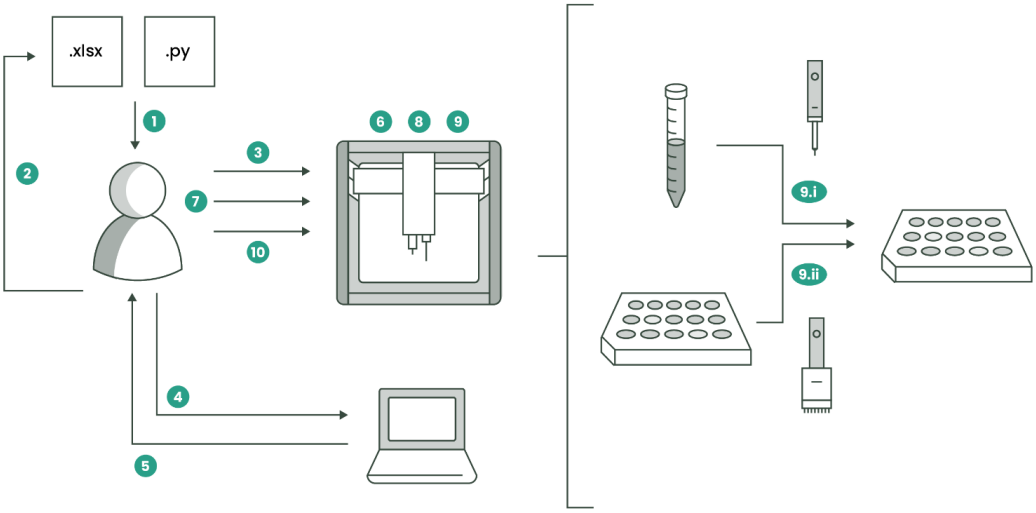
Instructions to use



1. Download the Excel template and Python script.
2. Fill the Excel file (variables file) to customise the experiment according to the guidelines at the end of this document.
3. Send the variables file to the robot that you are going to perform the protocol. If you are using a Unix-based or like system, you can send the file using the command `scp` to the folder `/data/user_storage`

   The command should look similar to
   ```
   scp -i [password file] VariablesPlateIncubation.xlsx root@[IP_Robot]:/data/user_storage
   ```

   For more information about sending files to an Opentrons robot visit https://support.opentrons.com/s/article/Copying-files-to-and-from-your-OT-2-with-SCP
4. Import the Python script to the Opentrons App. A warning will appear on the protocol simulation because the script is not designed to read the Excel variable file from your computer but from the robot system.
5. Start setup in the robot that has your customised Excel file.

   The Opentrons app will simulate the script in the chosen robot, providing you with an error message if something is wrong or, in case the run can be done, with the required labware layout and needed reagents. The

colour of the reagents given in the Opentrons app could change every run due to them being generated randomly, but the volumes will always be the same given the same Excel file.

The volumes of the reagents are exactly what the run needs, so it is recommended to pour between 10-20% more of that reagent either in the falcon tubes (if set like that) or wells of the plate. It is a good practice to have more volume in all wells/tubes than is needed due to the pipetting error that the chosen pipettes could have, and the fact that the OT-2 liquid handler does not have a sensor to check if the volume is picked or dispensed.

6. Set the different reagents and labware as the Opentrons App shows. Both reagents and different labware will have labels to help you recognise where every one of them should be placed.
7. Perform a labware offset, ensuring all the labware is calibrated correctly.
8. (Optional) Turn on the HEPA filter to lower the chances of contamination.
9. Close the door of the Opentrons and press the button 'Start run'. The Opentrons robot will follow the following stages:

    i) Creation of final plates with the single-channel pipette(s) from the falcon tubes
    ii) Creation of final plates with the multi-channel pipette(s) from the plates with reagents

    Both sub steps are optional, depending on the configuration of the final plates
10. Wait for the protocol to finish and, if needed, change the tip racks as stated in the Opentrons App.

Guidelines to fill in the Excel file

General Variables Sheet

API Name Labware with Reagent(s) in Tube(s)

- **Type of Value**: Text
- **Description**: Opentrons API name of the labware that will contain the falcon (15 or 50mL) or eppendorf tubes with the reagent(s) if some final plate is going to be created with single-channel pipette(s).
  In case that the tubes are falcon, the labware should only have 15 or 50mL tubes, this protocol does not admit mixed tube racks like "Opentrons 10 Tube Rack with Falcon 4x50 mL, 6x15 mL Conical".
  In case no plate is going to be created with a single channel, this variable will be ignored.
- **Example**: `opentrons_15_tuberack_falcon_15ml_conical`

Type of Reagent Tube

- **Possible Values**: eppendorf | falcon
- **Description**: This variable determines what type of tubes are the ones established in the labware set in 'API Name Labware with Reagent(s) in Tube(s)'. It is important that the 2 variables values are coherent, because if the tubes are set as *eppendorf* there will be no track of height while if it set as *falcon* there would be a track of height to prevent the pipette from getting wet with the reagent(s) during the aspiration of it.
- **Example**: `eppendorf`

API Name Labware with Reagent(s) in Plate(s)

- **Type of Value**: Text
- **Description**: This variable specifies the Opentrons API name of the labware that will contain wells, columns, or rows with the reagent(s), particularly if a final plate is going to be created using multi-channel pipette(s).
  The specified labware must have either 1 or 8 rows to ensure that the 8-channel multi-channel pipette can access it correctly, in other words, to ensure pipette-labware compatibility.
  If no plate is being created with a multi-channel pipette, this variable will be ignored.
- **Example**: `nest_12_reservoir_15ml`

API Name Final Plate

- **Type of Value**: Text
- **Description**: Opentrons API name of the labware that will contain the different reagents according to the maps given in 'Name Sheet Map Reagents' and 'Name Sheet Map Volumes'.
  Make sure that this labware has the same dimensions, rows and columns, that the maps provided in the latter variables.
  If any of the final plates is going to be created with a multi-channel, this labware should have 1 or 8 wells so the multi-channel can properly access it, i.e., to ensure compatibility pipette-labware.
- **Example**: `corning_96_wellplate_360ul_flat`

Number of Final Plates

- **Type of Value**: Whole Number
- **Description**: This variable specifies the number of columns in the Sheet 'FinalPlateVariables' that will be read. In other words, it would be the number of plates that are going to be created, not taking in account the replicas of each one of the specified plates.
- **Example**: `3`

Change Tip In Distribution

- **Possible Values**: never | reagent | aspirate | well
- **Description**: This variable determines how often the pipette tip is changed during the distribution of reagents. The pipette may be a multi-channel (distributing columns of reagents) or a single-channel (distributing from tubes of reagents). This setting controls the frequency of tip changes based on the level of sterility or contamination control required for your experiment.
  This variable can take one of the following 4 values and if left empty, it will be assumed as *well*:
  - **never**: the pipette tip is never changed throughout the entire distribution, i.e., the same tip is used to transfer all the reagent or column of reagents.
    1 tip will be used to distribute all the reagent.
    If multiple pipettes are used, the tip is changed each time the pipette is switched.
  - **reagent**: The pipette tip is changed every time the type of reagent being distributed changes. For a single-channel pipette, this means changing the tip when switching between different

tubes of reagent. For a multi-channel pipette, this means changing the tip when switching between columns with different reagent combinations. If multiple pipettes are used, the tip is also changed each time the pipette is switched.
Tip changes occur with each new reagent or column of reagents, as well as each pipette change.

- **aspirate**: the pipette tip is changed every time the pipette goes to a source tube or column to aspirate liquid. If multiple pipettes are used, the tip is also changed each time the pipette is switched.
- **well**: The pipette tip is changed every time the pipette dispenses liquid into a final well. If transferring the final volume requires multiple aspirations, the tip is changed for each dispense. If multiple pipettes are used, the tip is also changed each time the pipette is switched.

- **Example**: `aspirate`

Position Dispense Final Well

- **Possible Values**: top | bottom | center
- **Description**: This variable determines the position within the final well where the sample volume will be dispensed. This variable can take one of the following 3 values and if left empty, the default value is *bottom*:
  - **top**: the liquid will be dispensed at the top of the well.
  - **center**: the liquid will be dispensed at the center of the well.
  - **bottom**: the liquid will be dispensed 0.1 mm above the bottom of the well, which is the default position for transferring with the Opentrons.
- **Example**: `center`

Touch Tip After Dispense

- **Possible Values**: True | False
- **Description**: This setting helps ensure complete dispensing of the liquid and can reduce dripping or residual liquid left on the pipette tip. This variable determines whether the pipette will touch its tip to the top of the wells in the source and final columns during the transferring of volume from the source to the final plates. If set as *True*, the pipette will perform a touch tip in the source and final labware. By default, this value is set as *False*.
  Take in account that this variable is also dependent on the type of labware and the pipette that is going to be transferring and it is

completely dependent on the Opentrons robot ability to do the touch tip with the pipette in that specific labware. For example, in a lot of cases the touch tip with the multi-channel is not possible. For more information about the specific cases that a touch tip cannot be done, consult the Opentrons team and/or its documentation.

- **Example**: `False`

Internal Replicas

- **Possible Values**: True | False
- **Description**: This variable determines if the desired replicas for each final plate would be placed on the same plate or if another one will be created for each replica.
  If this variable is set as *True*, replicas will be placed on the same plate as the original map provided by the user. The replica will be placed from the last well or column with a reagent. Any empty cells before the first well with a reagent will be ignored, i.e., if the first well with reagents is the C1, those 2 empty places in A1 and B1 will be ignored.
  If the variable is set as *False*, a new labware with the same structure as defined in the maps associated to that plate (Name Sheet Map Reagents and Name Sheet Map Volumes sheets) will be created for each replica. In this case, everything will be maintained, any empty spaces before the first well with reagent, after the last well and any in between.
  In any case, the original structure of positions will be maintained. This means any empty spaces (either well or columns) between the first and last well with reagents will be preserved.
  If no replicas are desired for any of the final plates, i.e., all values for Number of Replicas in the sheet FinalPlatesVariables are set as 0 or left empty, this variable will be ignored.
- **Example**: `False`

Optimization Space Source Plate Reagents Disposition

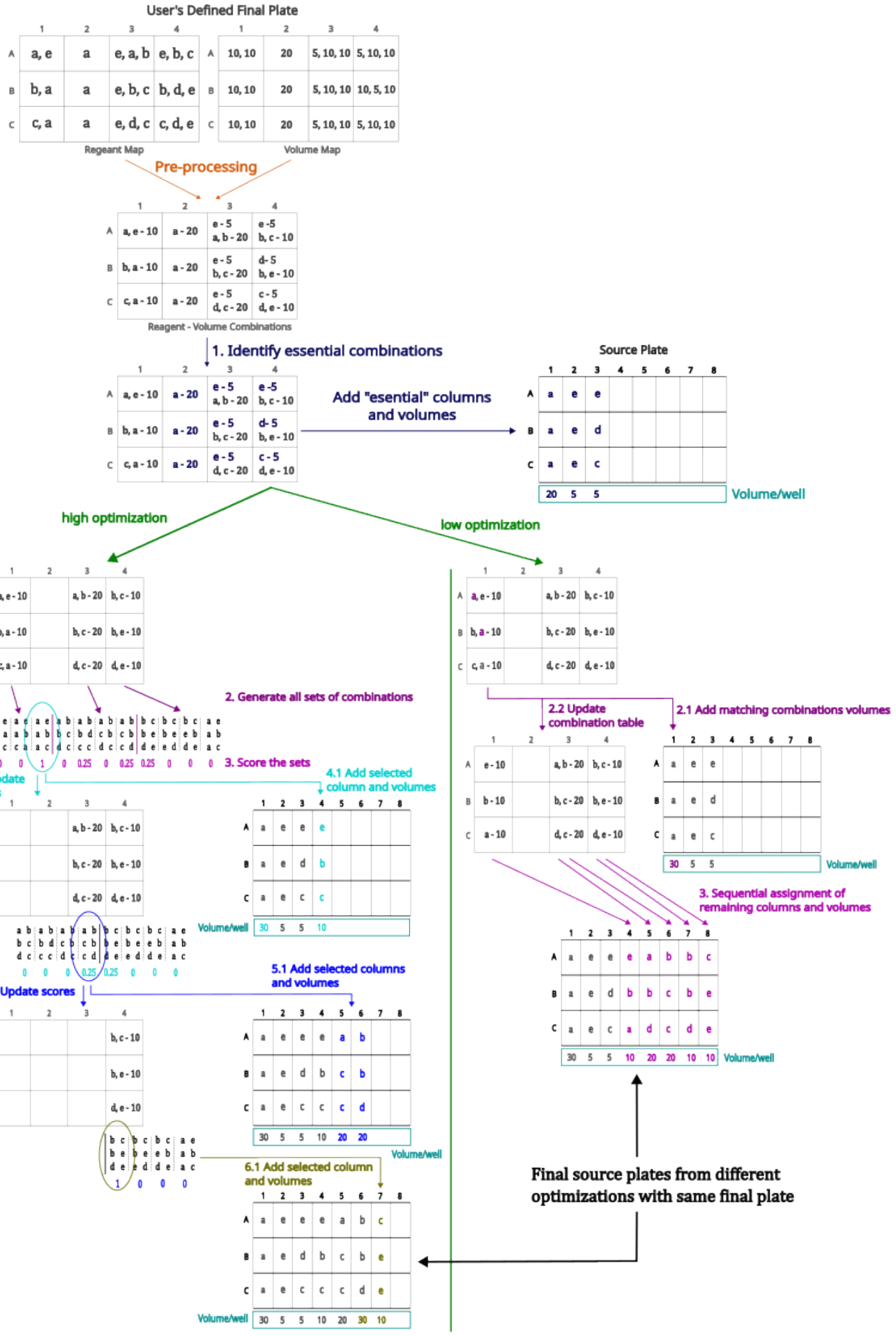
- **Possible Values**: low | high
- **Description**: variable that determines how reagents are distributed in the columns of the source plate, which are used to create the final plates. However, this variable will be ignored if no final plates are created with a multi-channel pipette.
  This variable can take on 2 values: *low* and *high*. If the source labware, set in 'API Name Labware with Reagent(s) in Plate(s)', has only 1 row, it will be directly assigned as *low*. Both optimisations could produce the same output, meaning the same distribution of reagent layouts.

The behaviour of the variable depends on its value as follows (both behaviours can be seen with an example in the picture situated on the next page):

- **low** (right part of the image): This optimisation of the source labware space (columns) guarantees that reagents will be assigned (unless it runs out of space), possibly at the cost of more source labware space. The steps of this optimisation are:
    1. Identify essential combinations: (step shared by both optimisations): Combinations of volume-set of reagents for all the final plate columns are analysed to find those that only have one option, i.e., 1 volume and 1 reagent or 1 column of reagents combination. These combinations are automatically assigned to columns in the source plate. We will call this kind of column an "essential" column.
    2. Check for matching combinations: The remaining combinations of reagents-volume are then checked to see if they match any of these essential columns. If they do, volume is added to that column.
    3. Sequential assignment: Any remaining combinations of reagents-volume are added sequentially based on their order of appearance in the final plate maps.
- **high** (left part of the image): While this method is more efficient regarding source labware space usage, it operates within a time cap. If the process takes too long, it may stop before achieving the optimal source labware combination, and the protocol will not be able to run; if this happens, the recommendation is to set this variable as low. The steps of this optimisation are:
    1. Identify essential combinations (step shared by both optimisations): Combinations of volume-set of reagents for all the final plate columns are analysed to find those that only have one option, i.e., 1 volume and 1 reagent or 1 column of reagents combination. These combinations are automatically assigned to columns in the source plate. We will call this kind of "essential" column.
    2. Generate possible sets: The remaining volume-reagent combinations are combined to form the user-defined column combinations, which create all possible sets of columns.
    3. Score analysis: Each set of columns is analysed and assigned a score based on the likelihood of being reused to form other columns in the final plates.

4. Prioritize and update scores: Combinations with the highest scores are prioritised, and scores are updated as combinations are used.
   This process continues until all necessary final plates have columns to be created.

Example: `low`


User's Defined Final Plate
Regeant Map
Volume Map
Pre-processing
Reagent - Volume Combinations
1. Identify essential combinations
Add "esential" columns and volumes
Source Plate
Volume/well
high optimization
low optimization
2. Generate all sets of combinations
3. Score the sets
4.1 Add selected column and volumes
4.2 Update scores
5.1 Add selected columns and volumes
5.2 Update scores
6.1 Add selected column and volumes
2.1 Add matching combinations volumes
2.2 Update combination table
3. Sequential assignment of remaining columns and volumes
Final source plates from different optimizations with same final plate

Pipette Variables Sheet

Name Right Pipette

- **Type of Value**: Text
- **Description**: Opentrons API name of the pipette that will be in the right mount of the Opentrons arm.
  If this variable is left empty both 'API Name Tiprack Right Pipette' and 'Initial Tip Right Pipette' will be ignored.
  Make sure to have at least 1 pipette that is single-channel if 1 or more final plates are created with it.
  Make sure to have at least 1 pipette that is multi-channel if 1 or more final plates are created with it.
- **Example**: `p300_multi_gen2`

API Name Right Pipette TipRack

- **Type of Value**: Text
- **Description**: Opentrons API name of the tip rack associated with the right mount pipette. This script does not check that the pipette and the tip rack are compatible.
  If the tip rack from the right and left pipette are the same, both pipettes will pick up from the same tip rack, and the initial tip should be the same.
  In case of pipette and tip rack incompatibility errors like "Arc out of bounds in the Z-axis" could happen
- **Example**: `opentrons_96_tiprack_300ul`

Initial Tip Right Pipette

- **Type of Value**: Alphanumeric characters
- **Description**: First tip that will be picked by the right pipette.
- **Example**: `A1`

Name Left Pipette

- **Type of Value**: Text
- **Description**: Opentrons API name of the pipette that will be in the left mount of the Opentrons arm.

If this variable is left empty both 'API Name Tiprack Left Pipette' and 'Initial Tip Left Pipette' will be ignored.
Make sure to have at least 1 pipette that is single-channel if 1 or more final plates are created with it.
Make sure to have at least 1 pipette that is multi-channel if 1 or more final plates are created with it.

- **Example**: `p20_single_gen2`

API Name Left Pipette TipRack

- **Type of Value**: Text
- **Description**: Opentrons name of the tip rack from which the left pipette will pick up tips from. This script does not check that the pipette and the tip rack are compatible.
  If the tip rack from the right and left pipette are the same, both pipettes will pick up from the same tip rack, and the initial tip should be the same.
  In case of pipette and tip rack incompatibility errors like "Arc out of bounds in the Z-axis" could happen.
- **Example**: `opentrons_96_tiprack_20ul`

Initial Tip Left Pipette

- **Type of Value**: Alphanumeric characters
- **Description**: First tip that will be picked by the left pipette.
- **Example**: `B3`

Replace Tiprack

- **Possible Values**: True | False
- **Description**: Value that will determine that, in case of more than one tip rack needed to run the protocol, the tip rack will be replaced and not added to the labware layout. If the right and left pipette tip racks are the same, and this variable is set to *True*, only one slot in the layout will be occupied by tips. If tips need to be changed while this variable is set as *True*, the protocol will stop, give a message in the app for the user to change the tip rack and then the user can resume the protocol when ready.
  This variable only accepts two values, *True* (tip racks will be replaced) or *False* (tip racks will be added to the layout).
- **Example**: `True`

Final Plates Variables Sheet*

Number of Replicas

- **Type of Value**: Whole Number
- **Description**: This variable indicates the number of replicas to be created for the final plate established in this column with the sheets established in 'Name Sheet Map Reagents' and 'Name Sheet Map Volumes'.
  The value entered represent the number of additional replicas of the final plate to be created. This number does not include the original plate.
  If 'Internal Replicas' in GeneralVariables is set as *True*, no additional physical plates will be created. Instead, the replicas will be organized on the same plate as the original map.
  On the other hand, if it is set as *False*, the number established in this variable + 1 will be the number of plates that are going to be crated for this column.
  If left empty, it defaults to *0*, meaning no replicas will be created and only the original plate will be created for this specific column.
- **Example**: `3`

Name Sheet Map Reagents

- **Type of Value**: Text
- **Description**: Sheet name inside the excel file that corresponds to the layout of the desired reagents distribution that will be obtained in the final labware. Each cell should contain a list of the desired reagents that the protocol will transfer to them. This sheet needs to have the same dimensions as the labware set in 'API Name Final Plate'.
  For more information about the guidelines, the characteristics and constraints depending on the value of 'Type of Pipette to Create Plate' go to the section Combination Reagents Sheet(s) of this document.
- **Example**: `Comb_plate1`

Name Sheet Map Volumes

- **Type of Value**: Text
- **Description**: Sheet name inside the excel file that corresponds to the layout of the desired volumes of the reagent distribution that will be obtained in the final labware. Each cell should contain a list of the desired volumes that the protocol will transfer to them. This sheet needs

to have the same dimensions as the labware set in 'API Name Final Plate'.
For more information about the guidelines, the characteristics and constraints depending on the value of 'Type of Pipette to Create Plate' go to the section Combination Volumes Sheet(s) of this document.

- **Example**: `Vol_plate1`

Type of Pipette to Create Plate

- **Possible Values**: single | multi
- **Description**: Variable that determines which type of pipette and the associated constraints for creating the final plate, only 2 values are allowed:
  - **multi**: the reagents will be dispensed with the possible 8-channel pipettes established in 'Name Right Pipette' and 'Name Left Pipette'.
    Reagents to be dispensed are stored in labware specified in API Name Labware with Reagent(s) in Plate(s), which should have either 1 or 8 rows. There are no constraints on the number of columns.
    The optimization of this source labware reagent distribution can be established in the variable 'Optimization Sace Source Plate Reagents Disposition' and additional constrains about the final plate reagents and volumes layout can be found in the sections Combination Reagents Sheet(s) and Combination Volumes Sheet(s) of this manual.
  - **single**: the reagents will be dispensed with the possible single channel pipettes established in 'Name Right Pipette' and 'Name Left Pipette'.
    There are no constrain in the number of reagents and their volumes, as long as they can be dispensed with the available single channel pipettes.
    Reagents will be stored in falcon or eppendorf tube(s) in the tube rack specified in 'API Name Labware with Reagent(s) in Tube(s)'

- **Example**: `2`

* If needed, this sheet can have more columns to the right, this meaning that if the Opentrons have more than 11 available slots, the sheet can be expanded and filled. Adding columns does not guarantee that more final plates can be created as that result depends on more variables, such as the tip rack replacement value or the number of replicas per plate.

The columns of the plate can have any name, these names will be included in the final layout that you will be able to see in the OT-App to help you recognize where each source plate must be placed.

Combination Reagents Sheet(s)

Sheet with the name set in the variable “Name Sheet Map Reagents” in the Sheet “FinalPlatesVariables”.

This sheet should outline the layout of the final labware that the user wants to create. Each cell should list the reagents to be placed in that position separating those reagents by commas.

This sheet should have the same dimensions (rows and columns) as the labware set in the variable “API Name Final Plate” in the sheet “GeneralVariables”.

In addition, it should have the names of the columns and rows of that labware as it is defined in the Opentrons labware definition. For example, a 96 well plate can have the columns named 1 to 12 and the rows named A to H.

Remember that this sheet is going to be read in combination with the corresponding volume sheet so some guidelines and constrains need to be considered:

- In the same cell for both sheets, the same number of elements should be listed
- The volumes will be assigned by order of appearance, i.e., the first element of the volume sheet cell will be assigned to the first reactive of the same cell in the reagent sheet, the second volume to the second reagent and consecutively.
- If using a multi-channel and a reservoir that has only columns (only 1 row) to create the final plate, the same reagent should be placed along the whole column. In other words, the same reagent-volume combinations should be set in the same column.
- Wells without any desired reagents should be left empty in both sheets.

Combinations Volumes Sheet(s)

Sheet with the name set in the variable "Name Sheet Map Volumes" in the Sheet "FinalPlatesVariables".

This sheet should outline the layout of the final labware that the user wants to create. Each cell should list of volumes to be transferred of their respective reagents in that position separated by commas.

This sheet should have the same dimensions (rows and columns) as the labware set in the variable "API Name Final Plate" in the sheet "GeneralVariables".

In addition, it should have the names of the columns and rows of that labware as it is defined in the Opentrons labware definition. For example, a 96 well plate can have the columns named 1 to 12 and the rows named A to H.

Remember that this sheet is going to be read in combination with the corresponding reagents sheet so some guidelines and constrains need to be considered:

- In the same cell for both sheets, the same number of elements should be listed
- The volumes will be assigned by order of appearance, i.e., the first element of the volume sheet cell will be assigned to the first reactive of the same cell in the reagent sheet, the second volume to the second reagent and consecutively.
- If a multi-channel pipette is used to create the final plate that is associated with this sheet, each column in the sheet must have the same combination of volumes. Different channels from the same pipette movement cannot aspirate different volumes.
- If using a multi-channel pipette and a reservoir that has only columns (only 1 row) to create the final plate, the same volumes should be placed along the whole column. In other words, the same reagent-volume combinations should be set in the same column.
- Wells without any desired reagents should be left empty in both sheets.