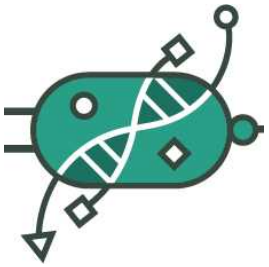
Oct 20, 2025 Version 3

OT-2 PCR sample preparation protocol V.3



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Ana Mariya Anhel[1], Lorea Alejaldre[1], Manuel Gimenez[1], Ángel oñi-Moreno[2]

[1]Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM)-Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA/CSIC), Madrid, Spain;
[2]Centro Nacional de Biotecnología (CNB-CSIC)

Ángel oñi-Moreno: angel.goni@cnb.csic.es;

Biocomputation Lab
CNB-CSIC

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

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Protocol Integer ID: 101396

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Abstract

This protocol is meant to create a PCR mastermix with 1 or more primer sets, distribute it in 1 or more 96-well plates, inoculate from 1 or more 96-well plates containing the template and run the reaction in an OT-2 thermocycler (if available).

This protocol is run by using a **LAP format** script and its corresponding .xlsx file were different customizable variables such as volumes of transfer, type of plates, number of primers per set, and availability of OT-2 thermocycler are indicated.
Specifically, this protocol provides a set of instructions or description of the **LAP repository** entry **LAP-PCR-OT2-2.0.0.** You can find the script and complementary information for this specific version of the protocol in the **LAP entry link** and **GitHub link to LAP entry documents**

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 arbitrary and spurious PCR samples.

The current version incorporates the following modifications:

- **Description of robot and protocol setup in a separate protocols.io entry** (Setting and Customizing OT-2 for LAP Entries)
- When transferring the polymerase and primers, **change tip every time it aspirates from source tubes**
- **Wells with controls can be anywhere in the plate**
- **New variable** in Sheet 'ModuleVariables' called 'Max Volume Per Mix Tube In Shaker'

Guidelines

This protocol has been tested with cultures from *Pseudomonas putida* KT2440 and PCR products as part of the High-throughput workflow for the genotypic characterization of transposon insertion library variants. Software requirements to run this protocol are the following:

- Python 3 (Developed with Python 3.7.1)
- Opentrons Protocol API version >= 2.14
- Opentrons App software Version >= 7.0.2
- Python packages needed (version moment of development): opentrons (7.0.2), pandas (0.25.3), numpy (1.15.1), openpyxl (3.1.2), math, random

Materials

Software

- python 3.7.1
- python packages: pandas, random, math, numpy, opentrons, openpyxl
- OT App
- Excel

OT-2 Labware

- Opentrons Tipracks

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

- PCR plates
- Opentrons Falcon tuberack

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

- 1.5mL eppendorfs (without the cap)
- 4ºC cold-block with adaptor (file attach)

Equipment

BRAND™ Centrifuge Tube Mini-Cooler	NAME
ColdBlock	TYPE
BRAND	BRAND
10141921	SKU
https://www.fishersci.es/shop/products/brandtech-scientific-brand-centrifuge-tube-mini-cooler-3/10141921	LINK

adaptor_OT_coldblock.stl

Reactives:

Phire Green Hot Start II PCR Master Mix **Fisher Scientific Catalog #**15391732 This is the reason why the transfering of the polymerase is done with a slower aspiration and dispensing rate, due to the viscosity of the reactive

Thermo Scientific™ Phire Hot Start II PCR Master Mix **Fisher Scientific Catalog #**15361732

MilliQ water

Oligonucleotides:

- For spurious integration control: PS3, PS4, PS5, PS6 (Silva-Rocha et al. 2013 doi: **10.1093/nar/gks1119**)
- For arbitrary PCR: ARB2, ME-O-Km-Ext-F, ME-O-Km-Int-F, ME-O-Sm-Ext-F, ME-O-Sm-Int-F, ME-O-Gm-Ext-F, ME-O-Gm-Ext-R (Martinez-Garcia et al. 2013 https://doi.org/10.3389/fbioe.2014.00046)
- For optional insert control: PSMCS

Equipment:

Equipment

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

Equipment

Single Channel Electronic Pipette (GEN2) 300uL	NAME
Opentrons Pipette	TYPE
Opentrons	BRAND
-	SKU
https://shop.opentrons.com/single-channel-electronic-pipette-p20/	LINK

Equipment

Single Channel Electronic Pipette (GEN2) 20uL	NAME
Opentrons Pipette	TYPE
Opentrons	BRAND
-	SKU
https://shop.opentrons.com/single-channel-electronic-pipette-p20/	LINK

Before start

Note that if more than one primer set wants to be used in the same final plate, the protocol should be re-run to create the new PCR master mix, the new starting position in the final plate should be specified.

Files Preparation

1 **Preparing Customized Template**

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

Here we attach one Excel 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. **SamplesPlateVariables:** variables related to the specifications of each source plate
3. **PipetteVariables:** variables related to the pipette(s) that are going to be used
4. **ReagentsPerReaction:** variables that will determine the final mix of the wells
5. **ModuleVariables:** variables related to the modules used in the protocol, the thermocycler and the heater-shaker
6. **TemperatureProfile (Optional):** PCR program that will be performed in the thermocycler if set as True in the ModuleVariables sheet
7. **Maps (Optional):** sheet(s) with the names of the samples in the source plates and will be reflected in the final plate map(s) → not included in the template but needed to be included if the variable Map IDs has a value.

PCRInstructions_v200.pdf

TemplateVariablesPCR.xlsx

1.1 *Fill the template* with the corresponding values (PCR conditions, number of primer pairs, output map, availability of OT-2 thermocycler etc...) and save file as ***VariablesPCR.xlsx*** (if the file has another name the LAP format script will not read the file).

Safety information

Verify volume range of the OT-2 pipettes available with the volume of a single reaction.

This can be a source of the errors displayed by the app in later steps

1.2 *Store it* with the name *VariablesPCR.xlsx*

Note

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

2 **Transferring variables file to Robot**

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

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

Note

Updated script versions can be found at our Github https://github.com/BiocomputationLab/LAPrepository/tree/main/LAPEntries or LAP repository **https://www.laprepo.com/repository/** in the directory and entry named **LAP-PCR-OT2** followed by the version.

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-PCR-OT2 was the 2.0.0 which script you can find attached

ScriptPCR_v200.py

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.

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. 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

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

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

Note

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

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

Safety information

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

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

Safety information

Make sure that the caps of these 1.5mL eppendorfors are removed (cut them) so they are not hanging and the pipette(s) do not have the possibility to crash with them

4.6 *Start Run*

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

1. Creation of primer sets combining water, primer(s) and polymerase
2. Mixing and distributing the primer set(s)
3. Transferring samples to the corresponding wells of all set of primers

Expected result

One or more final plates (usually PCR plates) with the mix for each set of primers combined with the colony samples and an excel file containig th emapping of each final plate in the folder */data/user_storage* of the robot

After-running

5 **Retrieve labware from the OT**

6 **Importing layout output maps from robot**

There will be 1 final excel file with the name set in variable 'Final Map Name' from sheet 'GeneralVariables' with as many maps as final plates with the following structure of names: *FinalMap* followed by the *PositionSlot* where that final plate was

Layout maps will be in directory */data/user_storage* of the robot where the protocol was run

For additional information on how to retrieve the maps from the OT-2 liquid handler:

Protocol

NAME

Setting and Customizing OT-2 for LAP Entries

CREATED BY

Biocomputation Lab

Preview

Example

2h 31m

7 **We want to perform a PCR mix preparation of 93 colonies and 2 controls that are in 2 source plates (40 colonies + 2 control samples in 1 plate and 54 colonies starting from the A4 well in the other plate but we dont want to take the sample in the well D6) with 2 primer sets. The first plate will have a map of identities(names) but the second one wont.**

We are going to have a heater-shaker module available but not a thermocycler. We are going to set the shaker to a high RPMs so we want the maximum volume on the mixing tubes, the tube sthat will contain the PCR mix with primers, polymerase mix and water, to be 1mL

We will use a computer with a Windows 10 system

7.1 *Prepare variable file*

10m

Excel temple filled and saved with the name *VariablesPCR.xlsx*

VariablesPCR.xlsx

7.2 *Upload custom labware to app*

2m

We are using a custom labware called *vwrblueprintdepth105_96_wellplate_390ul* and *3dprinted_opentrons_shaker_1.5mleppendorf* that has been created with the labware creator that opentrons offer (**https://labware.opentrons.com/create/**)

3dprinted_opentrons_shaker_1.5mle... vwrblueprintdepth105_96_wellplate...

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

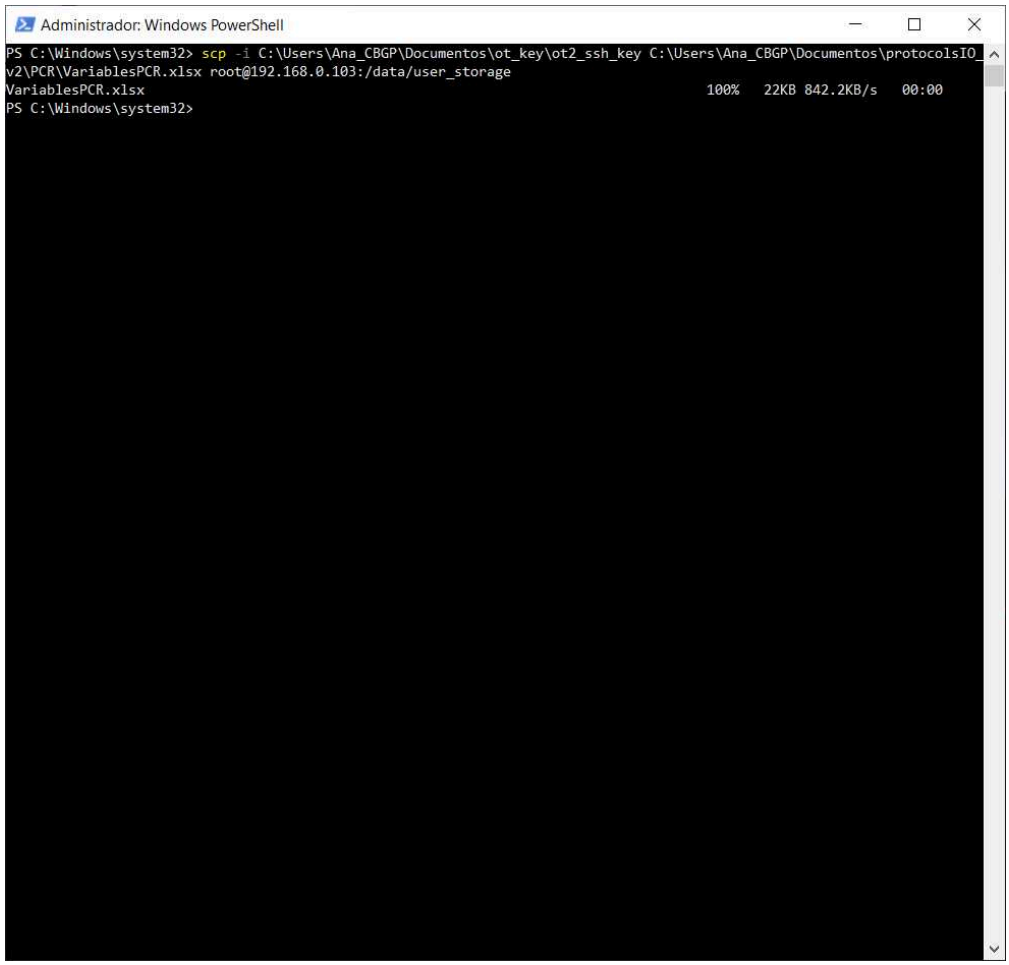
Setting and Customizing OT-2 for LAP Entries

vwrblueprintdepth105_96_wellplate...

3dprinted_opentrons_shaker_1.5mle...

7.3 *Export the variable file to the /data/user_storage folder in the robot*

30s



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

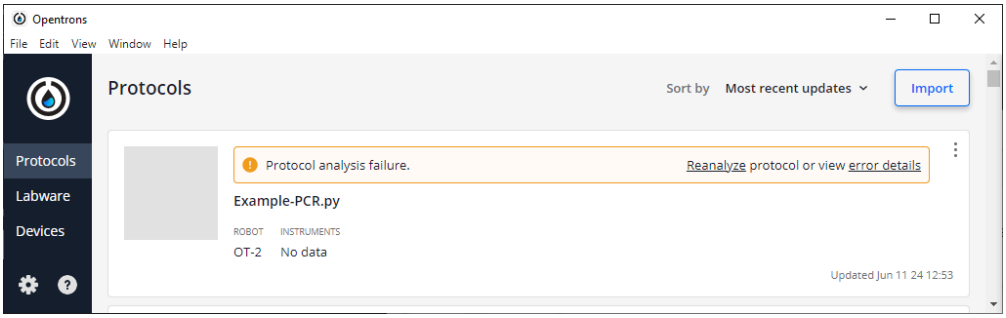
For more information about sending files to the OT-2

Setting and Customizing OT-2 for LAP Entries

7.4 *Import the script* 1m

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

Example-PCR.py



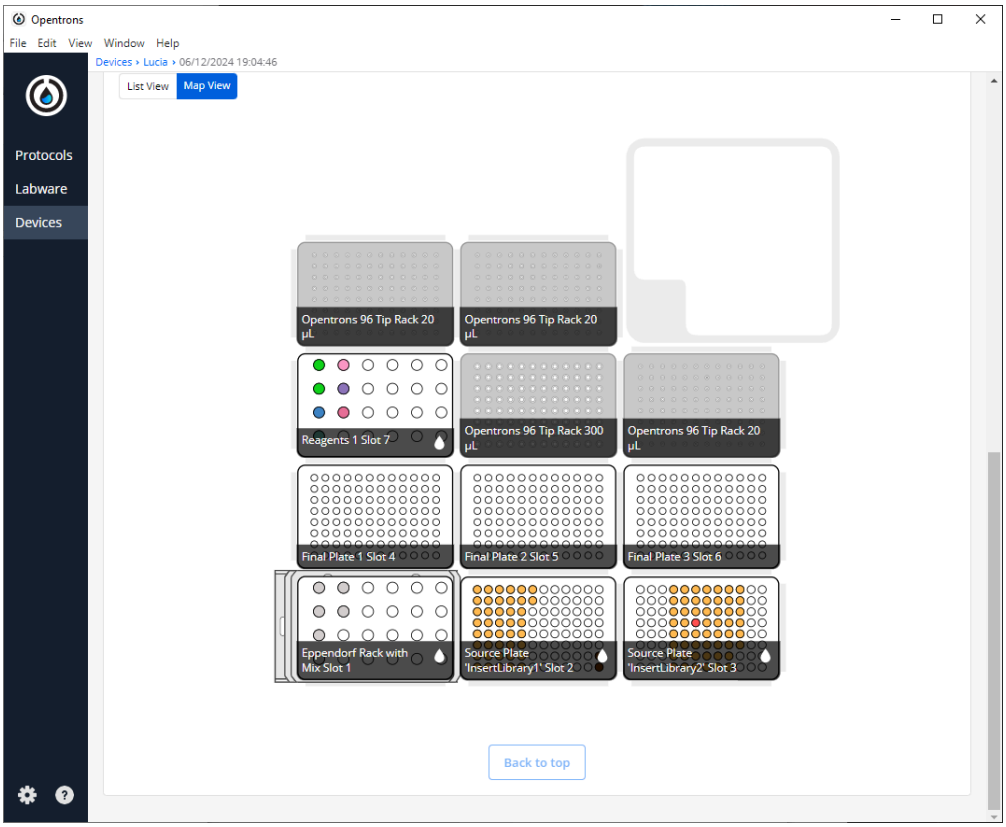
Result of importing the Python script in the OT-App

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

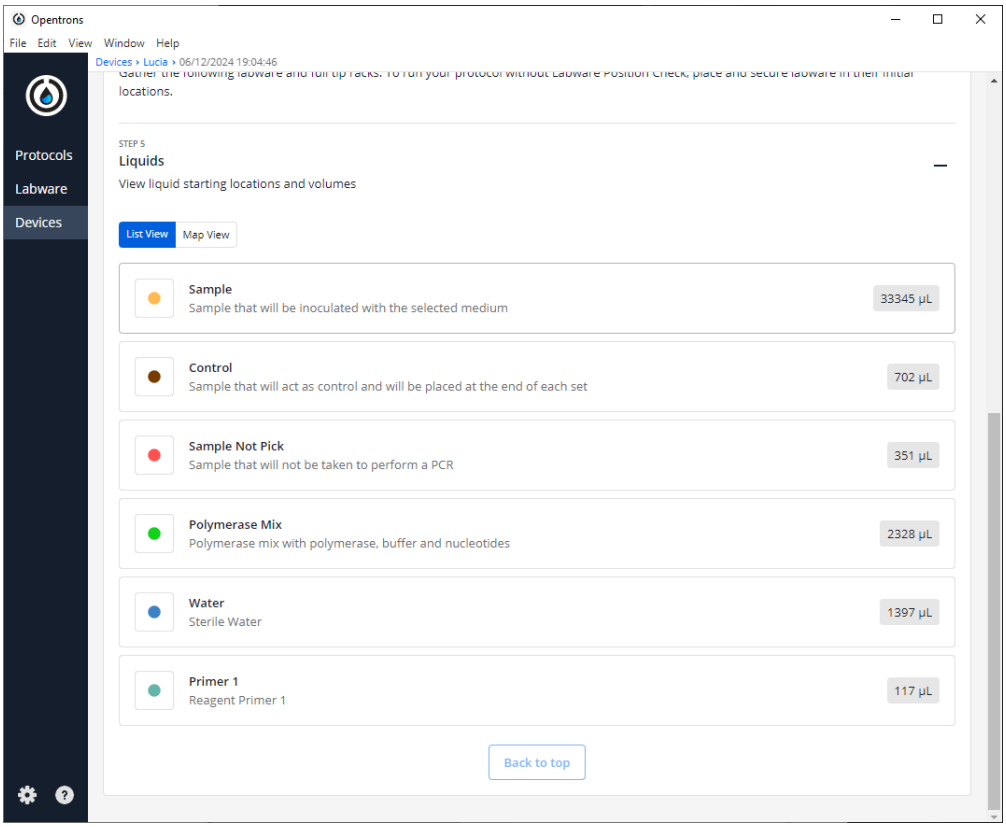
7.5 *Run the protocol in the robot that we have transferred the Excel file* 30s

Example-PCR.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

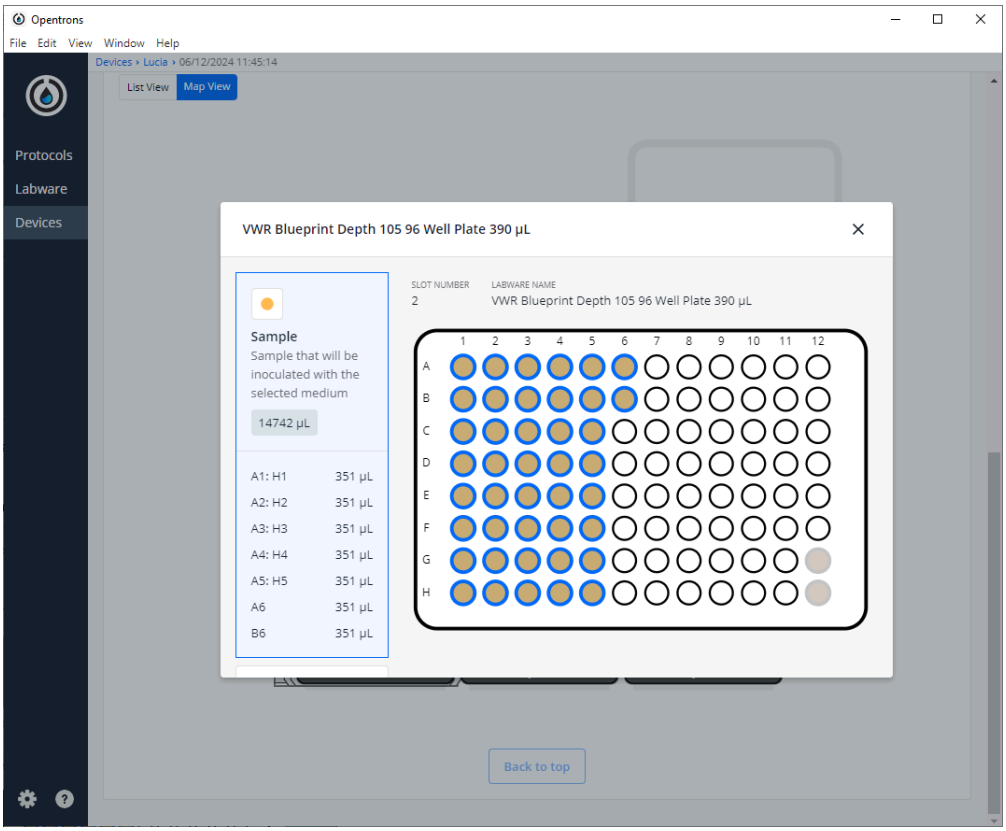


Labware and liquid set-up layout

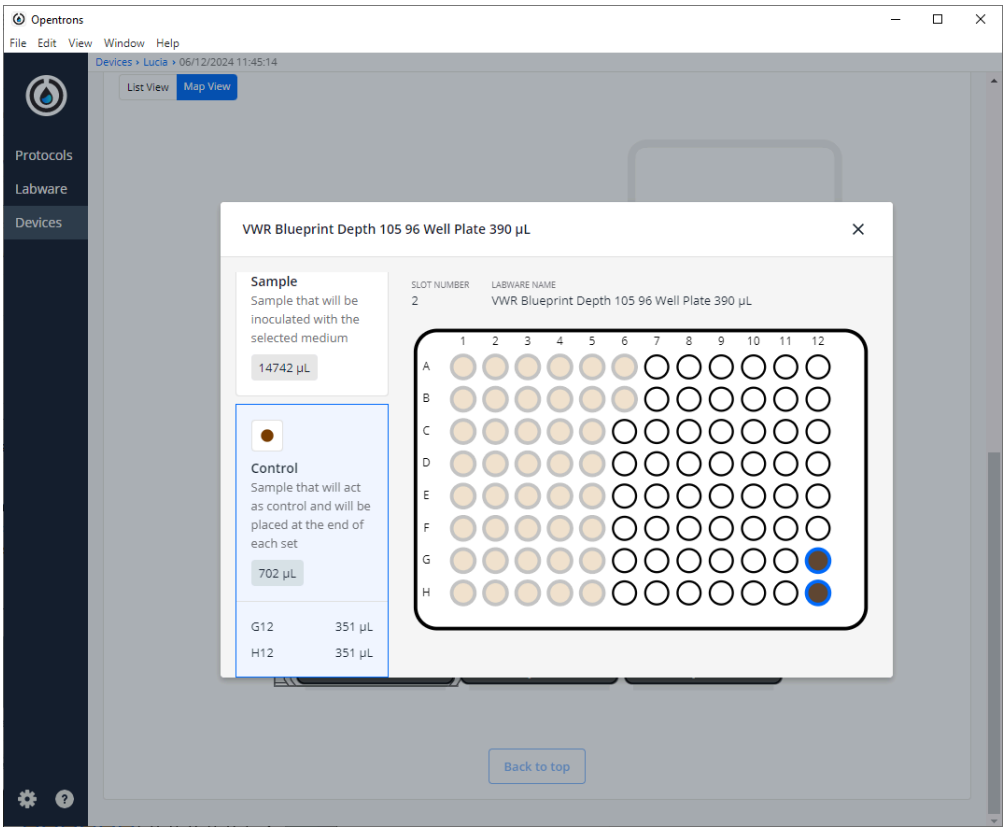


Volumes of the different reagents needed to perform the protocol

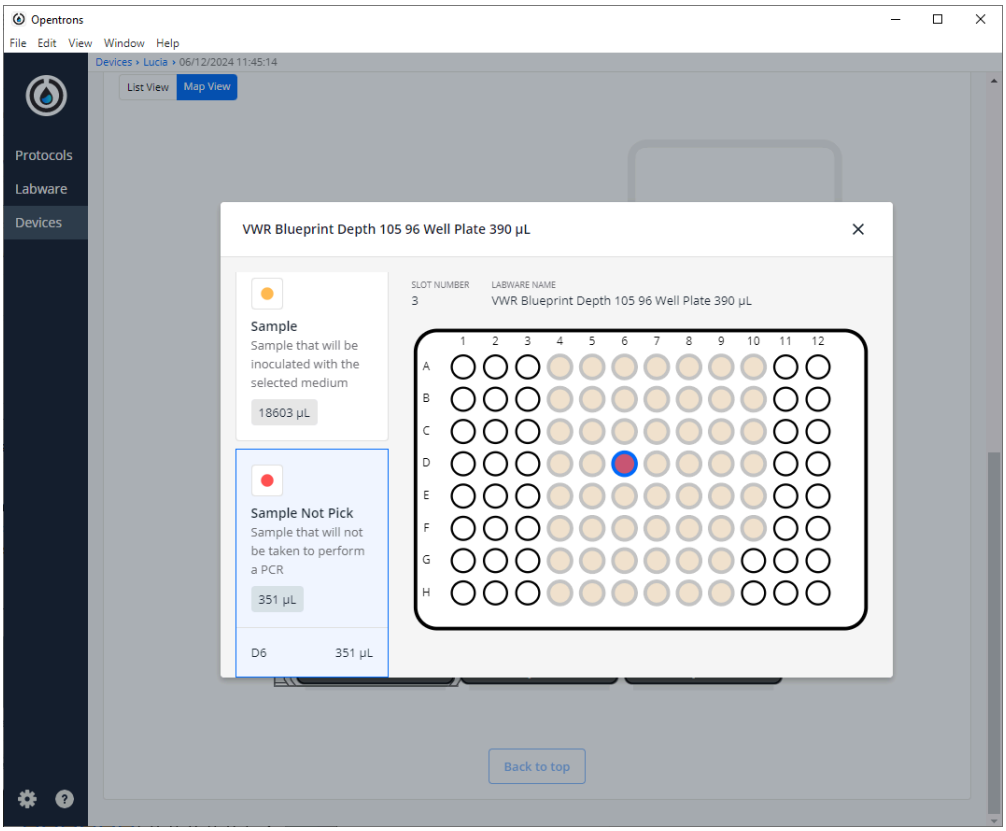
If we **click to the different reagents and the labware where they are** or go to **Map view** and click in the different labware with liquids we can see the distribution and volumes of each tube/well in the different labwares like the following pictures.



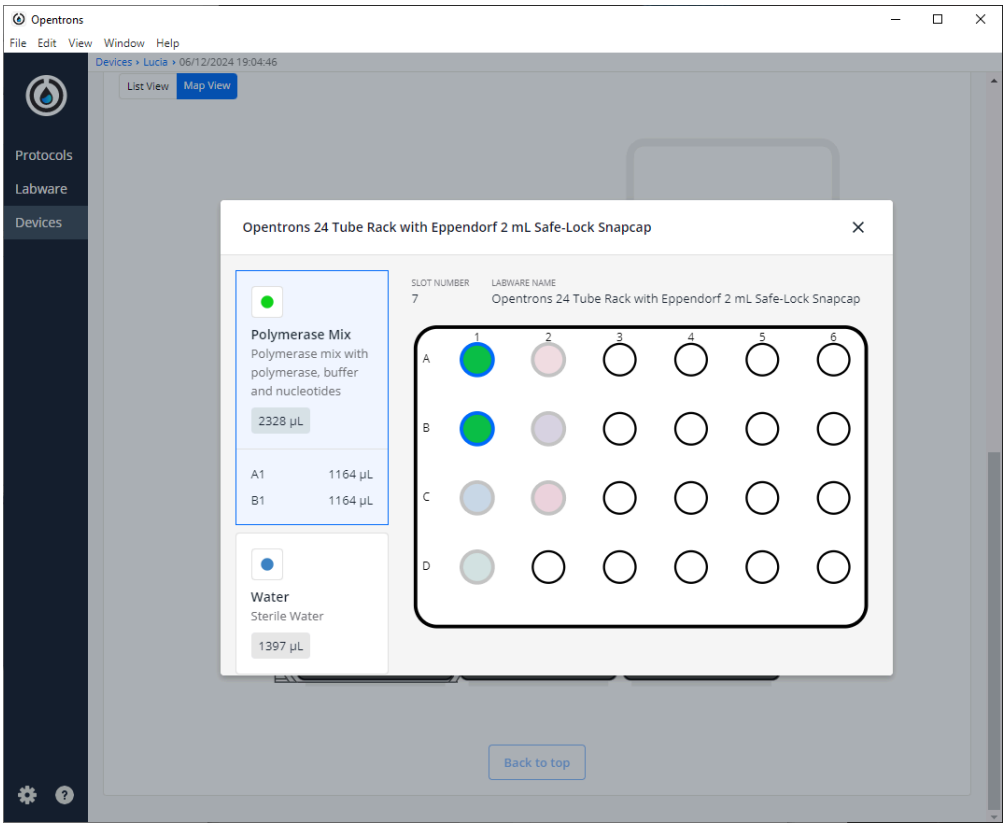
Position and volume of all the samples in the Plate in Slot 2 that are going to be picked to be mixed with the different sets of PCR mix



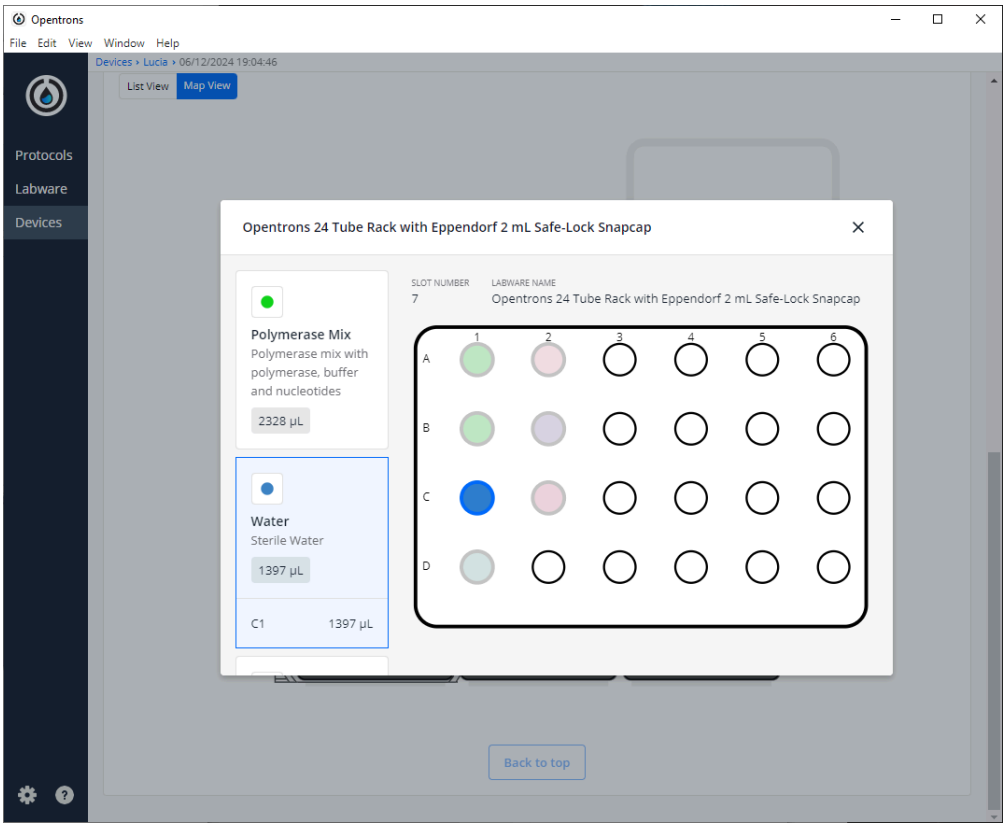
Positions and volume of the samples in the Plate in Slot 2 are going to be used as control



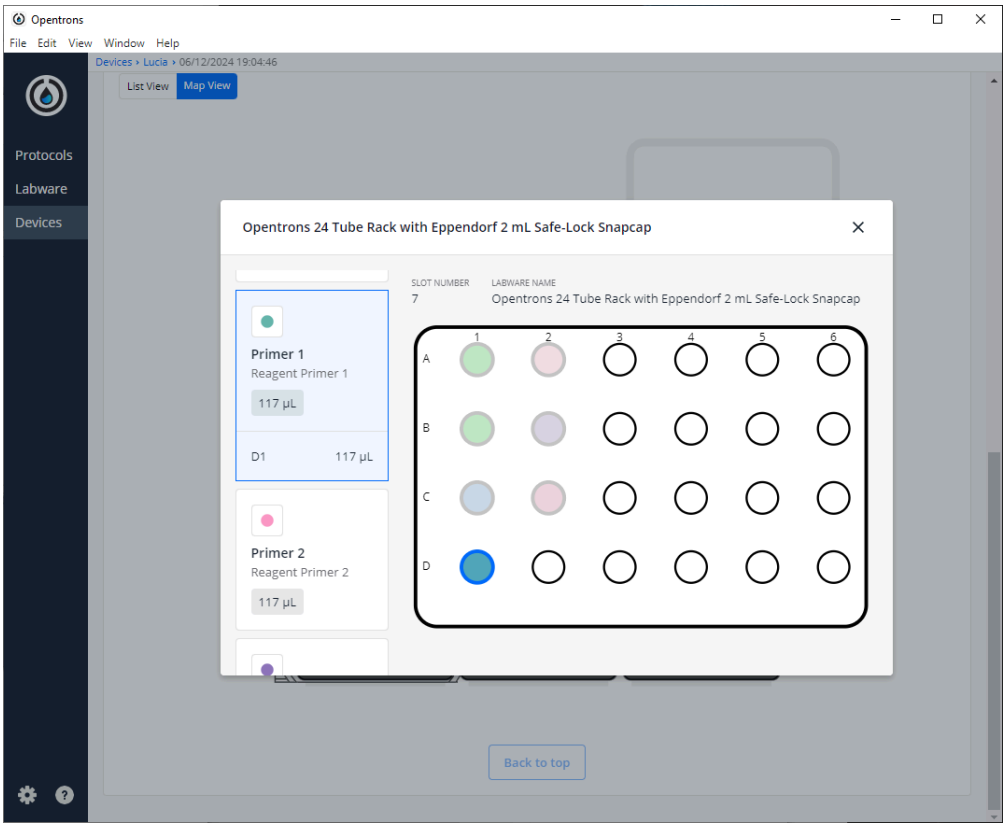
Position and volume of all the samples in the Plate in Slot 3 that are NOT going to be picked to be mixed with the different sets of PCR mix



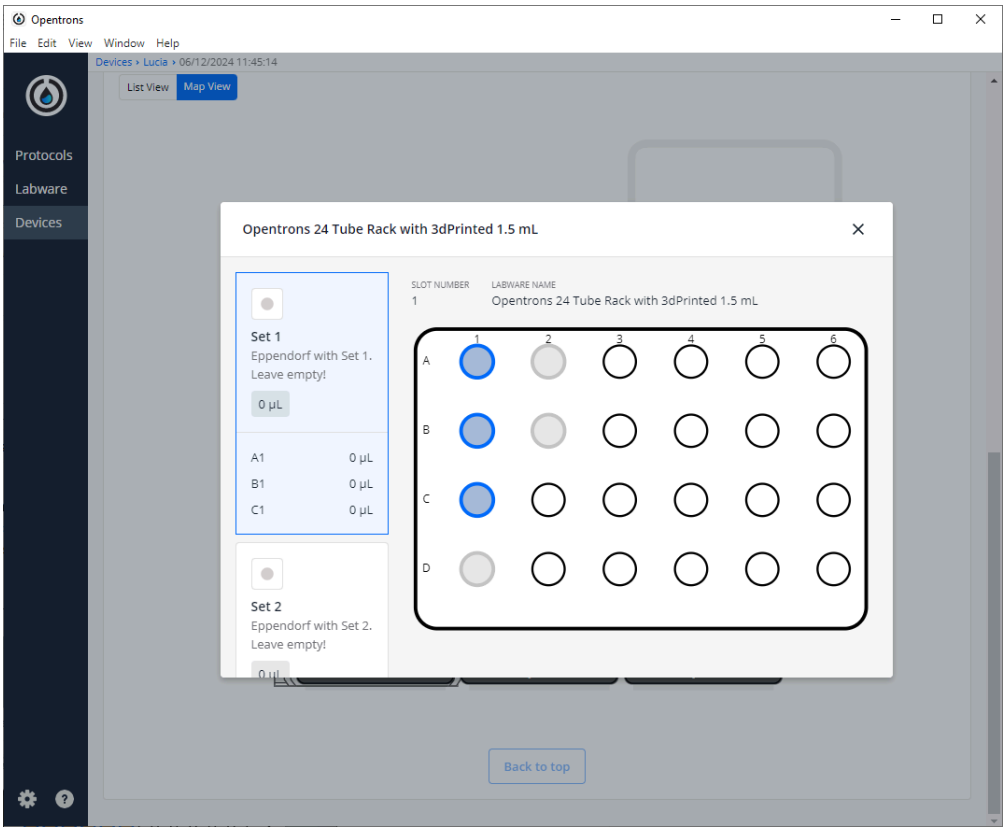
Position and volume of the tube that is going to contain the polymerase mix



Position and volume of the tube that is going to contain MiliQ water



Position and volume of the tube that is going to contain 1 of the primers that are going to be in the final PCR sets



Positions of the final tubes containing the set of primers, in this case Primer 1 and Primer 2, in the heater-shaker situated in the Slot 1

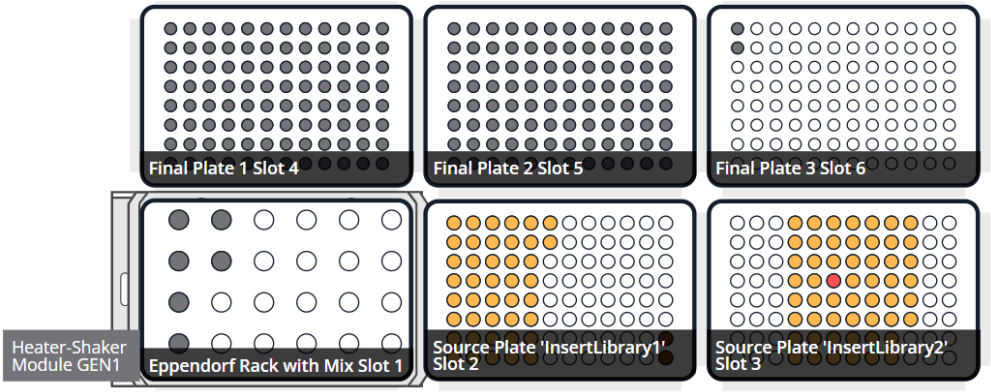
7.6 *Turn the HEPA filter module* 30s

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

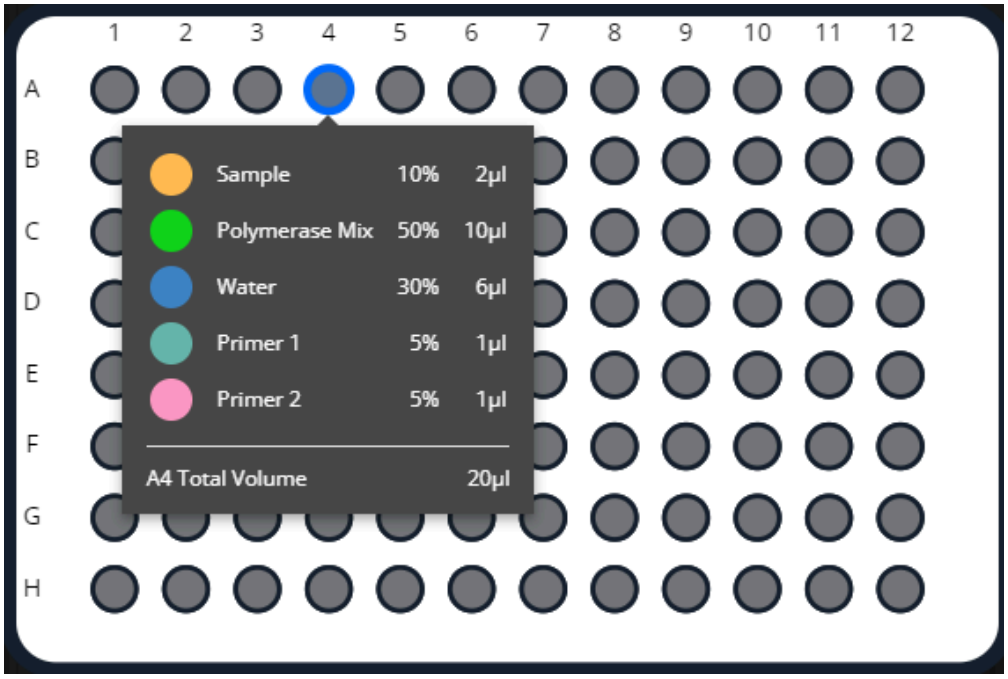
7.8 *Prepare all reagents and labware* in the places the App is showing and take into account the notes in step go to step #3.2 Notes 5m

7.9 *Start run* 2h

Expected result



Final layout of source and final plates in the run

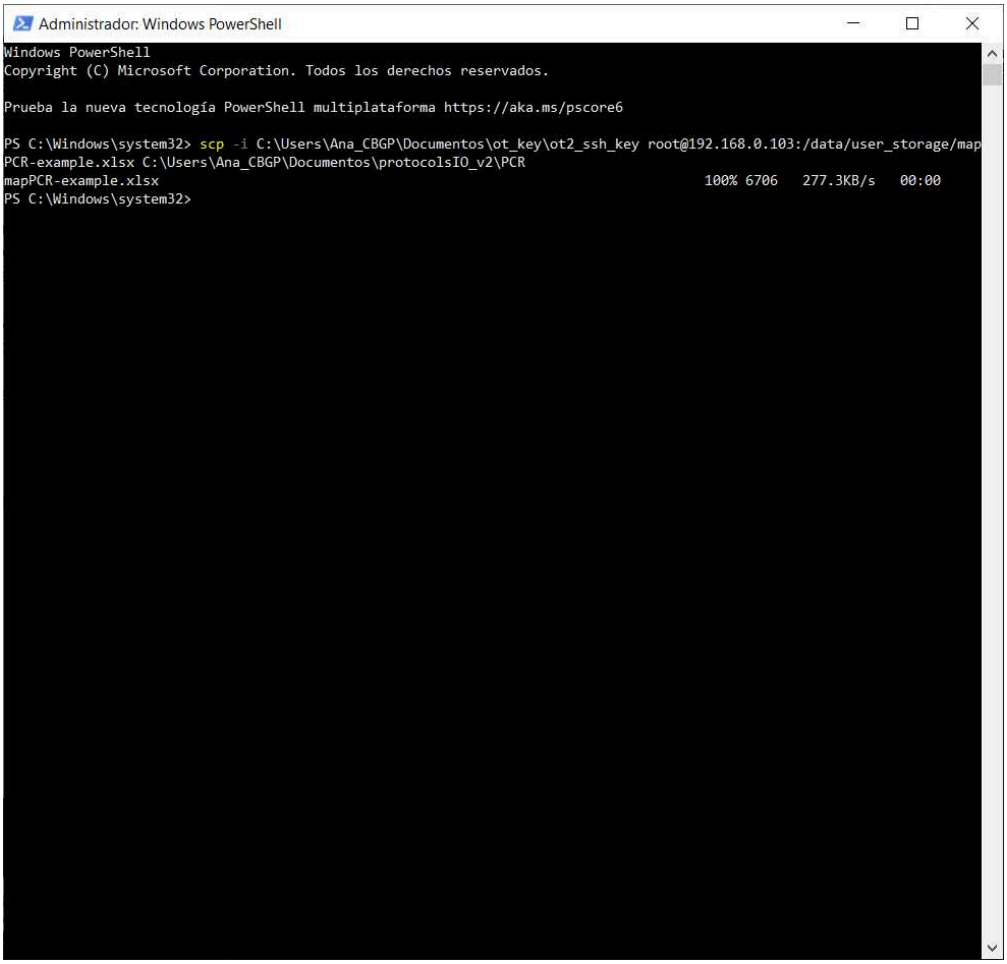


Content of A4 in the labware Final Plate 1 Slot 4 at the end of the run

Here, we will obtain the mix between the PCR mixes and the samples selected by the values in the variable file. These positions are seen in the images by the grey wells, and we can see the info of the plate and the media in the well A4 of the final plate on slot 4 in the second image.

7.10 *Retrieve labwares from the OT* 5m

7.11 Retrieve the final maps that are going to be in the file. In this case, they will be called *mapPCR-example.xlsx* (name that is stated in the variable file in the variable' Final Map Name') 1m



command line windows with the transfer command of the final file with the map(s) from the OT to our computer

mapPCR-example.xlsx

For more information about how to transfer files from one system to another one, in this case from the OT to your computer,

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

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