Windows 10 to change excel separator from ";" to "," you should go to **Region Setings > Change date, time, or number formats > Additional settings > change List separator from ; to ,**

Alternatively open the exported .csv file in an editor and replace all ; to , and remove the extra quotation marks

2 Transferring csv to Robot

Transfer the *Variables-ColonieScreening-OT.csv* to the directory */data/user_storage* of the OT system that we are going to use to perform the protocol.

Citation: Ana Mariya Anhel, Lorea Alejaldre, ÃÂngel GoÃÂ±i-Moreno OT-2 Counter-Selection https://dx.doi.org/10.17504/protocols.io.5qpvor5xdv4o/v1

Previous to transfering any file to the OT, we need to know the IP of the robot.

This can be obtained in the Networking section of the Device that we are going to use (In the OT-App > Devices > (three dots) Networking)

Here we present a summary of how to transfer the files in 3 OS: *Windows, Mac and Ubuntu* (applicable to most Linux)

Mac/Linux
We will use the command line with scp to transfer the file *Variables-ColonieScreening-OT.csv* to the OT system.

We need to perform the following line:

Passing Files to OT

```
scp -i [ot_key] [file] root@[IP_OT]:/data/user_storage
```

File passing from linux (our computer) to linux (OT raspberry)

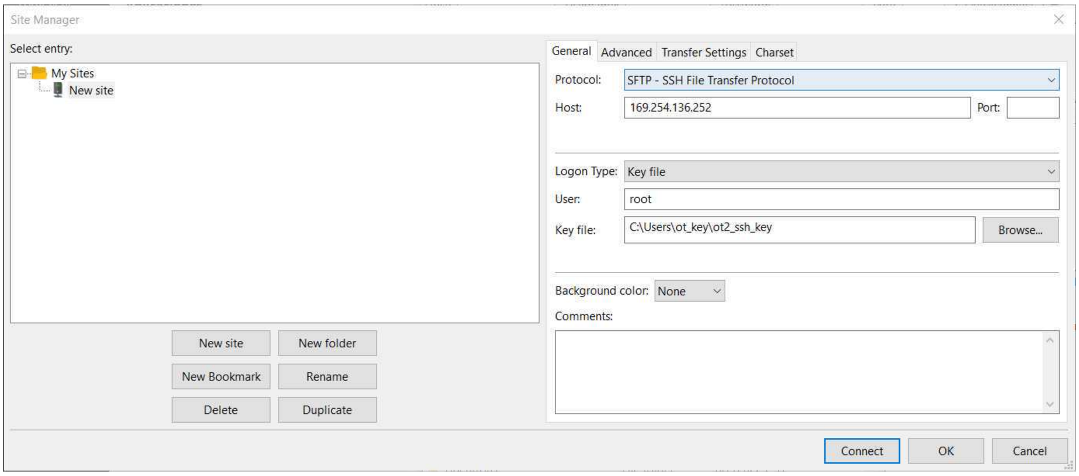
Ubuntu

The ot key should have been previously generated, for more info visit https://support.opentrons.com/s/article/Setting-up-SSH-access-to-your-OT-2

Windows
There are several ways to pass files from a windows to a linux (for example with a virtual machine).

Here we will use FileZilla (https://filezilla-project.org/download.php?type=client).

Go to **File > Site Manager > New Site > Change Protocol to SFTP**. Then introduce in Host the OT IP, change the Logon Type to key file, change the user name to root and give the directory were the ot key is. Should look something like this



Protocol: SFTP - SSH File Transfer Protocol

Host: 169.254.136.252

Port:

Logon Type: Key file

User: root

Key file: C:\Users\ot_key\ot2_ssh_key

Background color: None

Comments:

Example of setting the FileZilla to transfer files from windows (our computer) to linux (OT)

Then press connect and we will have a conection between our computer and the robot.

After this connection, we should be able to move out *Variables-ColonieScreening-OT.csv* (in our computer) to the directory */data/user_storage* in the robot.

Take in account that the IP of the robot could change, so it is possible that from time to time it will be needed to change the host in these connections.

The method described for the Windows system can be done as well in Ubuntu and Mac

3 ⊛ ⚠

Adding the custom labware

There is only a need to do this step when a labware that you are using is not OT official or your labware is not in the OT directory */data/labware/v2/custom_definitions/custom_labware*

⚠ This process/step is needed in the version 6.0.1 or below (when this protocol was developed).

Citation: Ana Mariya Anhel, Lorea Alejaldre, ÃÂngel GoÃÂ±i-Moreno OT-2 Counter-Selection https://dx.doi.org/10.17504/protocols.io.5qpvor5xdv4o/v1

According to their guide, this protocol should be possible only loading the labware in the OT-App (https://support.opentrons.com/s/article/Using-labware-in-your-protocols).

This bug has been reported and, hopefully, will be resolved as soon as possible. It is possible that you do not need to do this step if this error does not occur to you.

3.1 Creation of .json file

The description file can be obtained by describing the labware dimensions in https://labware.opentrons.com/create/

3.2 Creating description folder

We need to create for our custom labware a folder with the API name containing the description file (.json) called *1.json*

3.3 Transfer the description folder to the OT robot

We need to transfer the directory or directories created in Step 3.2

For that we need to introduce the following command (in Linux), for Windows process ⟲ **go to step #2**

Transfering directory to OT

```
scp -i [ot_key] -r [directory_custom_labware]
root@[IP_OT]:/data/labware/v2/custom_definitions/custom_bet
```

Transfering the custom labware to OT

Linux

We do not need to execute this part every time the protocol is used, only when that labware is not included in the OT official labware and these directories are not in the robot

4 Simulating and getting the output (user instructions)

Citation: Ana Mariya Anhel, Lorea Alejaldre, ÃÂngel GoÃÂ±i-Moreno OT-2 Counter-Selection https://dx.doi.org/10.17504/protocols.io.5qpvor5xdv4o/v1

For more information in how to setup the ssh visit the official OT support blog (https://support.opentrons.com/s/article/Setting-up-SSH-access-to-your-OT-2)

4.1 *Transferring python script*

To make sure that the protocol will work with the variables we have introduced we need to run the python script in the robot with the OT simulator.

The last script version can be found at https://github.com/Biocomputation-CBGP/OT2/tree/main/ColonySelection (name of this file is user's choice)

For that we can ↻ **go to step #2** but instead of passing the variables file, we pass the python script. In this case we can transfer it to the directory */data/user_storage* or we can directly pass it to the directory */root* (recommended)

> You can do this step only once and use always the same python script
>
> The pnly time that you need to change it is when there is an update in github, then you should download the new script version and do this step

4.2 *Simulating*

We should connect to the robot via ssh

Connect to Linux based OT via ssh

```
ssh -i [ot_key] root@[Robot_IP]
```

Simulate the protocol so we can know if the protocol is going to work with the given variables

Linux

Then we would move to the directory where the python script is with the **cd** command

Finally we are going to perform the simulation of the opentrons

Citation: Ana Mariya Anhel, Lorea Alejaldre, ÃÂngel GoÃÂ±i-Moreno OT-2 Counter-Selection https://dx.doi.org/10.17504/protocols.io.5qpvor5xdv4o/v1

Simulate OT protocol

```
opentrons_simulate -o nothing -e [name_script].py > [name_instruction_file].txt 2>&1
```

Simulate the protocol so we can know if the protocol is going to work with the given variables

Linux

Do not wory if it takes a little bit of time

4.3 In case that you want to see the instruction file before the next step, you can see the instruction file with cat

Show content file cmd

```
cat [name_instruction_file].txt
```

Show in the command line the instruction file content

Linux

4.4 **Retrieving output file (user instructions)**

To retrieve the output from the OT system you can **go to step #2** , replicate it but transfering the file from the OT to your computer

In this file we have 3 or more sections:

1. (Optional) ***Warnings***
2. ***General Information:*** pieces of information that are extracted from variables in the variables file and are only remainders
3. ***Deck Labware Position:*** this information will be provided by the OT as well
4. ***15mL Tuberack Reactive Positions:*** for each tuberack that is needed (with 15 positions) we have a table with the reactives and their respective volumes

It is recommended to have at least 10uL more of each reactive in each tube

Citation: Ana Mariya Anhel, Lorea Alejaldre, ÃÂngel GoÃÂ±i-Moreno OT-2 Counter-Selection
https://dx.doi.org/10.17504/protocols.io.5qpvor5xdv4o/v1

to make sure that the pipette does not take a bubble when aspirating

In the case that something went wrong, the variables are not consistents or there is not enough deck place to perform the experiments the instruction file will be sorter and will have a single section, ***Errors***.

Running Protocol

5 Setting Labware

5.1 Wipe the surface of the deck with 70% ethanol to clean and desinfect the surfaces

For more information in how to clean the robot go to the following link https://support.opentrons.com/s/article/Cleaning-the-robot-s-surfaces

5.2 Set the respective labwares in the slots as the OT App and the instruction file set

⚠ Make sure that the source and final A1 wells are at the left top (common mistake), and each labware fits appropierly.

⚠ Make sure that you are placing the source plate corespondant to the Antibiotic Transposition Genome File variable, i.e, the source plate where the colonies should grow (or the file where the value of the colonies that you want to select is higher than the variable *Grow OD* in the Template)

5.3 Set the different reactives in their respective coldblock positions, as stated in the instruction file

⚠ The protocol is going to aspirate from the falcon at a scpecific height which is set with the volume of the faqlcon, so make sure that you dont have a falcon with more than 1mL more than the instruction file specific volume.

This way, the pipette will not end wet in the running of the protocol

6 Load script in OT-App

Now that we have made sure that the protocol is going to work we import the python script in the OT-App.

⚠ This whole step has been developed with version 6 of the OT-App

Indications may vary from version to version

6.1 Load the script

Protocols > Import > Drag the python script

The last script version can be found at https://github.com/Biocomputation-CBGP/OT2/tree/main/ColonySelection (name of this file is user's choice)

⚠ The App with version 6 analyzes your protocol before setting a robot to run, so the labware will not be shown before assigning the protocol to a specific robot.

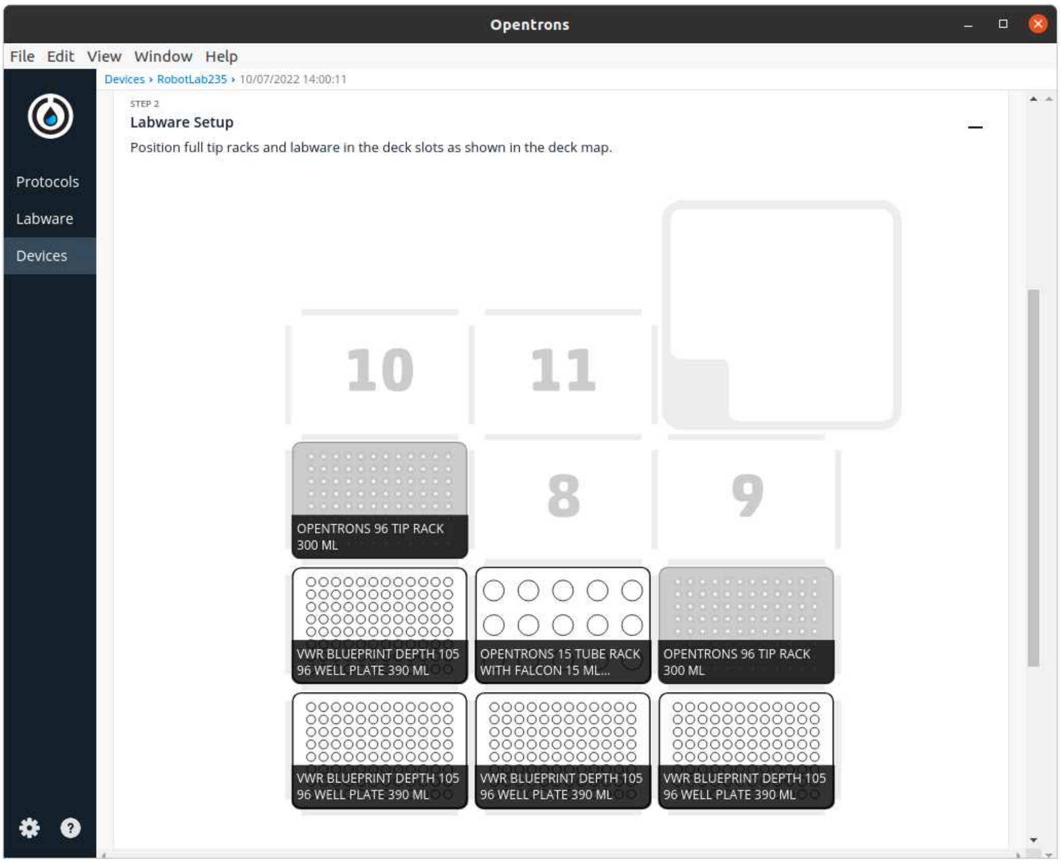
6.2 Select Robot to Perform Script

Click in the protocol > Run Protocol > Choose the OT where the file *Variables-ColonieScreening-OT.csv* is > Proceed To Setup

After clicking in Proceed to Setup you should obtain the setup of the labware as instructed in the Deck Labware Positions section of the instructions file.

The Labware Setup tab should look similar to the following image

Citation: Ana Mariya Anhel, Lorea Alejaldre, ÃÂngel GoÃÂ±i-Moreno OT-2 Counter-Selection https://dx.doi.org/10.17504/protocols.io.5qpvor5xdv4o/v1


Opentrons
File Edit View Window Help
Devices › RobotLab235 › 10/07/2022 14:00:11
Protocols
Labware
Devices
10
11
8
9
OPENTRONS 96 TIP RACK 300 ML
VWR BLUEPRINT DEPTH 105 96 WELL PLATE 390 ML
OPENTRONS 15 TUBE RACK WITH FALCON 15 ML...
OPENTRONS 96 TIP RACK 300 ML
VWR BLUEPRINT DEPTH 105 96 WELL PLATE 390 ML
VWR BLUEPRINT DEPTH 105 96 WELL PLATE 390 ML
VWR BLUEPRINT DEPTH 105 96 WELL PLATE 390 ML

STEP 2

Labware Setup

Position full tip racks and labware in the deck slots as shown in the deck map.

Example of a Labware Setup for a colonie counter selection protocol

It is recommended that you perform a labware position check.

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

7 Run Protocol in OT

7.1 Make sure the needed calibrations are done

7.2 Labware position check is performed (if needed)

7.3 Start Run

The procedure that the robot is going to do is mainly divided in 3 parts:

1. Select colonies that comply with the selected parameters
2. Distribute reactives to respective plates
3. Distribute to all plates the selected culture samples
4. Generate identity maps (to be exported in following steps)

Several plates, with different reactives but the same samples in the same order in adittion toa map (csv file) that will give the position in these plates with their identifiers (location in the original source plate)

This protocol can be done only for one source plate at a time

For more plates, re-run the script. To re-run the protocol make sure that the filled template is in the OT system and **go to step #6.2**

After-Running

8 Retrieve labware from the OT

9 Importing maps from robot

This can be done if in Step 1 the variable Optional Map was set to True

There will be as many maps as source plates with the following structure of names: *VariableNameMap.csv*

To retrieve we can **go to step #2** and reproduce it with transfering the files to the computer.

They will be in the directory */data/user_storage*

Citation: Ana Mariya Anhel, Lorea Alejaldre, ÃÂngel GoÃÂ±i-Moreno OT-2 Counter-Selection https://dx.doi.org/10.17504/protocols.io.5qpvor5xdv4o/v1

The maps contains the identity (position in original plate) of the samples selected in the places that they have been placed/distributed

Example

10 **We want to select the samples that have an OD_{600nm} higher than 0.07 in the Antibiotic Transposition genome file** (*ODvalues_ant_library1.csv*) **and have an OD_{600nm} lower than 0.07 in the Antibiotic ampicillin plasmid file** (*ODvalues_amp_library1.csv*). This corresponds to the example in the Template excel file in **go to step #1**

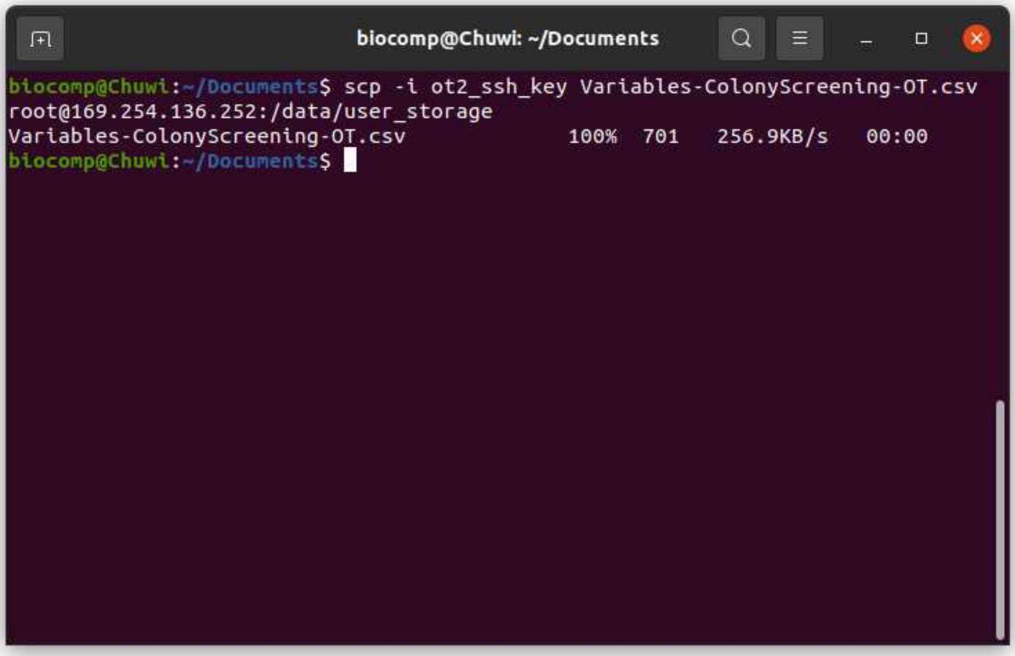
ODvalues_ant_library1.csv

ODvalues_amp_library1.csv

10.1 Excel temple filled and exported to csv with the name *Variables-ColonyScreening-OT.csv*

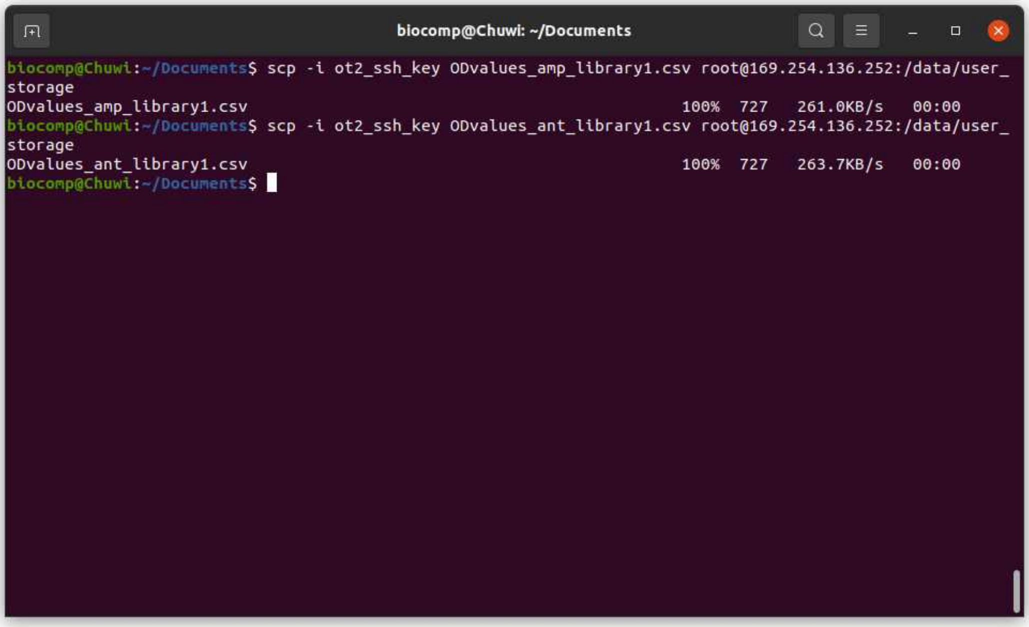
Variables-ColonyScreening-OT.csv

10.2 Export the variables csv and the OD csv files to */data/user_storage*



```
biocomp@Chuwi:~/Documents$ scp -i ot2_ssh_key Variables-ColonyScreening-OT.csv
root@169.254.136.252:/data/user_storage
Variables-ColonyScreening-OT.csv              100%  701    256.9KB/s   00:00
biocomp@Chuwi:~/Documents$ 
```

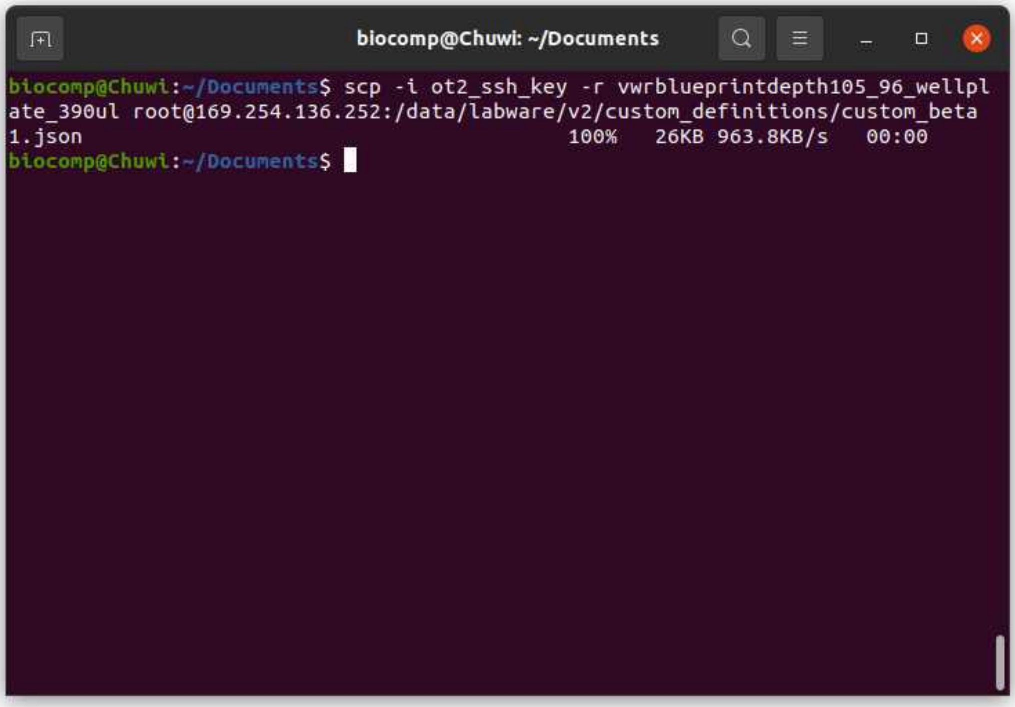
cmd window with scp commands to transfer the variables .csv from our computer to the OT



```
biocomp@Chuwi:~/Documents$ scp -i ot2_ssh_key ODvalues_amp_library1.csv root@169.254.136.252:/data/user_
storage
ODvalues_amp_library1.csv                                             100%  727   261.0KB/s   00:00
biocomp@Chuwi:~/Documents$ scp -i ot2_ssh_key ODvalues_ant_library1.csv root@169.254.136.252:/data/user_
storage
ODvalues_ant_library1.csv                                             100%  727   263.7KB/s   00:00
biocomp@Chuwi:~/Documents$ 
```

cmd window with scp commands to transfer the OD values .csv from our computer to the OT

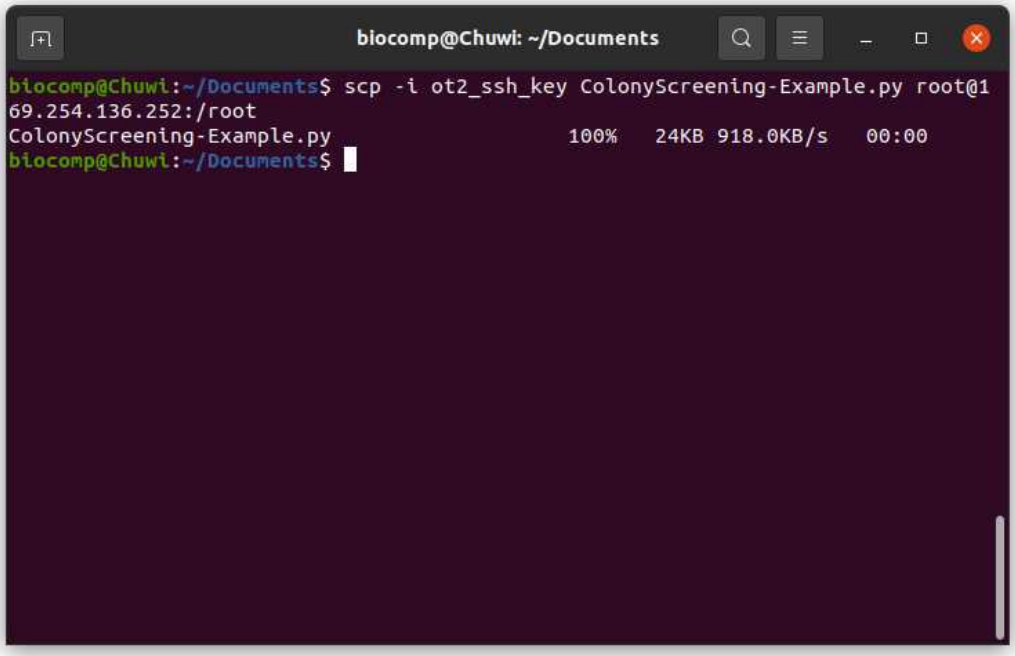
10.3 We have never used the labware vwrblueprintdepth105_96_wellplate_390ul, so we must pass the directory regarding this labware to the robot



```
biocomp@Chuwi:~/Documents$ scp -i ot2_ssh_key -r vwrblueprintdepth105_96_wellpl
ate_390ul root@169.254.136.252:/data/labware/v2/custom_definitions/custom_beta
1.json                                      100%    26KB 963.8KB/s    00:00
biocomp@Chuwi:~/Documents$ 
```

cmd window with scp commands to transfer the custom labware directory from our computer to the OT

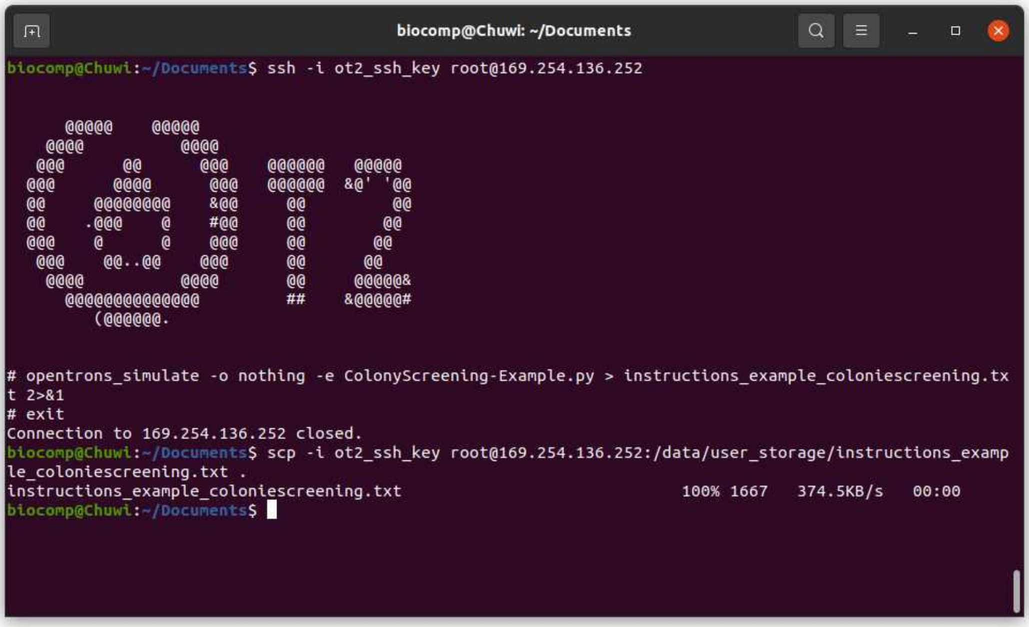
10.4 Pass the scipt that I have downloaded from https://github.com/Biocomputation-CBGP/OT2/tree/main/ColonySelection (I name it *ColonyScreening-Example.py*)



```
biocomp@Chuwi:~/Documents$ scp -i ot2_ssh_key ColonyScreening-Example.py root@1
69.254.136.252:/root
ColonyScreening-Example.py                    100%   24KB 918.0KB/s   00:00
biocomp@Chuwi:~/Documents$
```

cmd window with scp commands to transfer the python script from our computer to the OT

10.5 Connect to OT computer, simulate, exit OT system, store the output in *instructions_example_coloniescreening.txt* and importing it to the computer



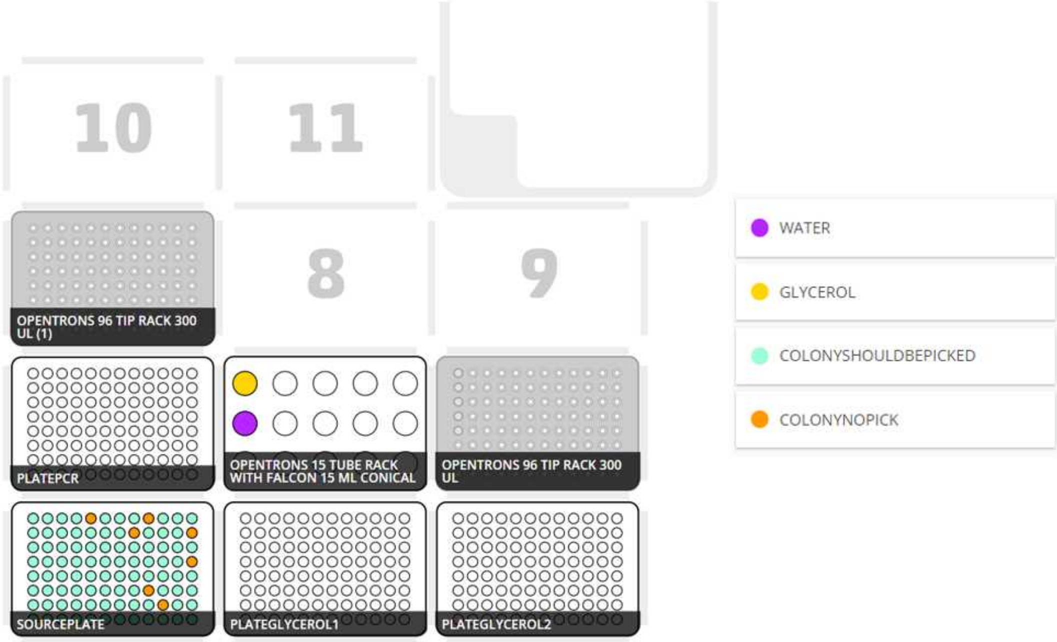
```
biocomp@Chuwi:~/Documents$ ssh -i ot2_ssh_key root@169.254.136.252


      @@@@@    @@@@@
    @@@@          @@@@
   @@@      @@      @@@    @@@@@@   @@@@@
  @@@      @@@@      @@@   @@@@@@  &@' '@@
  @@     @@@@@@@@    &@@     @@         @@
  @@    .@@@    @    #@@     @@        @@
  @@@    @      @    @@@     @@       @@
   @@@    @@..@@    @@@      @@      @@
    @@@@          @@@@       @@     @@@@@&
      @@@@@@@@@@@@@@         ##    &@@@@@#
         (@@@@@@.


# opentrons_simulate -o nothing -e ColonyScreening-Example.py > instructions_example_coloniescreening.tx
t 2>&1
# exit
Connection to 169.254.136.252 closed.
biocomp@Chuwi:~/Documents$ scp -i ot2_ssh_key root@169.254.136.252:/data/user_storage/instructions_examp
le_coloniescreening.txt .
instructions_example_coloniescreening.txt                             100% 1667    374.5KB/s    00:00
biocomp@Chuwi:~/Documents$
```

cmd window with connection to OT, simulation of python script, exiting and transfering .txt from OT to our computer

instructions_example_coloniescreening.txt

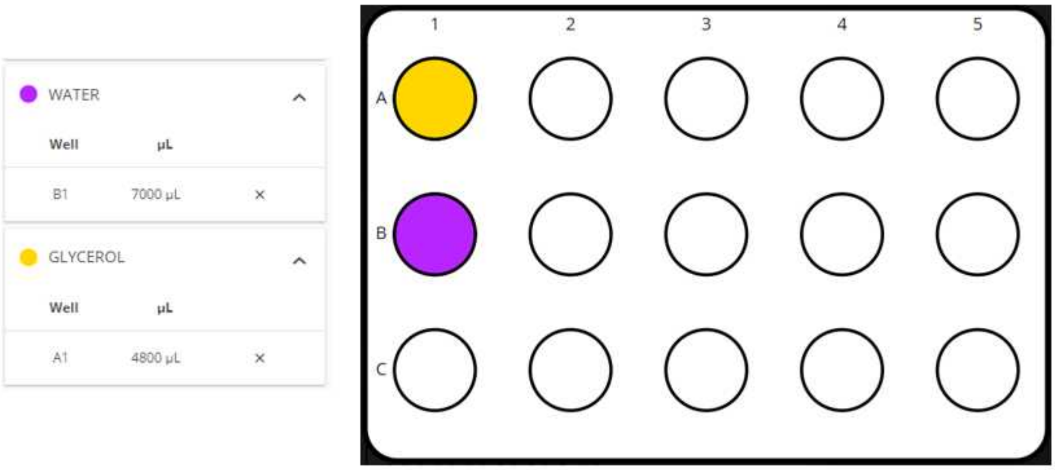
10.6 Prepare all reactives and labwares

Initial Labware Setup and Coldblocks setup should look like the following picture



10
11
8
9
OPENTRONS 96 TIP RACK 300 UL (1)
PLATEPCR
OPENTRONS 15 TUBE RACK WITH FALCON 15 ML CONICAL
OPENTRONS 96 TIP RACK 300 UL
SOURCEPLATE
PLATEGLYCEROL1
PLATEGLYCEROL2
WATER
GLYCEROL
COLONYSHOULDBEPICKED
COLONYNOPICK

Deck sketch with labware and reactives leyend



WATER

Well	µL	
B1	7000 µL	×

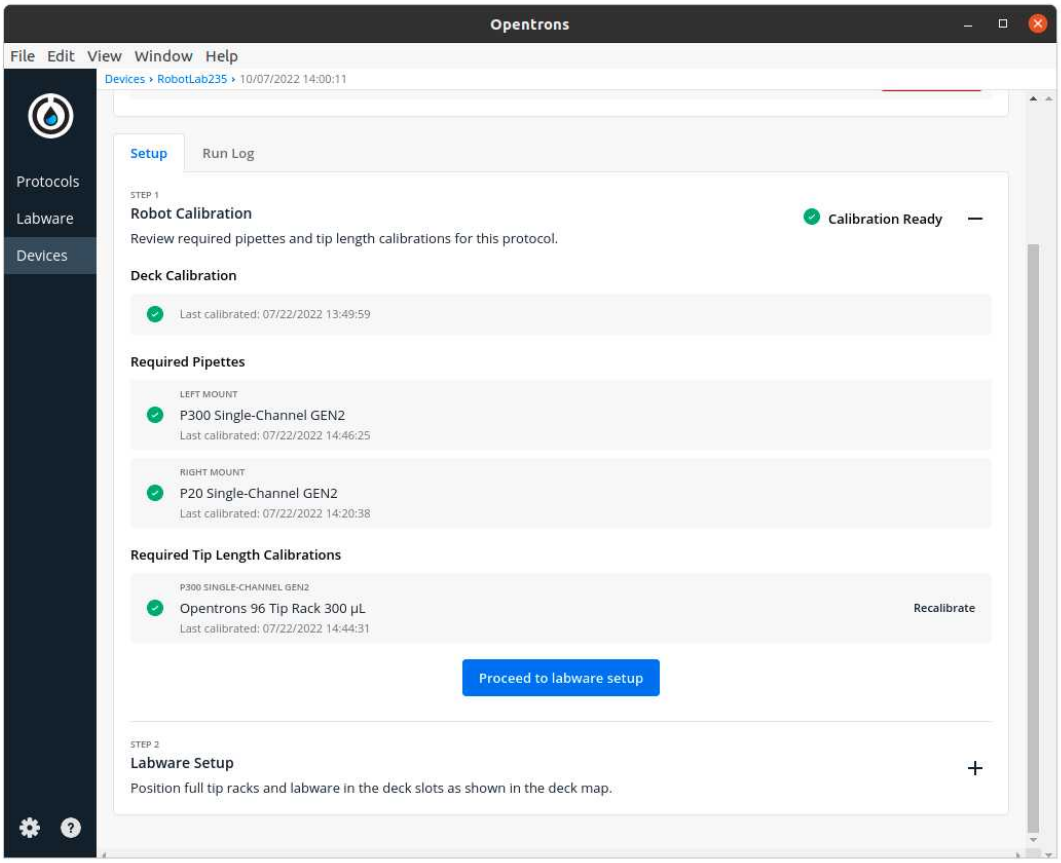
GLYCEROL

Well	µL	
A1	4800 µL	×

ColdBlock with reactives (positions and volumes)

10.7 Load to OT-App the ***ColonyScreening-Example.py*** **> Run > Select robot** in which we are going to run the protocol

The window and tabs of the OT-App should look like the following pictures



Opentrons
File Edit View Window Help
Devices › RobotLab235 › 10/07/2022 14:00:11
Protocols
Labware
Devices
Setup
Run Log

STEP 1

Robot Calibration

Calibration Ready

Review required pipettes and tip length calibrations for this protocol.

Deck Calibration

Last calibrated: 07/22/2022 13:49:59

Required Pipettes

LEFT MOUNT
P300 Single-Channel GEN2
Last calibrated: 07/22/2022 14:46:25

RIGHT MOUNT
P20 Single-Channel GEN2
Last calibrated: 07/22/2022 14:20:38

Required Tip Length Calibrations

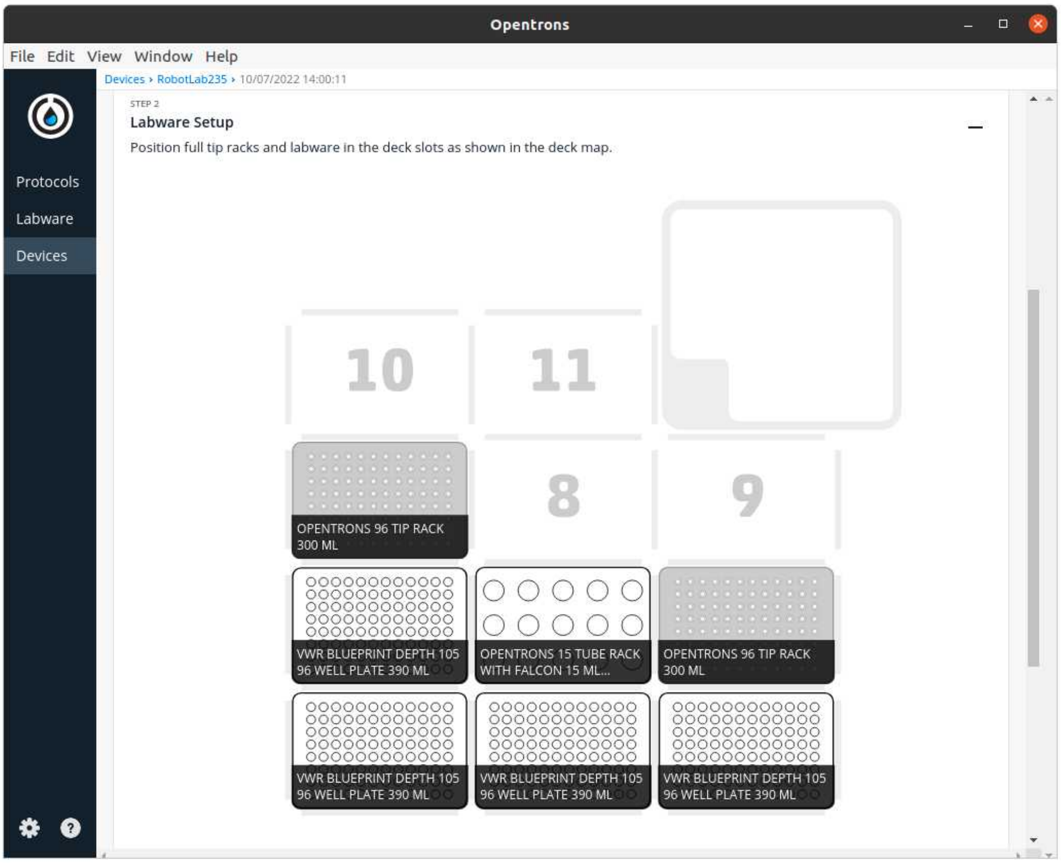
P300 SINGLE-CHANNEL GEN2
Opentrons 96 Tip Rack 300 µL
Last calibrated: 07/22/2022 14:44:31

STEP 2

Labware Setup

Position full tip racks and labware in the deck slots as shown in the deck map.

Calibration Tab OT with Example1 variables



Opentrons
File Edit View Window Help
Devices › RobotLab235 › 10/07/2022 14:00:11
Protocols
Labware
Devices
STEP 2

Labware Setup

Position full tip racks and labware in the deck slots as shown in the deck map.

10
11
8
9
OPENTRONS 96 TIP RACK 300 ML
VWR BLUEPRINT DEPTH 105 96 WELL PLATE 390 ML
OPENTRONS 15 TUBE RACK WITH FALCON 15 ML...
OPENTRONS 96 TIP RACK 300 ML
VWR BLUEPRINT DEPTH 105 96 WELL PLATE 390 ML
VWR BLUEPRINT DEPTH 105 96 WELL PLATE 390 ML
VWR BLUEPRINT DEPTH 105 96 WELL PLATE 390 ML

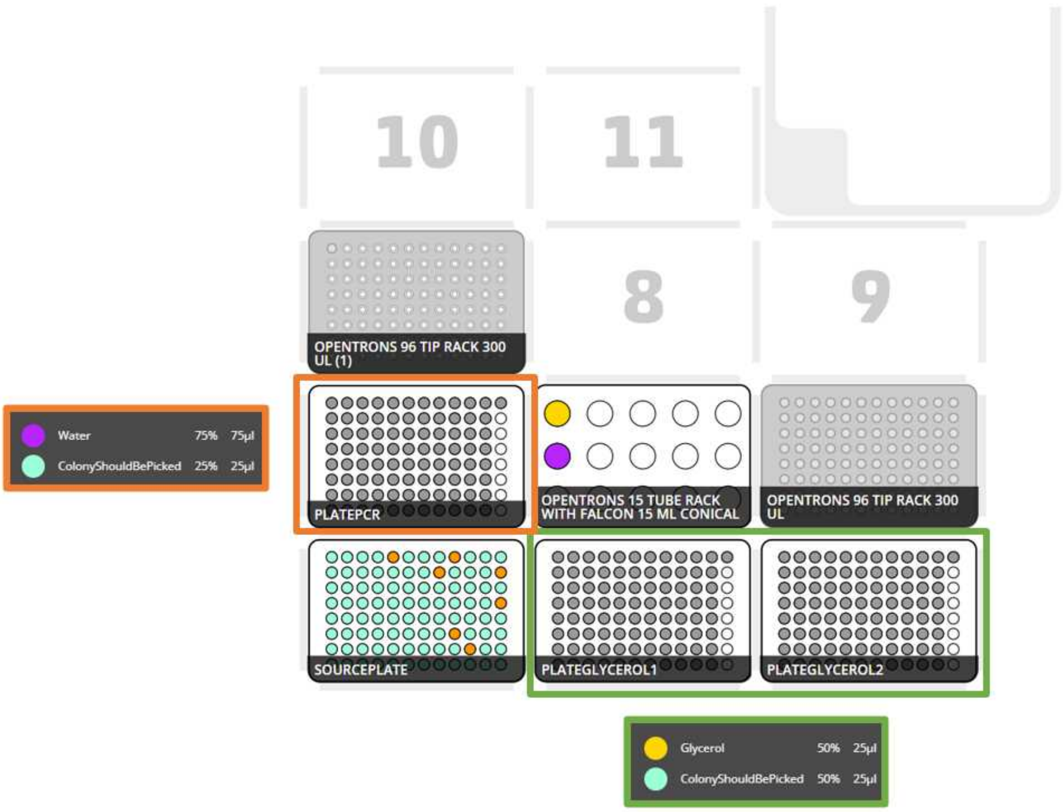
Labware Setup Tab OT with Example1 variables

10.8 Turn HEPA module on and wipe OT with 70% ethanol

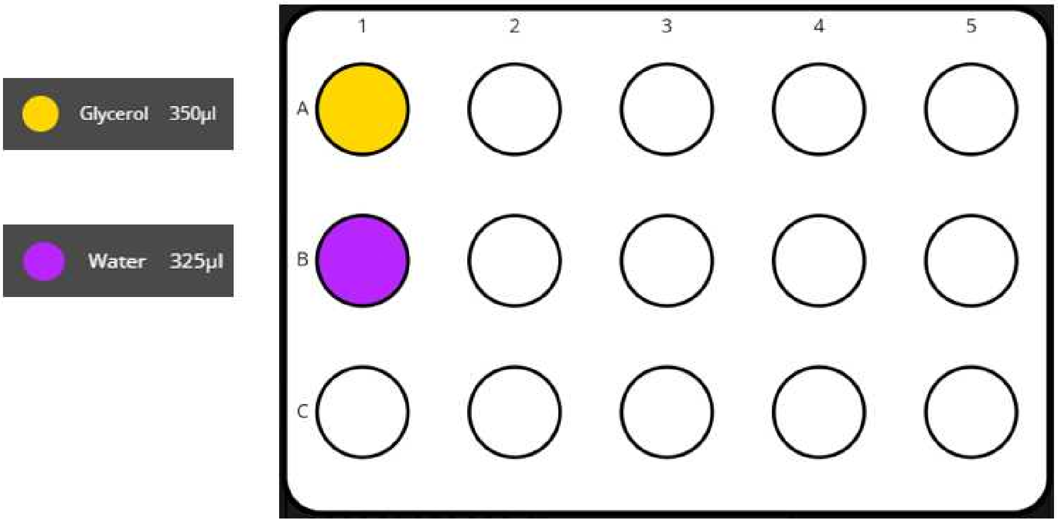
10.9 Load protocol labware as instructed in Step 10.6

10.10 Run protocol

The final results should look like the following pictures


10
11
8
9
OPENTRONS 96 TIP RACK 300 UL (1)
Water 75% 75µl
ColonyShouldBePicked 25% 25µl
PLATEPCR
OPENTRONS 15 TUBE RACK WITH FALCON 15 ML CONICAL
OPENTRONS 96 TIP RACK 300 UL
SOURCEPLATE
PLATEGLYCEROL1
PLATEGLYCEROL2
Glycerol 50% 25µl
ColonyShouldBePicked 50% 25µl

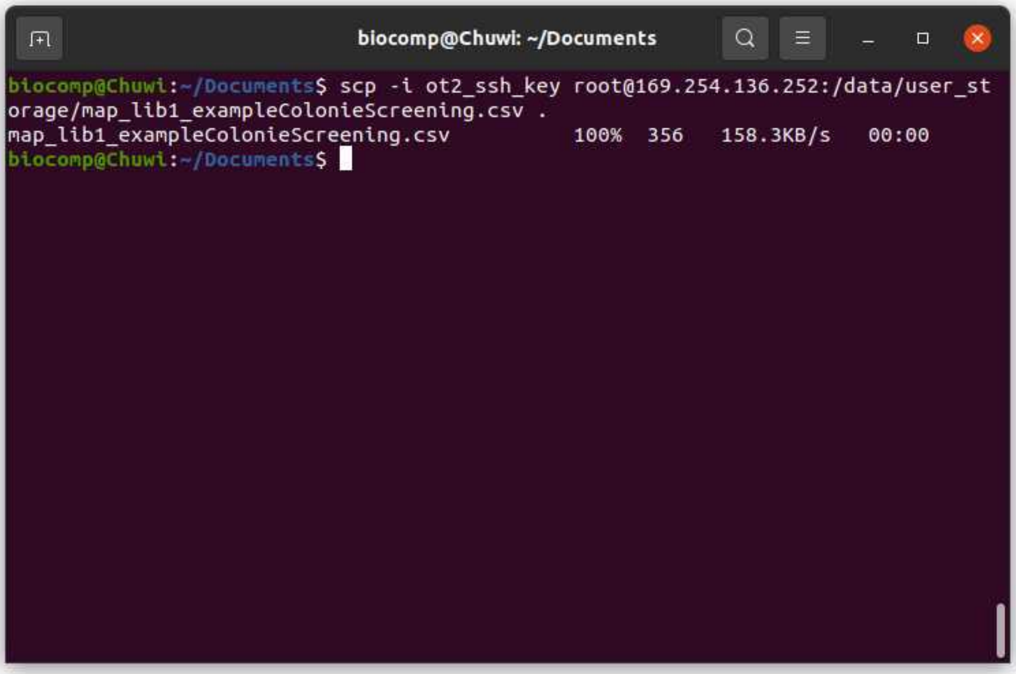
Final labware and final plates (PCRs plates) compositions



Falcon tube rack reactives and their respectives volumes at the end of the running

10.11 Retrieve labwares from the OT

10.12 Retrieve the final map (in this case will be called *map_lib1_exampleColonieScreening.csv*) of the IDs of the samples that fulfilled the requisites



```
biocomp@Chuwi:~/Documents$ scp -i ot2_ssh_key root@169.254.136.252:/data/user_st
orage/map_lib1_exampleColonieScreening.csv .
map_lib1_exampleColonieScreening.csv          100%  356   158.3KB/s   00:00
biocomp@Chuwi:~/Documents$ 
```

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

map_lib1_exampleColonieScreening.csv

Citation: Ana Mariya Anhel, Lorea Alejaldre, ÃÂngel GoÃÂ±i-Moreno OT-2 Counter-Selection https://dx.doi.org/10.17504/protocols.io.5qpvor5xdv4o/v1