Webserver – Experiment Assistant

2021/07/23

Table of Contents

1. Overview

The webserver serves as a support our lab automations. At the moment, there are four types of operation-oriented applications and one measurement pre-processor application. The main directory of the server can be accessed through https://vanhasseltlab.lacdr.leidenuniv.nl/ot2/home. The different applications can be accessed from this homepage (Fig. 1), or by using their respective address.

[1] Single Plate Processor
Type : OT2 protocol designer
Accessible from : https://vanhasseltlab.lacdr.leidenuniv.nl/ot2/SingleplateMIC/
Accessible from main directory as : SingleplateMIC
Processor for filling ONE 96-well plate with drug solutions. The processor can only process a maximum of ONE DRUG PER-WELL, even though there is a less stringent limit to how many drugs can be included per-plate (this depend more on the tube rack availability).

[2] Multiple Plate Processor
Type : OT2 protocol designer
Accessible from : https://vanhasseltlab.lacdr.leidenuniv.nl/ot2/MVPlate/
Accessible from main directory as : MVPlate
Processor for filling MULTIPLE 96-well plate with drug solutions. The processor can only process a maximum of ONE DRUG PER-WELL, even though there is a less stringent limit to how many drugs can be included per-plate (this depend more on the tube rack availability).

[3] Drug Combination Processor (Multiple Plate)
Type : OT2 protocol designer
Accessible from : https://vanhasseltlab.lacdr.leidenuniv.nl/ot2/CQ_Plate/
Accessible from main directory as : CQ_Plate
Processor for filling MULTIPLE 96-well plate with drug solutions. The processor can process a maximum of MULTIPLE DRUGS PER-WELL. However, the complex dilution performed in this operation may significantly increase robot run time.

[4] Custom Medium Mixer
Type : OT2 protocol designer
Accessible from : https://vanhasseltlab.lacdr.leidenuniv.nl/ot2/M9MixR/
Accessible from main directory as : M9MixR
Processor for mixing custom medium solutions. Each medium may have a maximum of 50 mL size. The effective limit of the number of components depend on tube rack availability.

[5] Plate Measurement Preprocessor
Type : Measurement preprocessor
Accessible from : https://vanhasseltlab.lacdr.leidenuniv.nl/ot2/PlateAnalysis/GrowthCurve/
Accessible from main directory as : PlateAnalysis > GrowthCurve
Takes plate map and spectrophotometer readout file(s) as input. This pre-processor combines measurement data from all measurement files and combine them into a single long-format file. Actual measurement time (as noted by the machine, not by the file name) will be used for tagging time. Measurement values from wells with identical drug-medium condition will be combined as a single measurement based on its average.

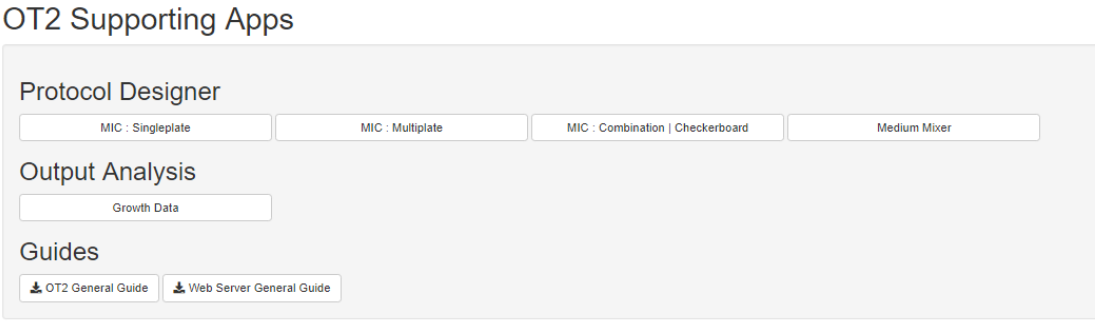

Fig. 1 | Homepage of the Webserver

2. OT2 Protocol Designers

a. General Operating Procedure

OT2 protocol designers are used to translate experiment designs into a set of commands that can be recognized by the OT2 robot. In general, the operation of the protocol designer application consists of the following four steps.

1. Download and adjust the input file
2. Upload the input file to the web application
3. Run the application
4. Download the output files

b. Input Files

Single Plate Processor

The single plate processor requires one input, the **plate map** (.xlsx). Templates/examples for the input files can be downloaded from each respective web server (Fig. 2). After downloading, the input files can be opened and adjusted on Excel or other spreadsheet editors. The plate map describes the desired composition of each wells in the 96-well plate. It is important to note that this operation will not leave any empty wells. Empty wells will automatically filled with the medium to the maximum intended volume. The plate map mainly consists of four sections, 1) stock drug specifications, 2) volume specifications, 3) well specifications, and 4) final plate overview. The first three sections are to be filled by the user.

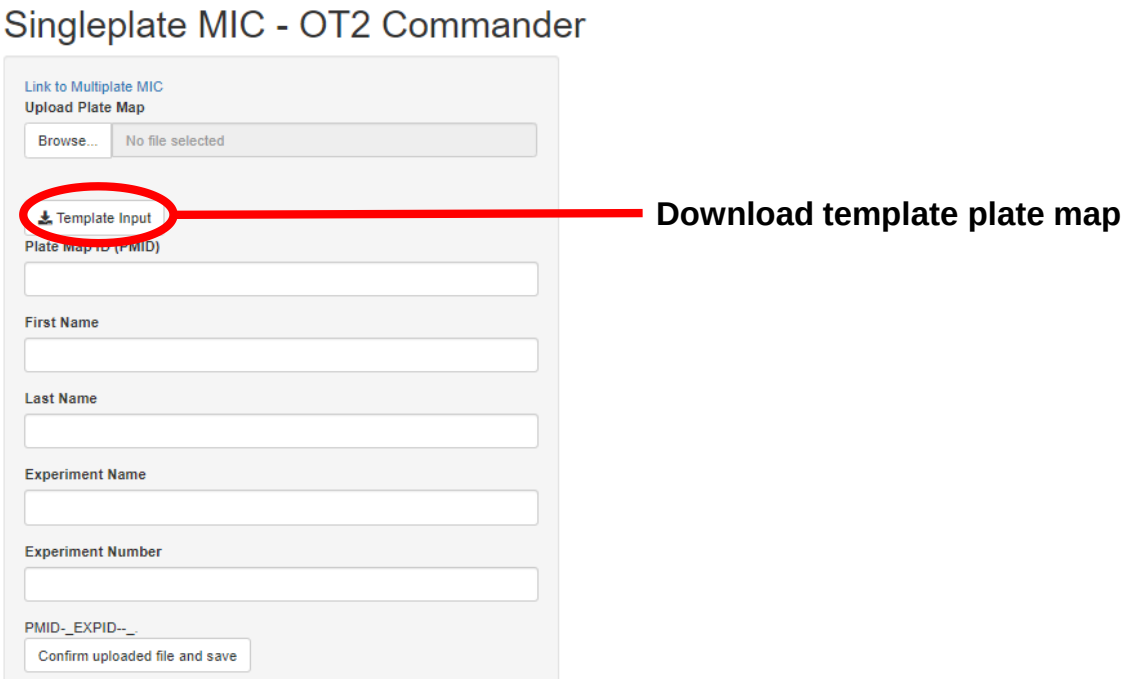

Fig. 2 | Download Button for Plate Map Input Template

Drug Stock Specification

The stock drug specification section can be found on rows 1-3 of the plate map (Fig. 3). The first row is to be filled with drug name. The same drug name will be used in subsequent sections. Drug name is case-sensitive, so make sure to use the same cases throughout the plate map. **The drug name cannot contain spaces or dashes (" - ")**. The second row should indicate the concentration of the drug stock. Do not use thousands separator. The third row indicates the units of concentration used on the concentration. Description of each drug stocks has to be written in its own column. Additional drug stocks can be added by simply adding additional description in its own column. In theory, if stock dilution is not required, the processor can take up to 24 different drug stocks.

Fig. 3 | Drug Stock Specification Section in the Plate Map Input Spreadsheet

Volume Specification

The volume specification section can be found on rows 5-6 (Fig. 4). This section is important for the server to accurately calculate the drug concentration. On cell 5C, the user should indicate the final intended volume of the well (that is, the final volume after all the intended components, including the inoculum, are added into the well). Common 96-well plates have a maximum volume of 200 to 300 µL. On cell 6C, the user should indicate how much of the final volume will be filled with inoculum culture. In the example on Fig. 4, the final volume was set to 200 µL in which 100 µL of this volume is intended for inoculation. On cell 6F, the user is given the option to perform the inoculation inside the robot. The inoculation option is currently removed to ensure the sterility of the robot environment. Thus, the cell 6F should be filled with "No". After the run, users should thus manually inoculate the wells by adding the pre-determined

inoculum volume. In this example, the inoculum volume would be 100 µL. Remember to adjust the inoculum concentration to account for this dilution!

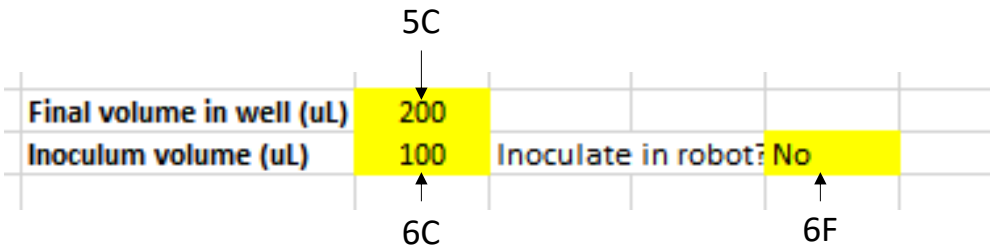

Fig. 4 | Volume Specification Section in the Plate Map Input Spreadsheet

Well Specification

The well specification consists of four maps, the drug map, concentration map, media map, and strain/inoculum map. Each cells in these maps represents the well coordinate of a 96-well plate.

To add a drug solution to a well, the user can indicate the name of the drug into the drug map (Fig. 5, top). With the Single Plate MIC application, you can only add one drug to one well. Alternatively, the cells can be left blank to fill the wells with only the medium and leave it out of the analysis. Drug concentration to be added into these wells should be indicated on the concentration map (Fig. 5, bottom). Naturally, the units of concentration used in the concentration map has to be the same as that indicated on the stock description section. Zero concentration controls and empty wells should be filled with zeros, as shown on the template.

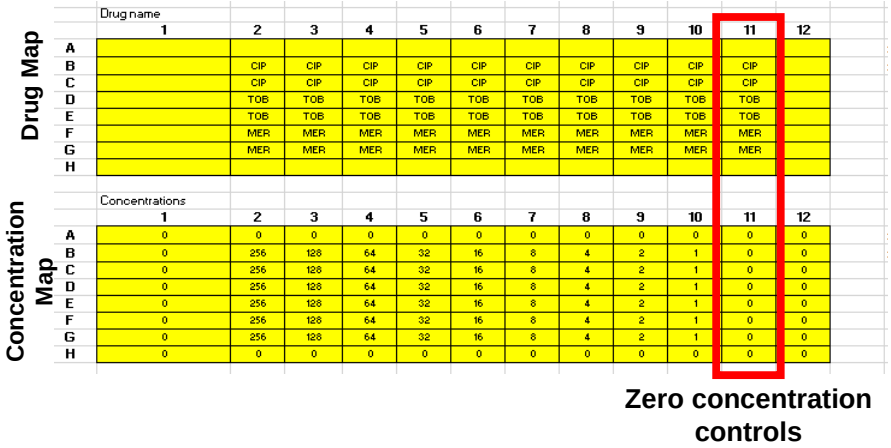

Fig. 5 | Drug and Concentration Maps of the Well Specification Section

Important! Zero concentration controls should also include the name of the drug (in the template, look at wells located on B11-G11). Otherwise, the well will be filled to the final volume, leaving no room for the inoculum. Wells with blank drug names will also be skipped during the pre-processing.

In the medium map (Fig. 6, top), users can indicate the type of medium to be used in each wells. In the current format, users can use up to 6 different mediums in one plate. Medium name does not need to be declared elsewhere in the input file, but it is important to note that the **medium names are case-sensitive**. Similarly, the strain/inoculum map (Fig. 6, bottom) can be filled without declaring the strain name elsewhere in the input file. There is no limit to how many strains you can use in one plate. **Non-inoculated wells should be left blank**. Non-inoculated wells will be used in the pre-processor webserver as an option to apply measurement controls.

	1	2	3	4	5	6	7	8	9	10	11	12
A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A
B	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A
C	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A
D	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A
E	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A
F	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A
G	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A
H	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A	MED_A

Strain name (inoculum)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		STR_A	STR_A	STR_A	STR_A	STR_A	STR_A	STR_A	STR_A	STR_A	STR_A	
C		STR_B	STR_B	STR_B	STR_B	STR_B	STR_B	STR_B	STR_B	STR_B	STR_B	
D		STR_A	STR_A	STR_A	STR_A	STR_A	STR_A	STR_A	STR_A	STR_A	STR_A	
E		STR_B	STR_B	STR_B	STR_B	STR_B	STR_B	STR_B	STR_B	STR_B	STR_B	
F		STR_A	STR_A	STR_A	STR_A	STR_A	STR_A	STR_A	STR_A	STR_A		
G		STR_B	STR_B	STR_B	STR_B	STR_B	STR_B	STR_B	STR_B	STR_B		
H												

Fig. 6 | Medium and Strain Maps of the Well Specification Section

Final Plate Overview

The final plate overview is a non-editable zone. This section is to be used for checking the resulting plate based on the descriptions that you have given above.

Multi-Plate Processor

The multi-plate processor creates up to 6 identical plates. The multi-plate processor requires one input, the **plate map** (.xlsx). Just like the single plate processor, each wells in the multi-plate setting can only receive one type of drug. The plate map for multi-plate processor is slightly different from the plate map of the single plate processor. The operation is mostly identical with that of the single plate processor, with exception to the volume specification section. Unlike the single plate processor, the multiple plate processor no longer has the option for in-robot inoculation. In place of the inoculation option, the input on cell 6F now receives the number of plates to be made in a single run.

Final volume in well (uL)	200		
Inoculum volume (uL)	100	Number of Plate?	2

Fig. 7 | Volume Specification Section of Multi-Plate and Drug Combination Processors

Drug Combination Processor (Multiple Plates)

Unlike the previous plate processors, the drug combination processor can be used to design more complex experiments where a well contains more than 1 type of drug. The multi-plate processor requires one input, the **plate map** (.xlsx). The input operations are mostly identical with the input operation of multi-plate processor, with exception to the well specification section, namely the drug map and the concentration map.

In the combination processor input, the drug map can be filled with multiple drugs, separated with an underscore ("_"). Similarly, drug concentration of each intended drug types should also be separated with an underscore. The order in which the drug types are written defines the order of concentration of the specific drug type. For example, let us take a look at the well C2 in the example on Fig. 7. In the drug map, the well is said to be filled with "AB1_AB2_AB3", which means that it is filled with three types of drugs, namely AB1, AB2, and AB3. On the concentration map, we can see "32_16_0". This means that the concentration of AB1, AB2, and AB3 in this well will be 32, 16, and 0 (units) on the final plate.

Drug name

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	
C		AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	
D		AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	
E		AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	
F		AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	
G		AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	AB1_AB2_AB3	
H												

Concentrations

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0	0	0	0	0	0	0	0	0
B	0	32_32_0	32_32_0	16_32_0	16_32_0	0_0_0	32_0_32	32_0_32	16_0_32	16_0_32	0_0_0	0
C	0	32_16_0	32_16_0	16_16_0	16_16_0	0_0_0	32_0_16	32_0_16	16_0_16	16_0_16	0_0_0	0
D	0	32_8_0	32_8_0	16_8_0	16_8_0	0_0_0	32_0_8	32_0_8	16_0_8	16_0_8	0_0_0	0
E	0	32_4_0	32_4_0	16_4_0	16_4_0	0_0_0	32_0_4	32_0_4	16_0_4	16_0_4	0_0_0	0
F	0	32_2_0	32_2_0	16_2_0	16_2_0	0_0_0	32_0_2	32_0_2	16_0_2	16_0_2	0_0_0	0
G	0	32_1_0	32_1_0	16_1_0	16_1_0	0_0_0	32_0_1	32_0_1	16_0_1	16_0_1	0_0_0	0
H	0	0	0	0	0	0	0	0	0	0	0	0

Fig. 7 | Drug and Concentration Maps of the Drug Combination Processor

Just like in the previous two processors, "empty" wells are to be left blank on the drug map, and filled with zeros on the concentration map. Remember "empty" in this case means that the wells will be filled with a type of medium to the maximum volume determined on the volume specification section.

In application, there is no limit to how many drug types can be added to a single well, but the pipetting accuracy may be affected in highly complex experiment. To illustrate, for a 3-drug experiment with a final volume of 200 µL and inoculum size of 50 µL, the robot will aliquot 50 µL of each drug solutions into the well. Should this be a 5-drug experiment, the robot will instead aliquot 30 µL of the drug solution. Since 30 µL is the lower limit of the P300 pipette, an experiment with more than 5 drugs in a well would thus lower the pipetting accuracy.

Custom Medium Mixer

The custom medium mixer receives one input file (.xlsx). The file contains two main sections, the stock details, and the custom solution details.

Stock details

	Item Category	Item	Stock Concentration	Unit
3	Salts	Salt mixture A	1.00	x
4	Salts	MgSO4 (7H2O)	1.00	mM
5	Salts	CaCl2	0.10	mM
6	Salts	KNO3	280.00	mM
7	Metals	Trace metal mixture A	1.00	x
8	Vitamins	BME vitamin mixture	1.00	x
9	Amino acids	Alanine	720.00	mM
10	Amino acids	Arginine - HCl	120.00	mM
11	Amino acids	Aspartate	106.67	mM
12	Amino acids	Cysteine	160.00	mM
13	Amino acids	Glutamate	30.00	mM
14	Amino acids	Glycine	960.00	mM
15	Amino acids	Histidine-HCl-H2O	25.00	mM
16	Amino acids	Isoleucine	88.00	mM
17	Amino acids	Leucine	80.00	mM

Custom solution details

Custom Solutions	A	B	C	D
Final Volume (mL)	30	30	30	30
	0.2	0	0.2	0.2
	0	0.001	0.001	0.001
	0.0001	0.0001	0	0.0001
	0.35	0.35	0.35	0
	0.0025	0.0025	0.0025	0.0025
	0.01	0.01	0.01	0.01
	1.8	1.8	1.8	1.8
	0.3	0.3	0.3	0.3
	0.8	0.8	0.8	0.8
	0.2	0.2	0.2	0.2
	1.5	1.5	1.5	1.5
	1.2	1.2	1.2	1.2
	0.5	0.5	0.5	0.5
	1.1	1.1	1.1	1.1
	1.6	1.6	1.6	1.6

Fig. 8 | Preview of Input Template of Medium Mixer Processor

Stock Details

The stock details consists of four columns, Item Category, Item, Stock Concentration, and Unit. The "Item Category" column is an arbitrary filler for categorizing the types of materials added to the medium. The "Item" column defines the exact name of the solution to be added into the medium mix. The "Stock Concentration" defines the concentration of the stock solution, with the unit defined on the "Unit" column. New (additional) items can be added to the mixture by expanding the rows of the stock details table.

Custom Solution Details

The custom solution details consists of an (***m+2) x n*** table where ***m*** represents the number of items defined in the stock details table, and ***n*** represents the number of custom medium mix to be made in the run (currently capped to 6 solution mixtures). The first row, "Custom Solutions", should be filled with the name of the solution mix (arbitrary; in the example, the names are simply A, B, C, and D). The second row, "Final Volume (mL)", should be filled with the intended final volume of each solution mix. The remaining ***m*** rows are to be filled with the concentration of the m^{th} item listed on the stock details table to be included in the current custom solution. In the example, custom solution A would receive Salt mixture A to a final concentration of 0.2x (with respect to the stock concentration). The rows in the custom solution details can be filled with zeros to skip an item from the stock details.

c. Uploading and Downloading

After adjusting the input file (remember to save the changes), users would need to upload the input file to the web server. This can be done by clicking the "Browse" button on the web server (Fig. 9). Once the upload is completed, the plate summary will be shown on the right hand side of the display (Fig. 10). User can then fill all the optional experiment details (PMID, name, experiment name, and experiment number) and click "Confirm uploaded file and save".

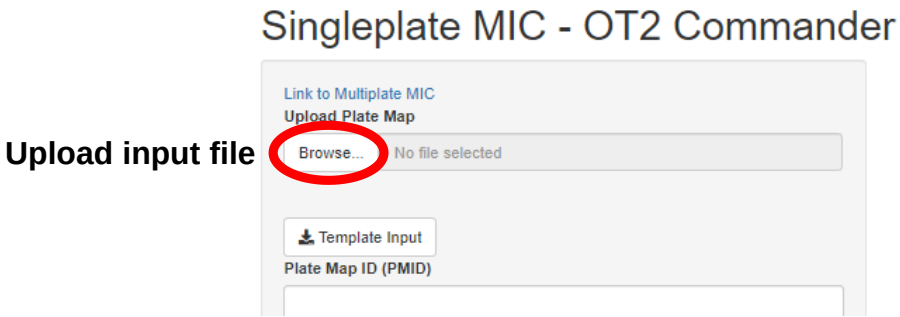

Fig. 9 | Upload Button for Plate Map Input

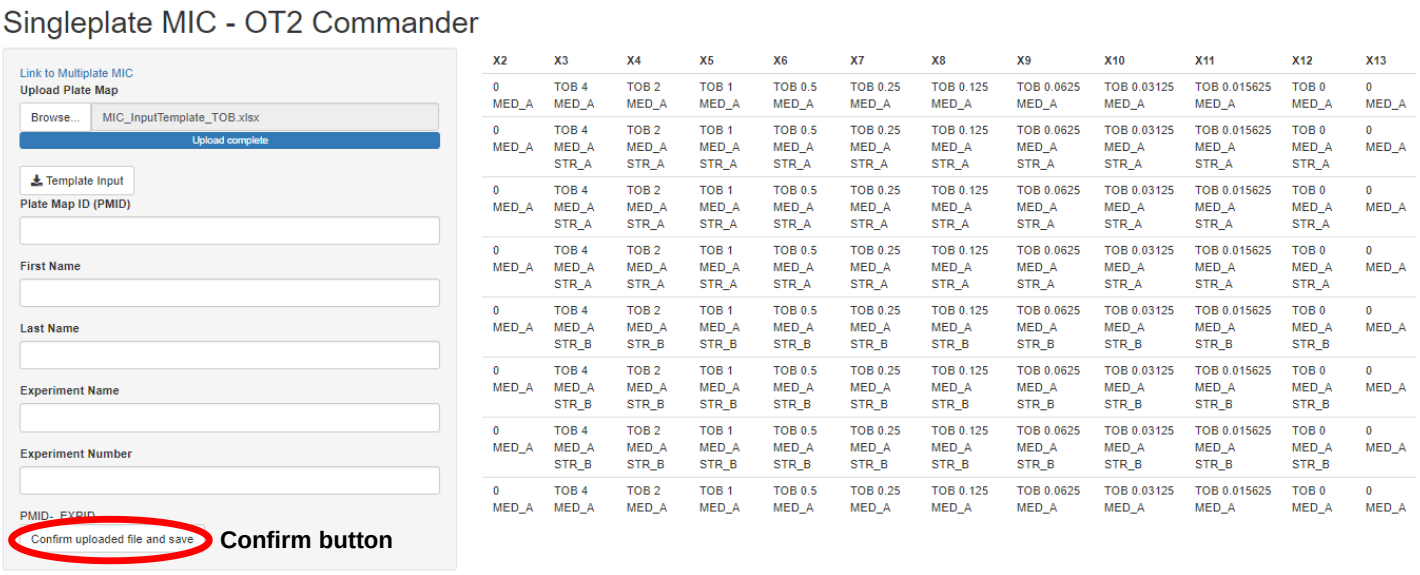

Fig. 10 | Webserver Preview after Successful Input Upload

After clicking the confirm button, the webserver may take a few seconds to design the required OT2 commands. If the run is successful, the display on the right hand side of the screen will change to a table showing required items/solutions and its respective locations in the OT2 robot (Fig. 11). This information is needed to execute the OT2 experiment, but is also available in the output file. There are two output files, 1) the robot commands, and 2) the robot setup guide. The robot commands are to be uploaded to the OT2 robot, whereas the robot setup guide provides information required to setup the robot to run the specific experiment (see guide: **OT2 General Guideline and Maintenance**). These output files can be obtained by clicking the two download buttons, which will only show up after a successful run (Fig. 11, red circle).

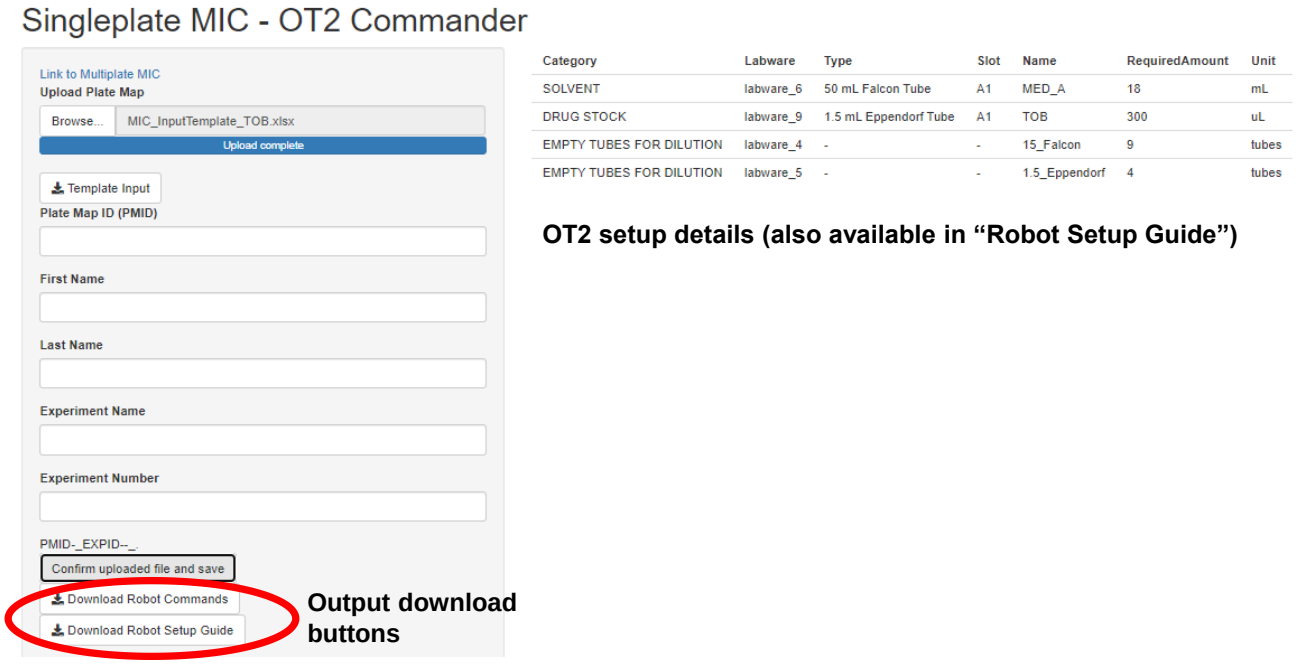

Fig. 11 | Webserver Preview after Processing Run

c. Common error: decimal separator

The plate webserver uses dots (".") as decimal separator. This might not always be the case with some PCs where commas (",") will instead be used as decimal separator. This can result to an error. Make sure your PC accepts dots as decimal separator before opening and editing the input template. Alternatively, you can use the PC attached to the OT2 robots in the lab to edit the input templates (use OpenOffice instead of Microsoft Excel).

3. Operations for Plate Measurement Preprocessor

a. General Operating Procedure

The growth curve preprocessor can be used to combine multiple output files from the plate reader into a single table. The application can also be used to normalize measurement values to pre-determined controls. Operation of the preprocessor application consists of three main steps, 1) file upload, 2) control selection, and 3) output download.

File Upload

The preprocessor receives two types of main inputs, measurement files (.csv, multiple files allowed) and one plate map file (.xlsx, one file allowed). Currently, the processor can only accept measurement files produced by the incubator-plate reader robot (Newton). The plate map input is identical to the plate map input given in either multiple plate or single plate processor. Adjustment of this plate map input was discussed on section 2.b.

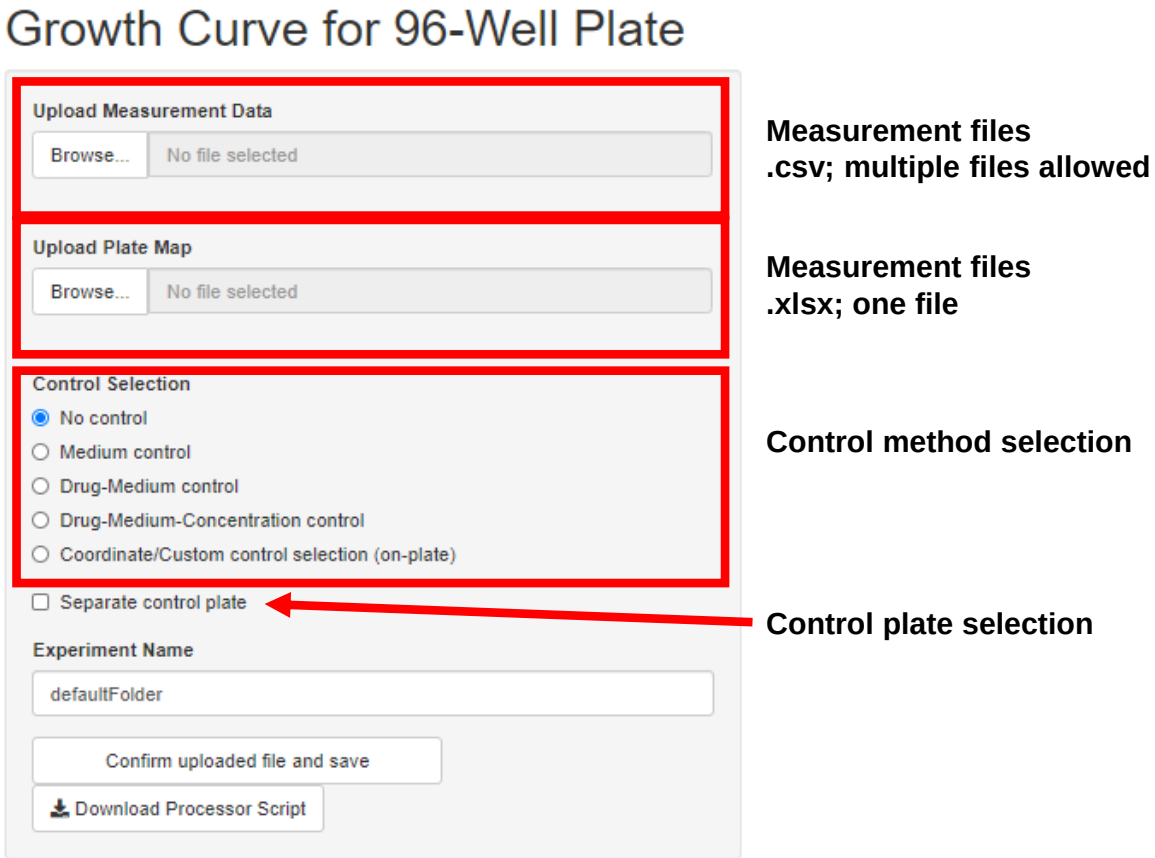

Fig. 12 | Preview of Measurement Preprocessor Webserver

Caution: at the moment, the pre-processor is not yet optimized for accepting drug combination plate maps. The application seem to run successfully but its operation in different use cases is yet to be evaluated. Additional troubleshooting might still be needed.

Control Selection

During the control selection, users are prompted to select the control method as well as the plate in which control wells are located. There five types of control method. The difference between these methods are summarized in the following table.

Table 1. Available Control Methods

Control Method	Description
No control	Measurement values reported as is without correction
Medium control	Measurement values corrected to the values of non-inoculated and zero drug concentration wells containing the same type of medium.
Drug-Medium control	In addition to medium control, specific controls will be selected for each drug type
Drug-Medium-Concentration control	In addition to drug-medium control, specific controls will be selected for each drug concentrations
Coordinate/Custom control selection (on-plate)	Additional control plate map used to define control selection. This option is not available in separate control plate option.

With exception to the custom control selection, wells that are selected for controls have the following characteristics.

1. Not inoculated (no strain name given on the plate map)
2. Have a drug name assigned on the drug map (otherwise, the well will be recognized as 'filler' wells)

Wells to which no applicable control can be found will not be corrected. No value will be given on its 'corrected value' column.

Custom Control Selection

This option will not be shown when the 'separate control plate' option is NOT selected (using a separate control plate will inherently provide a similar function). When this option is selected, an additional upload interface will appear. An additional download button will also appear for downloading the template for the custom control map.

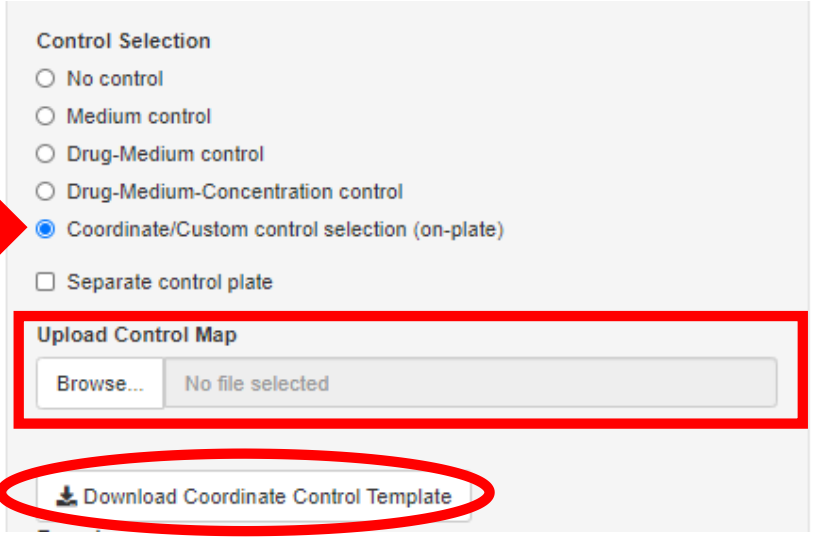

Fig. 13 | Control Plate Map Upload for Coordinate Control Option

The custom control map is written in a 96-well plate map format in which each cells represents a well in a 96-well plate (Fig. 14). The cells are to be filled with group indicator and control/variable indicator, separated by an underscore. In the example, wells A2 to A11 (group 1 controls) will be used as control for wells B2 to D11 (group 1 variables, or 'var'). Ingored wells should also be indicated by filling the respective cells with 'ignore'. Careful not to confuse column/row nomenclature of a 96-well plate and Microsoft Excel's column/well nomenclature.

	1	2	3	4	5	6	7	8	9	10	11	12
A	ignore	1_control	1_control	1_control	1_control	1_control	1_control	1_control	1_control	1_control	1_control	ignore
B	ignore	1_var	1_var	1_var	1_var	1_var	1_var	1_var	1_var	1_var	1_var	ignore
C	ignore	1_var	1_var	1_var	1_var	1_var	1_var	1_var	1_var	1_var	1_var	ignore
D	ignore	1_var	1_var	1_var	1_var	1_var	1_var	1_var	1_var	1_var	1_var	ignore
E	ignore	2_var	2_var	2_var	2_var	2_var	2_var	2_var	2_var	2_var	2_var	ignore
F	ignore	2_var	2_var	2_var	2_var	2_var	2_var	2_var	2_var	2_var	2_var	ignore
G	ignore	2_var	2_var	2_var	2_var	2_var	2_var	2_var	2_var	2_var	2_var	ignore
H	ignore	2_control	2_control	2_control	2_control	2_control	2_control	2_control	2_control	2_control	2_control	ignore

Fig. 14 | Plate Map Template Preview for Custom Control Selection

Separate Control Plate

User may use measurements from a separate plate as control for the current measurement by selecting the option 'separate plate control'. If 'separate control plate' option is selected, user will be required to upload additional measurement and plate map file for the control plate (Fig. 15). The plate map, in this case, will also be the plate map provided by one of the OT2 protocol designers, adjusted according to the control plate design. Drug and medium names used in the control plate map has to be identical to that given in the measurement plate's plate map. When relevant, drug concentration of indicated on the control plate map should also be indicated in the same unit as that indicated in the measurement plate map. Just like the measurement files of the main plate, multiple .csv files should be uploaded for the control plate. When separate control plate is used, main measurement will be normalized to its control's measurement at the closest time point.

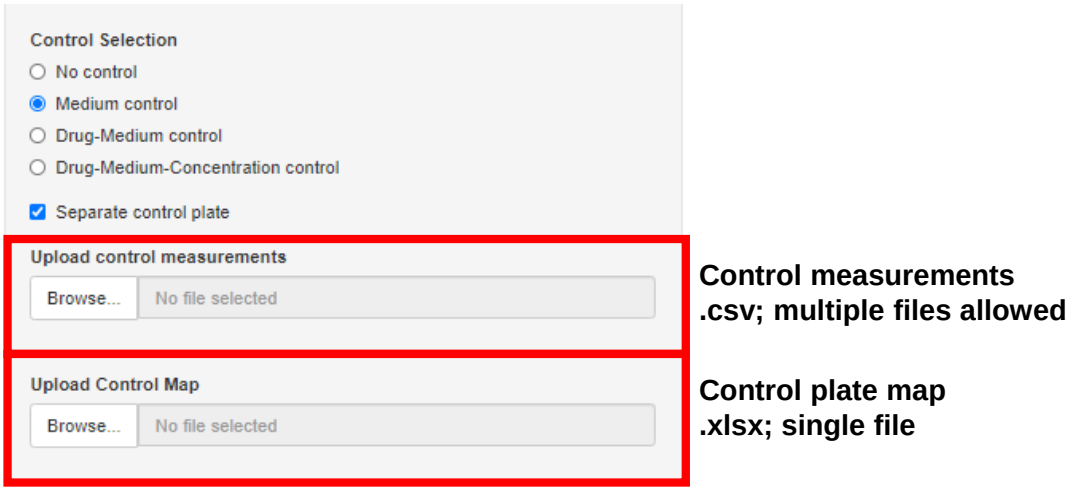

Fig. 15 | Control Measurements and Plate Map Upload for Separate Control Plate

Caution: time points are calculated as elapsed time by using the first measurement as the zero time point. Time points for control and main measurements are calculated separately, using each of its corresponding first measurement as the zero time point.

Running the Preprocessor

After uploading all the required input files, the preprocessor can be run by clicking the "Confirm uploaded file and save" button. The application may take a couple of seconds to complete the operation, which may extend depending with increasing number of measurement files. If the run is successful, a data table will be displayed on the right hand side of the screen (Fig. 16). Additionally, two download buttons will appear at the bottom-left of the display (Fig. 16, red circles). You can download the preprocessed data or the non-processed control datasets (individual control measurement values may be excluded from the preprocessed dataset in some control options) by using these download buttons.

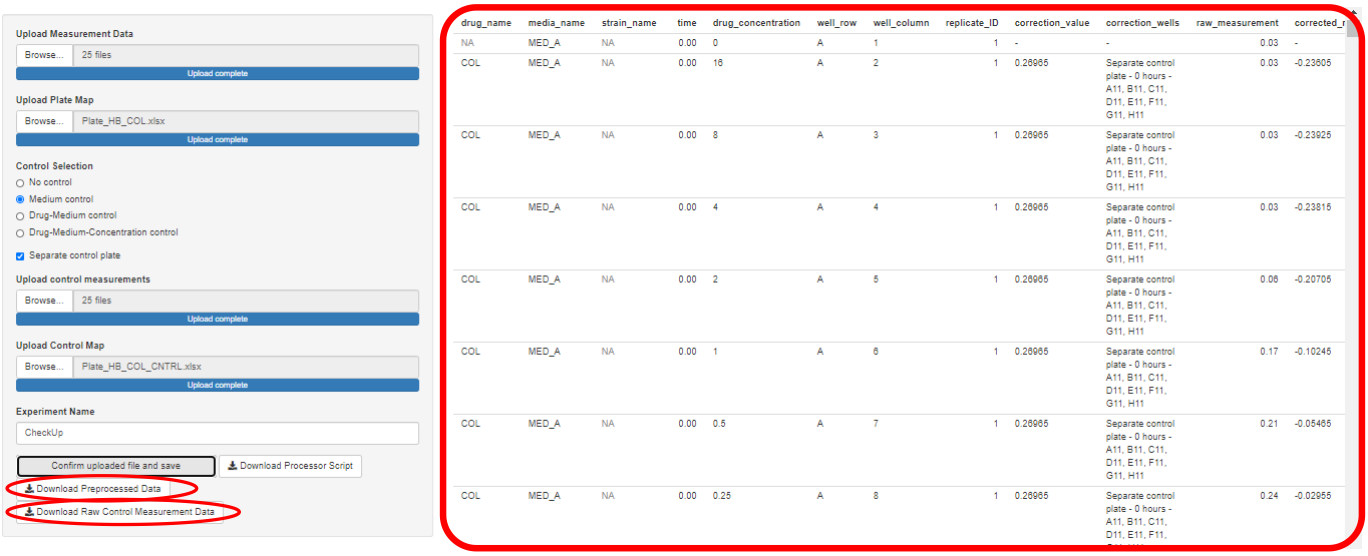

Fig. 16 | Plate Preprocessor Webserver Preview after a Successful Preprocessing Run