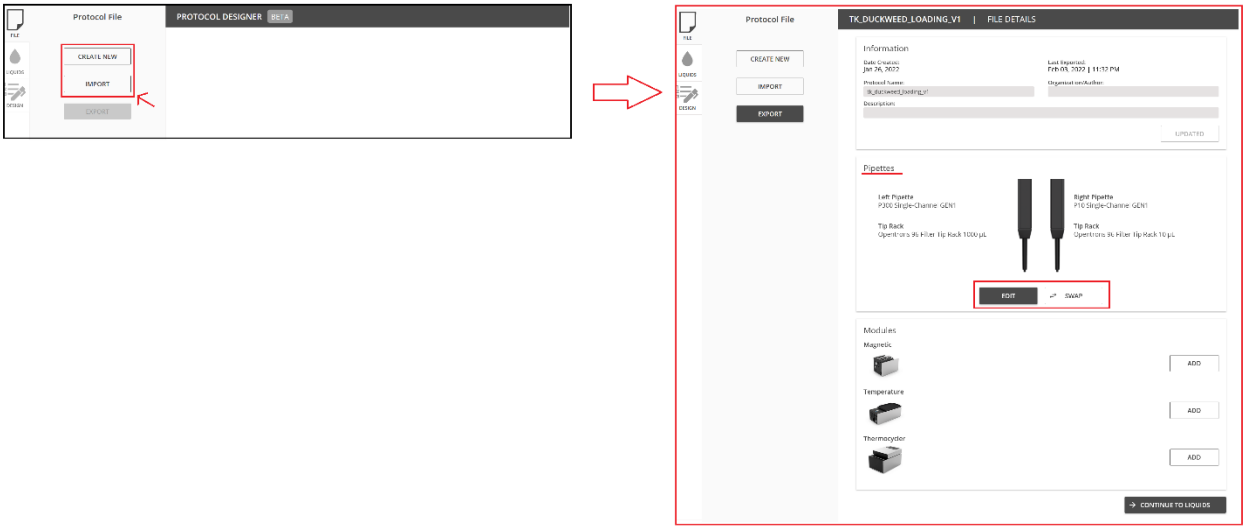
How to load duckweed using OT-2

OT-2 is a liquid handler robot that follows protocols defined and loaded by the user. Currently, we have achieved utilization of OT-2 to pick up and drop duckweed using specific inoculation loops into well-plates. Here, the procedures to be followed are presented.

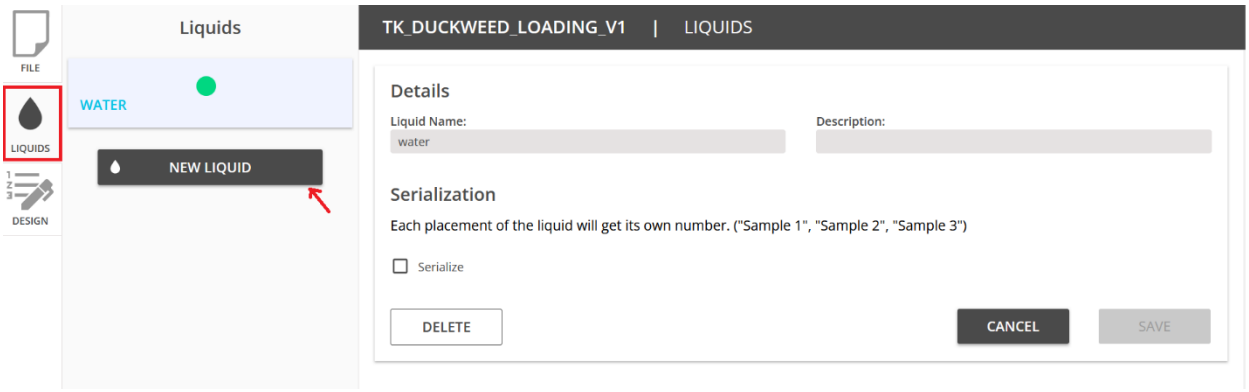
1- **Preparing your protocol**

There are two options to create a protocol for OT-2: the protocol designer tool and writing your own code using Python. Here, we focused on how to use the designer tool since we are not using any complex commands for our duckweed loading operation.

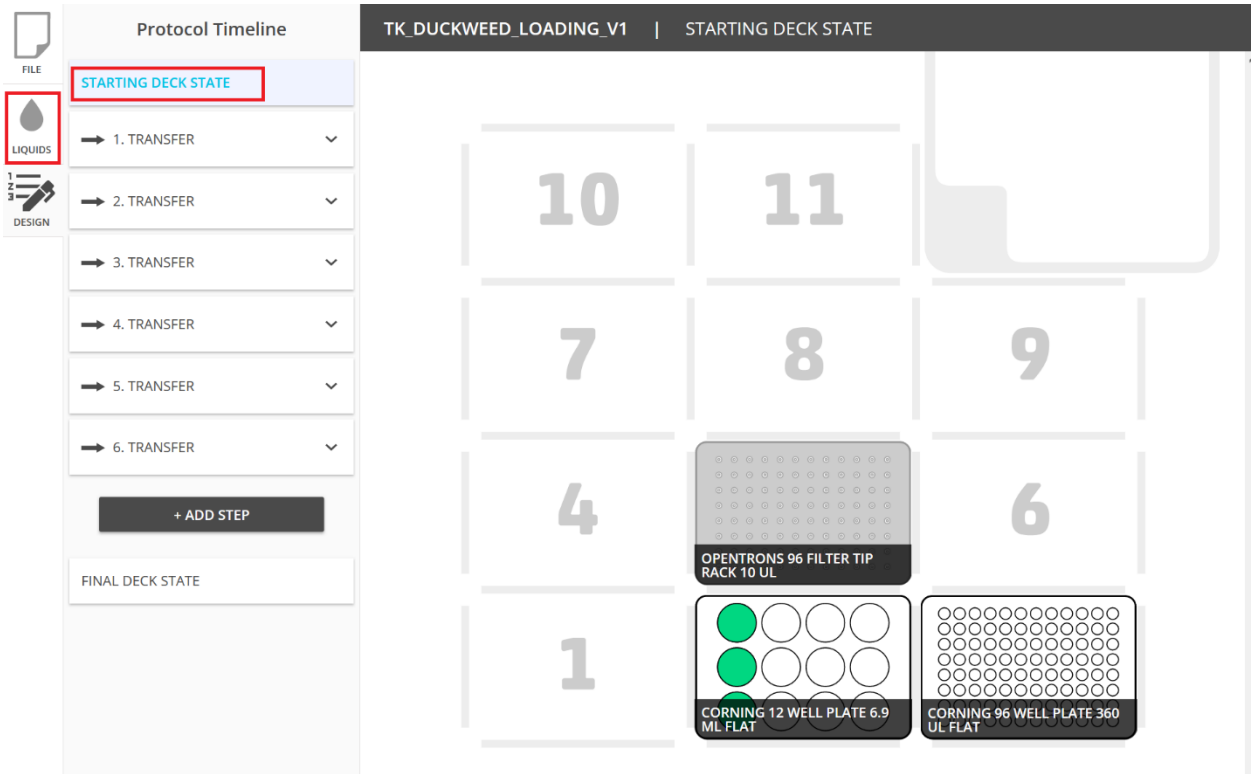
a. Using your browser, open https://designer.opentrons.com. Then, click on Create New if you intend to create your own protocol. However, there are readily available ones created for duckweed loading purposes. You can import one of them and change only some settings if you like. No matter which option you choose, you have to define the Pipettes loaded on the robot. If the defined pipette and the physically loaded pipettes are not the same, you will see errors while loading your protocol, hence, make sure you correctly select the right Pipette head matching the loaded pipette heads. **Note that even though you only use one pipette in your protocol, both pipette slots have to be defined properly.**

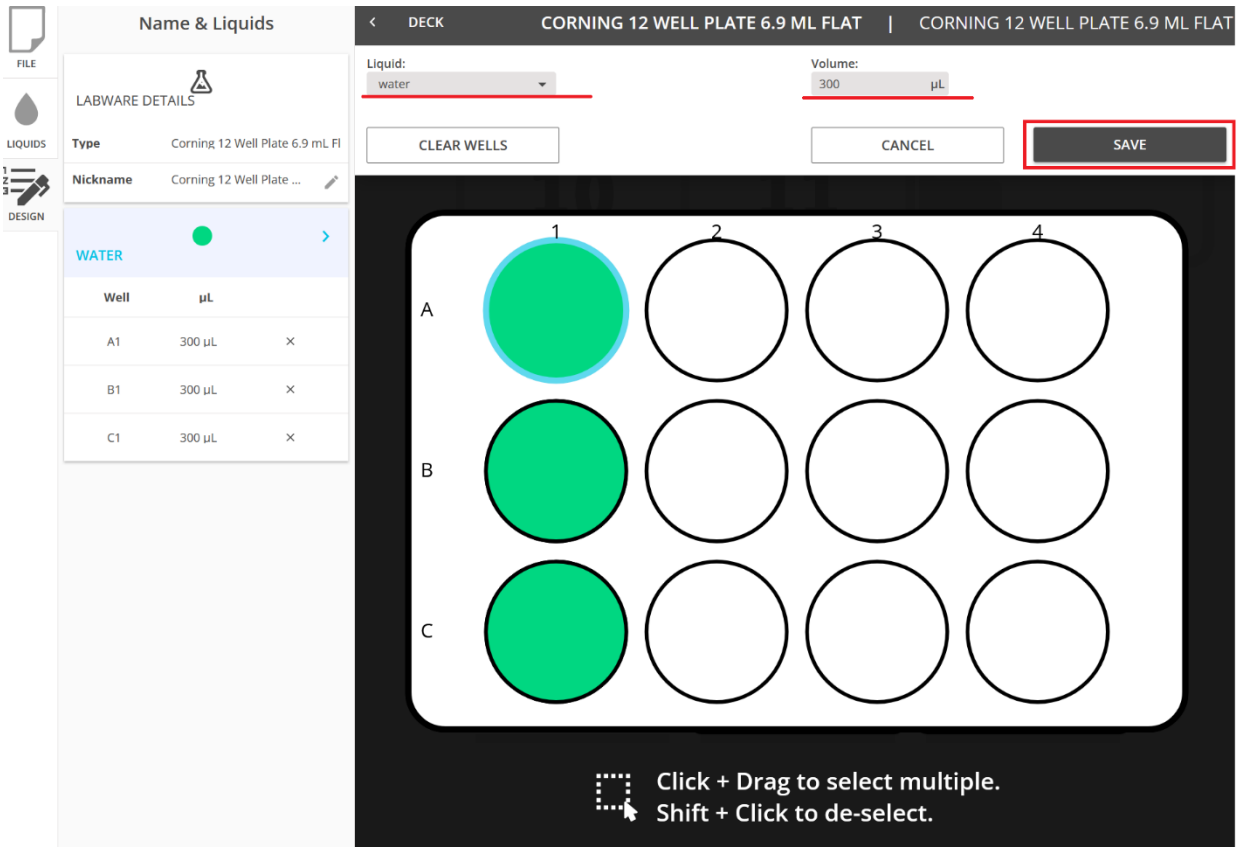


b. Once set the pipettes, you can move to the second tab which is Liquids tab. On this tab, you should define the liquids you are going to use in this protocol. Please note that since OT-2 is a liquid handler robot, this is a typical step for making a protocol. Although we won't use any liquids in our actual operation, we create the protocol as if the robot carries liquid around. Hence, we define a New Liquid and give it a name so that we have liquid to move around.

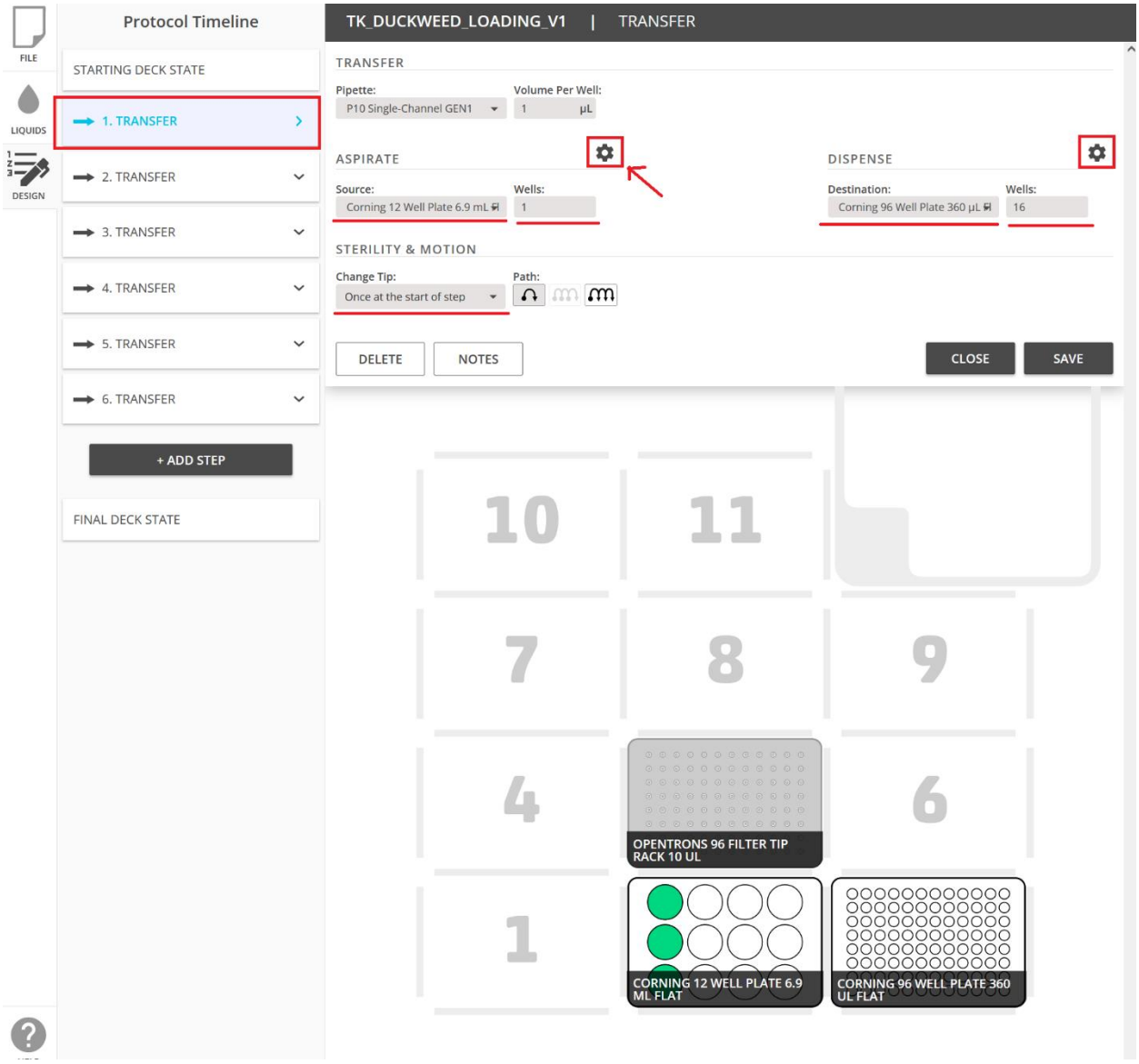


c. After setting our liquid, we can move to the Design tab. This tab shows the overview of the footprint of the OT-2 which has 11 slots and one thrash bin on the top right. One can add any labware from their pre-defined library. In our duckweed loading setup, we selected one rack tip placed on Slot#5 (Opentrons 96 Filter Tip Rac 10 uL), a source wellplate where we placed our duckweed (Corning 12 Well Plate 6.9 mL Flat) on Slot#2 and a destination well plate (Corning 96 Well Plate 360 uL Flat) on Slot #3. At the starting deck state, the liquids are supposed to be defined in the wells where you placed your duckweed. In the given example, the first column is used as the source wells. To add a liquid to your source wells, click on your source well-plate and follow the instructions of the visuals appeared.



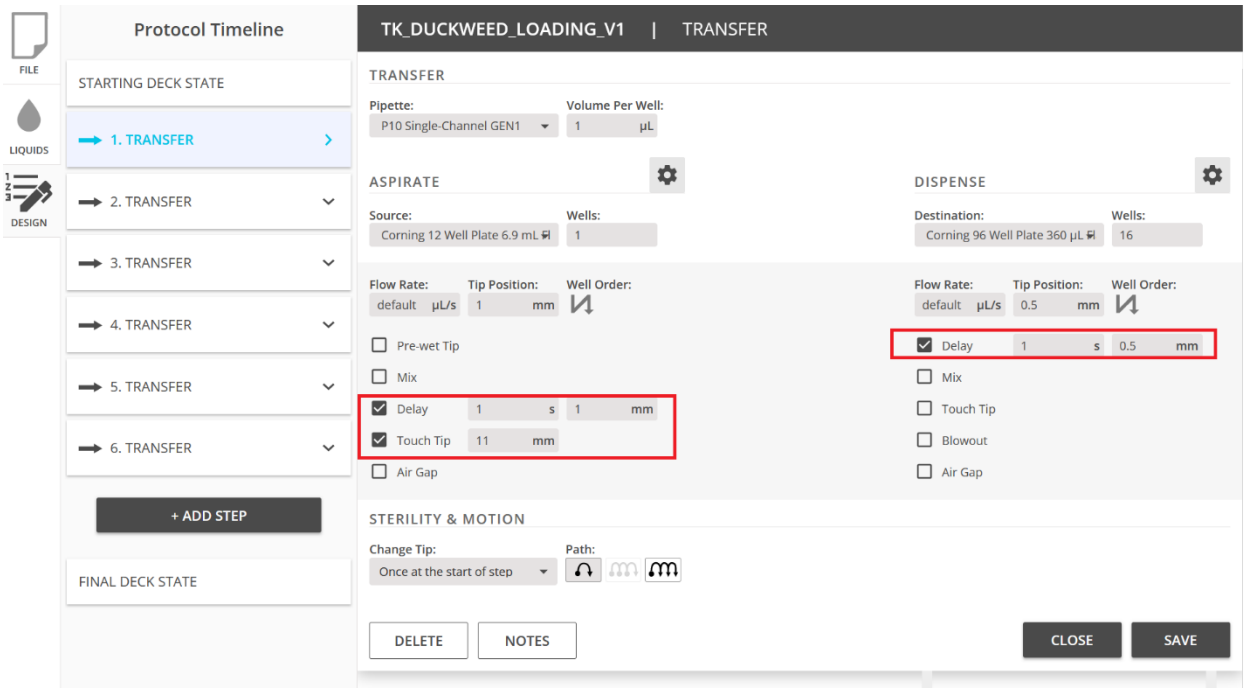


d. After setting up the labware and the liquids inside the source well-plate, one can add protocol steps by clicking on the *Add Step* button. Transfer is the typical step used in duckweed loading.
For the example shown, we defined 6 transfer steps. For each 2 columns in the destination well-plate (corresponding to 16 wells in total), a transfer step is used. After filling out the first two columns, we switched the source well from A1 to B1, and B1 becomes the source well for the 3rd and 4th columns in the destination well-plate. By switching the source wells after two columns, we aimed at preserving the density of duckweed in the source well, which is a critical parameter. When switched to other source well, you can add up some liquid to the previously used source well in case you realize the liquid level changed. Similarly, you can top up some duckweed in case the duckweed layer gets less dense on the surface of the liquid.


FILE
LIQUIDS
DESIGN
Protocol Timeline
STARTING DECK STATE
1. TRANSFER
2. TRANSFER
3. TRANSFER
4. TRANSFER
5. TRANSFER
6. TRANSFER
+ ADD STEP
FINAL DECK STATE
TK_DUCKWEED_LOADING_V1 | TRANSFER
TRANSFER
Pipette:
P10 Single-Channel GEN1
Volume Per Well:
1
µL
ASPIRATE
Source:
Corning 12 Well Plate 6.9 mL
Wells:
1
DISPENSE
Destination:
Corning 96 Well Plate 360 µL
Wells:
16
STERILITY & MOTION
Change Tip:
Once at the start of step
Path:
DELETE
NOTES
CLOSE
SAVE
10
11
7
8
9
4
6
1
OPENTRONS 96 FILTER TIP RACK 10 UL
CORNING 12 WELL PLATE 6.9 ML FLAT
CORNING 96 WELL PLATE 360 UL FLAT

e. The critical parameters for each step can be tuned by clicking on the Transfer step. The source and destination wells can be selected using the graphical interface pops up when clicked on the related areas. In this protocol, one source well is selected while destination includes 16 wells (two columns in the destination well-plate).
The settings menu pops up when the gear icon is clicked for Aspiration or Dispense as shown below.

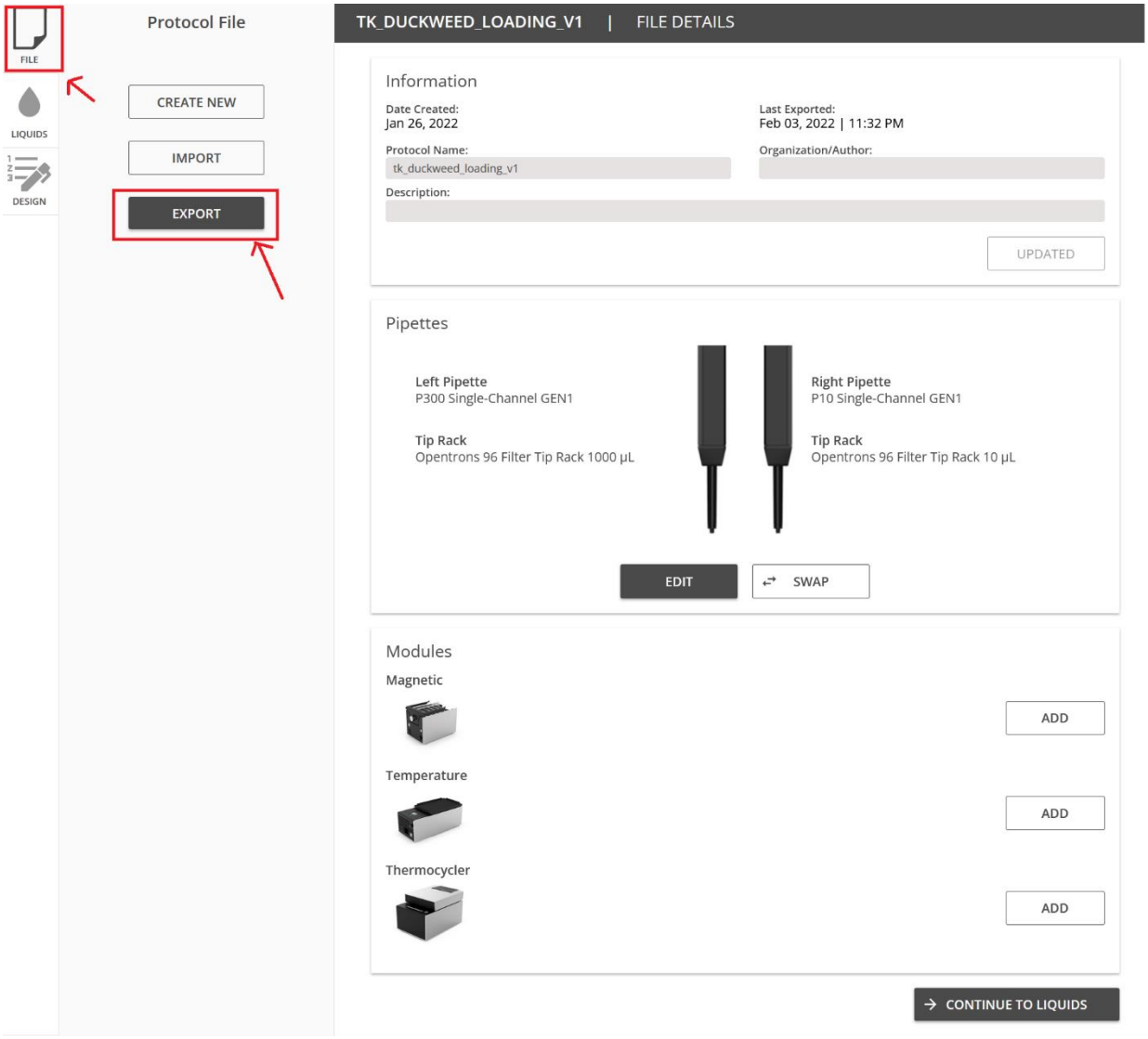
After clicking on the gear icon (Settings):



The special commands used in the duckweed pick up from the source wells (Aspirate step) are the Delay and Touch Tip commands according to our trials. **The Delay settings are used as 1 s and 1 mm. The setting of the touch tip command is really critical. For a fully loaded source well, we observed 11 mm works fine in the first three transfer steps (assuming you are also using 12 well-plate, and calibrated the robot properly). Touch tip position is changed to 10.5 mm for the Transfer Steps 4-5 and 6 assuming there is some liquid level change. This can be kept as 11 mm if the researcher observe no issue after couple of trials.**
The special command used in the duckweed drop to the destination wells (Dispense step) is the Delay command only according to our observations. **The Delay settings are used as 1 s and 1 mm or 0.5 mm. We do not add Touch Tip command in the dispense settings.**
The change of the tip can also be set for each transfer step by clicking the Change Tip drop down menu. In the protocol presented, it is set to pick up a tip and use the same tip for the entire well-plate (Change tip is set to *Never* in Transfer 2-6). This may cause

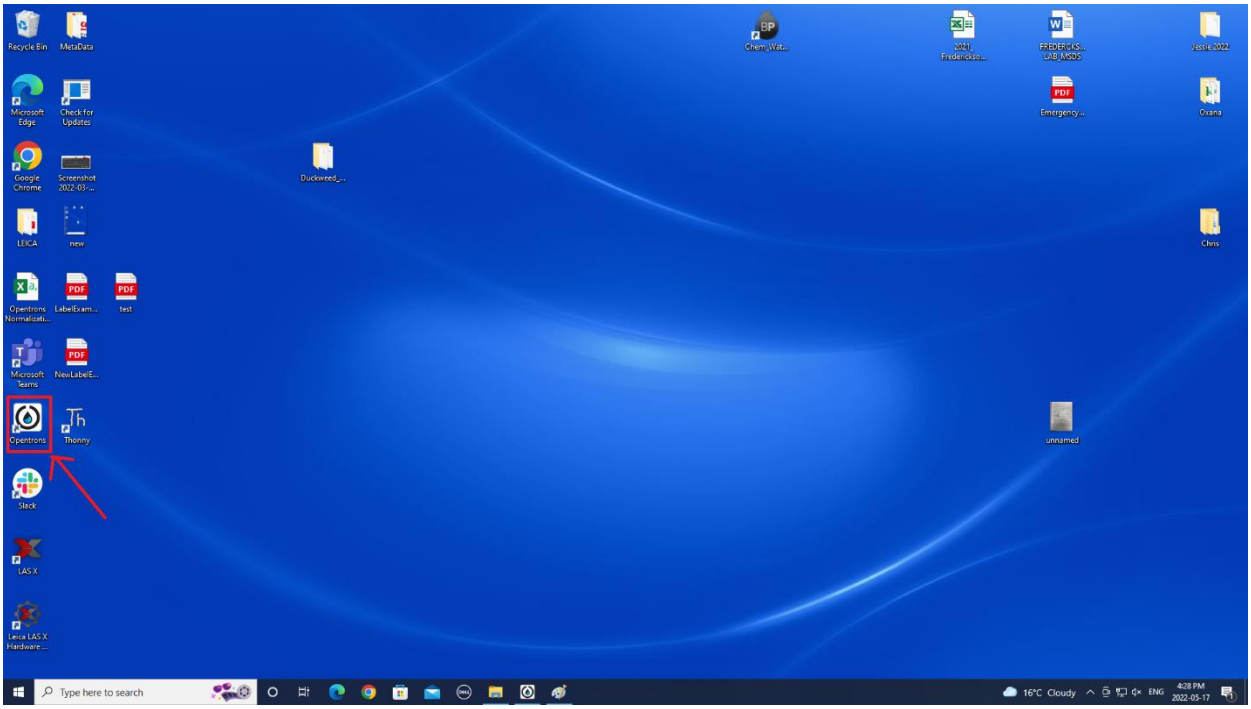
some contamination issue since the same tip will be dipped into every well successively. Thus, please be aware of this while designing your protocol.

f. Once finishing entering the relevant settings for each Transfer step, you can click on the File Tab and export your protocol file in terms of .json file.
Please note that for any changes required for the designed protocol, you can come back to the designer and import your existing protocol to edit instead of setting everything from scratch.

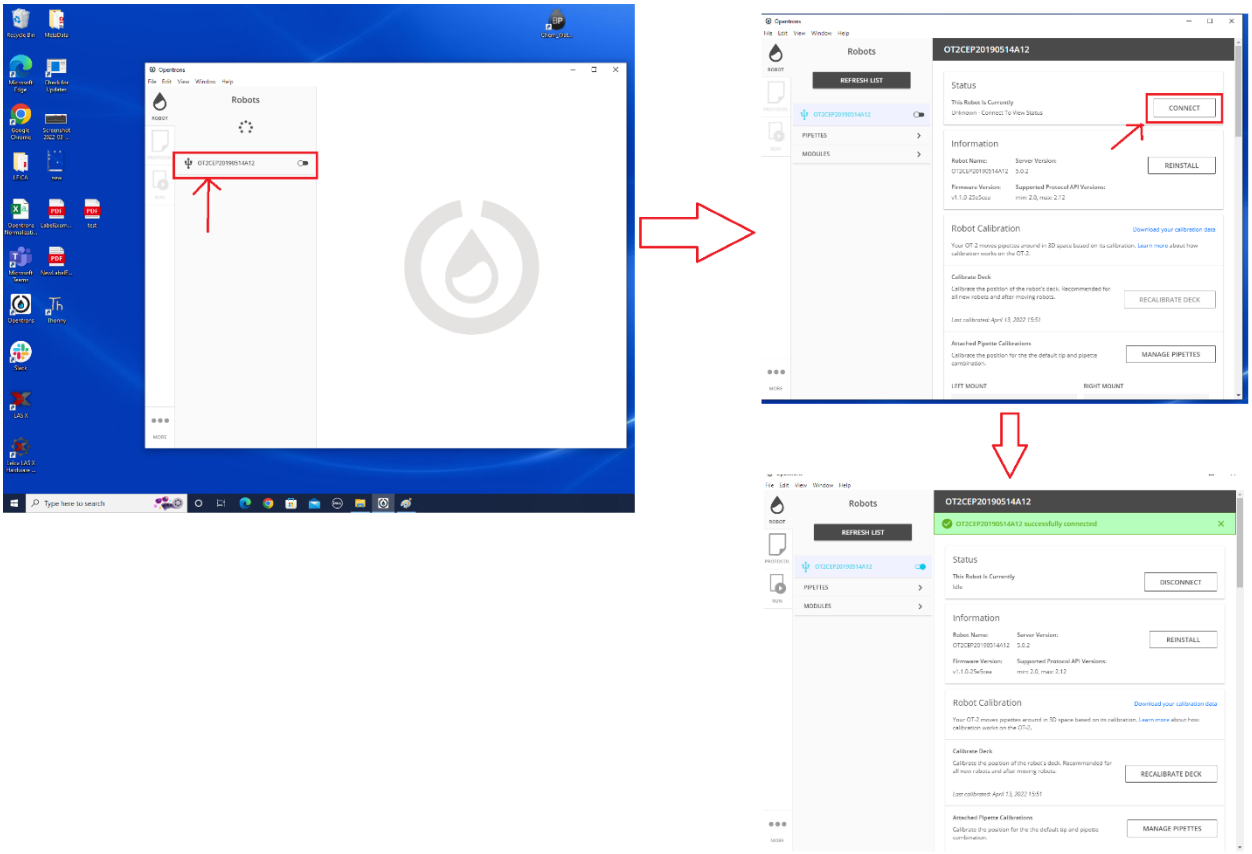


2- Loading your protocol

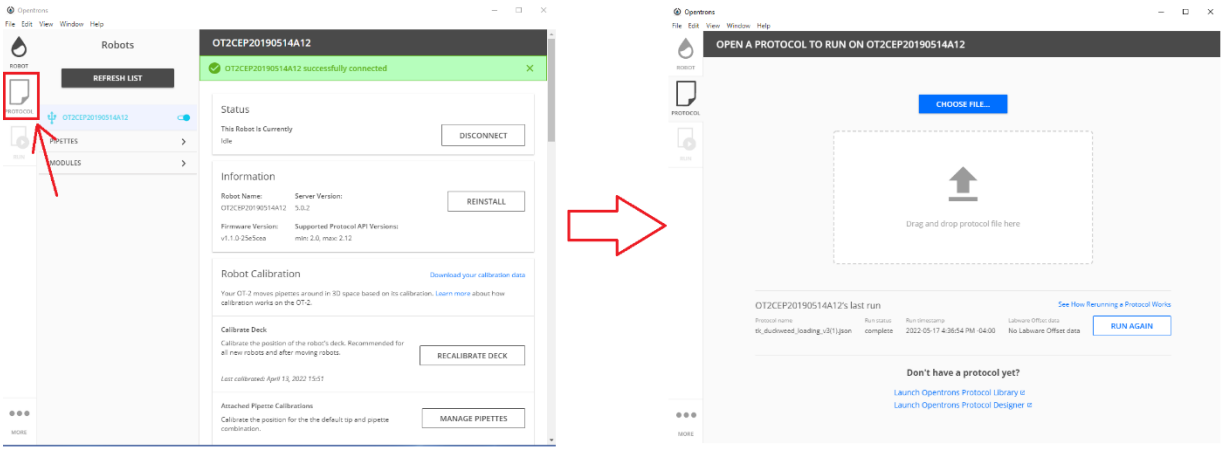
a. Run Opentrons software using the icon on the desktop.



b. Click on the robot listed and press Connect in order to establish the connection between the robot and the computer. Once connected, you'll see the successful connection message on the software.



c. To load the prepared protocol or your customized Python code, click on “Protocols” tab and select or drag your protocols file on the denoted area on the software.

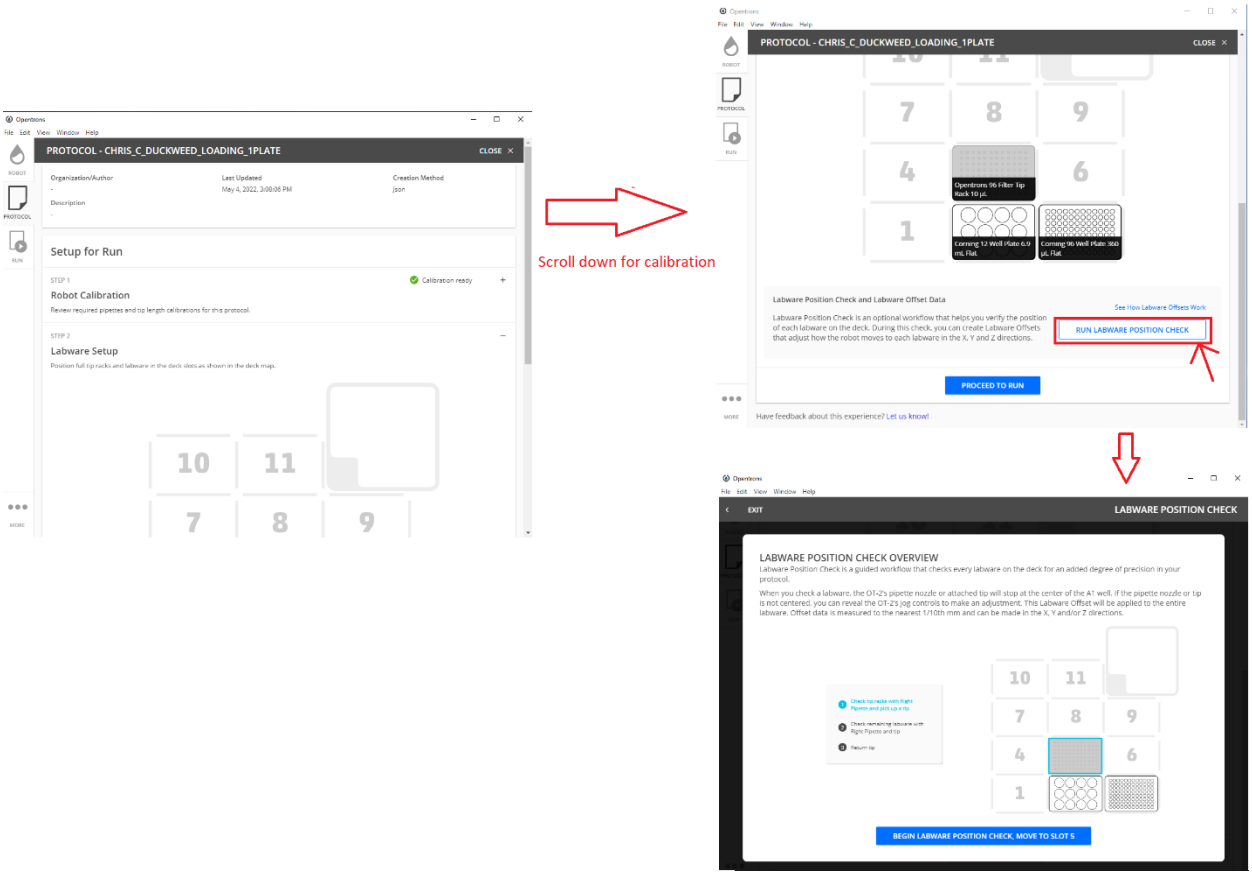


3- Calibration of Labware

After you successfully open your protocol using Opentrons software, you’ll need to calibrate the robot based on the hardware used in the protocols. Our inoculation loops to load duckweed is not designed to be employed in OT-2, hence, we adapted our inoculation loops so that it can be picked up by the readily available OT-2 Pipette heads (P10 Single Channel GEN-1 is compatible).

These inoculation loops are placed in readily available pipette tip racks. Currently, we are using *Opentrons 96 Filter Tip Rack 10 uL* as the accommodating platform for our inoculation loops. Since the actual pipette tips of 10 uL and the inoculation loops have different physical properties, the calibration will be essential to execute the protocols without any crashes. Therefore, one cannot skip calibration step as long as the readily available labware is defined in the protocols, which is common practice if you utilized the designer tool. Here, you'll find the details and how you go through the calibration process.

a. On your protocols tab, find the STEP 2 – Labware Setup and scroll down to Click on *Run Labware Position Check*. Please refer to the screenshots below in order to avoid confusion regarding the proper calibration process.
Note: There is also STEP 1 - Robot Calibration just above Step 2, which helps user calibrating the pipette properties which are not related with our Duckweed loading protocols. Please don't try to run calibration through STEP 1.



b. After clicking the Run Labware Position Check, software will inform you regarding the entire process. It will follow through all hardware available on your protocols. In the example shown above, you'll see 3 different labware is used: a tip rack, a 12 well-plate as the source of duckweed and a 96-well-plate as the destination of duckweed. Whenever you click Begin Labware Position Check, Move to Slot 5, the robot head will move to the Slot 5 which is the first listed labware in the protocol. The robot head will

proceed to the A1 tip (Located on the top left corner when look at the rack from the top) in order to calibrate the position of the head with respect to the tip it will use. **Before clicking that blue button, you need to remove the inoculation loops near position A1 since the inoculation loops have more protrusion than the original filter tips as seen below. Failure to remove the inoculation loops will result in the Pippette head crashing the inoculation loops physically and may damage the robot.** After removing the inoculation loops from the position A1, you can let the robot move to Slot 5 by clicking on *Begin Labware Position Check, Move to Slot 5.*

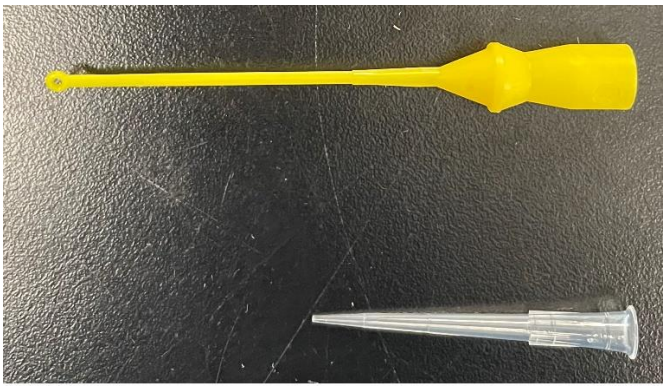
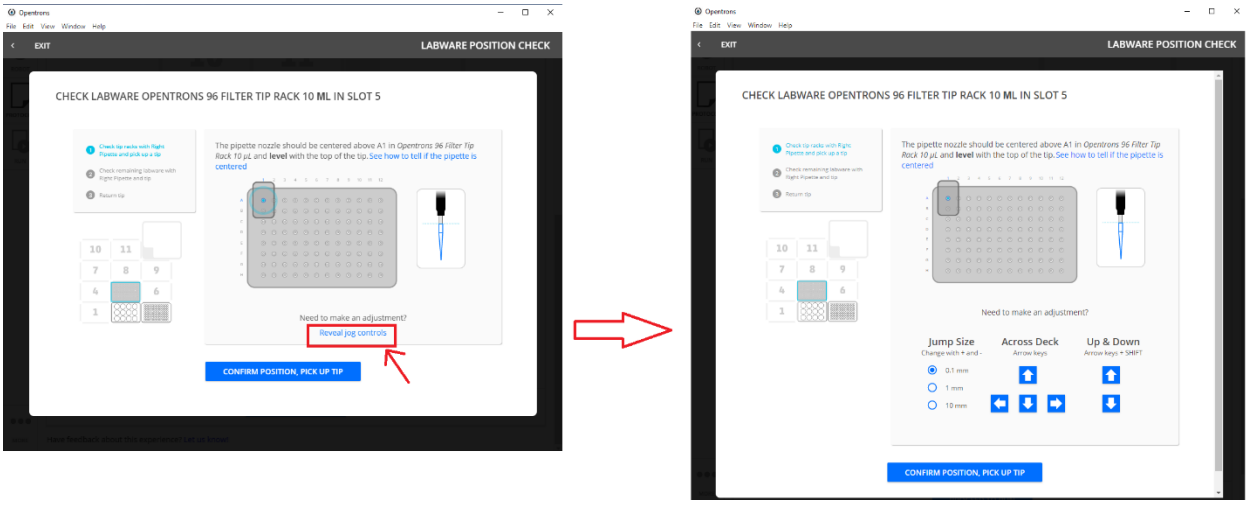


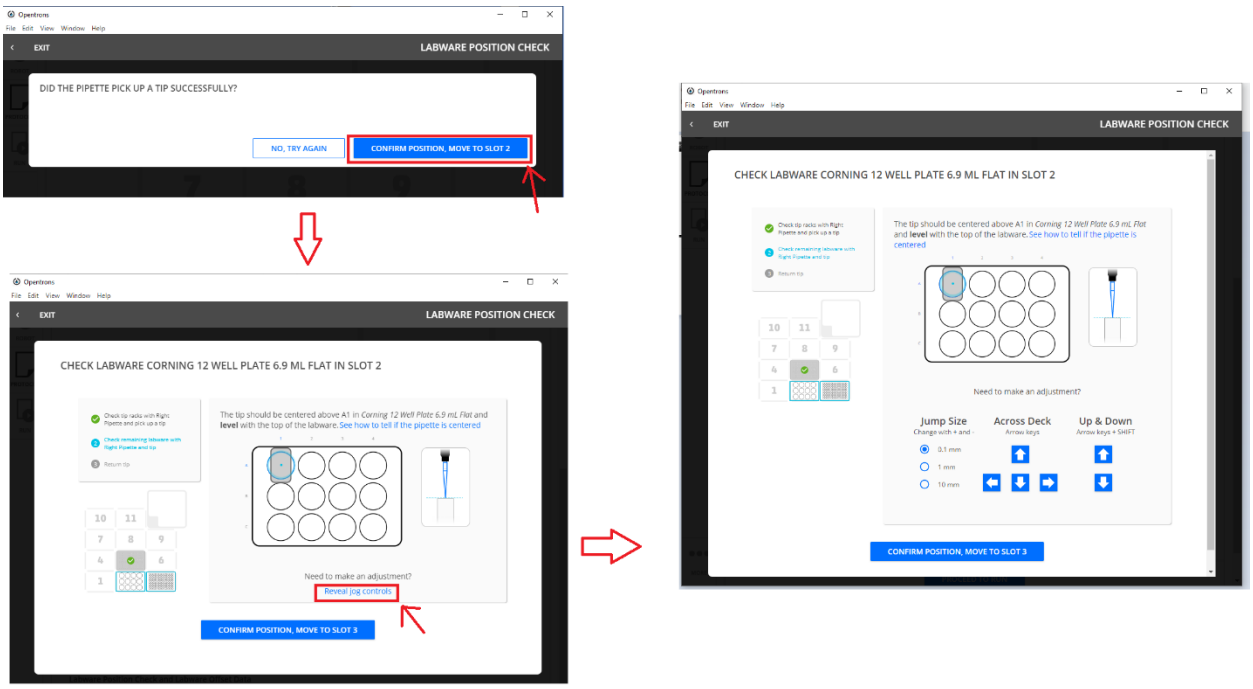
Figure 1: Physical difference between an inoculation loop and a regular 10 uL pipette tip.

c. You will see the robot head moving to the Tip Rack as expected. After that, you can find how to align the robot head with respect to the tips. In order to move the pipette heads manually, you should click on the *Reveal jog controls* which pops 6 arrows and 3 movement step options (Jump sizes). If you need large movements of pipette head you can select 10 mm which indicates that you would see the head moving by 10 mm based on whichever arrow you clicked. In order to make the head move up or down you'll use the arrows on the very right, while for the in-plane motion of the head (at the same height) you can use the four arrows shown on the software.
Move the pipette head up using the jog control tools (On the very right, top arrow button). After elevating the head around 4 cm, you can place one of the inoculation loops into the A1 position so that you will make the alignment between the robot head and the inoculation loop. Use the jog control buttons to align the hardware and press *Confirm Position, Pick up Tip* when you think the head and the inoculation loop is aligned based on the visual shown on the software. The software will record this new position as the Tip Pick-up position.

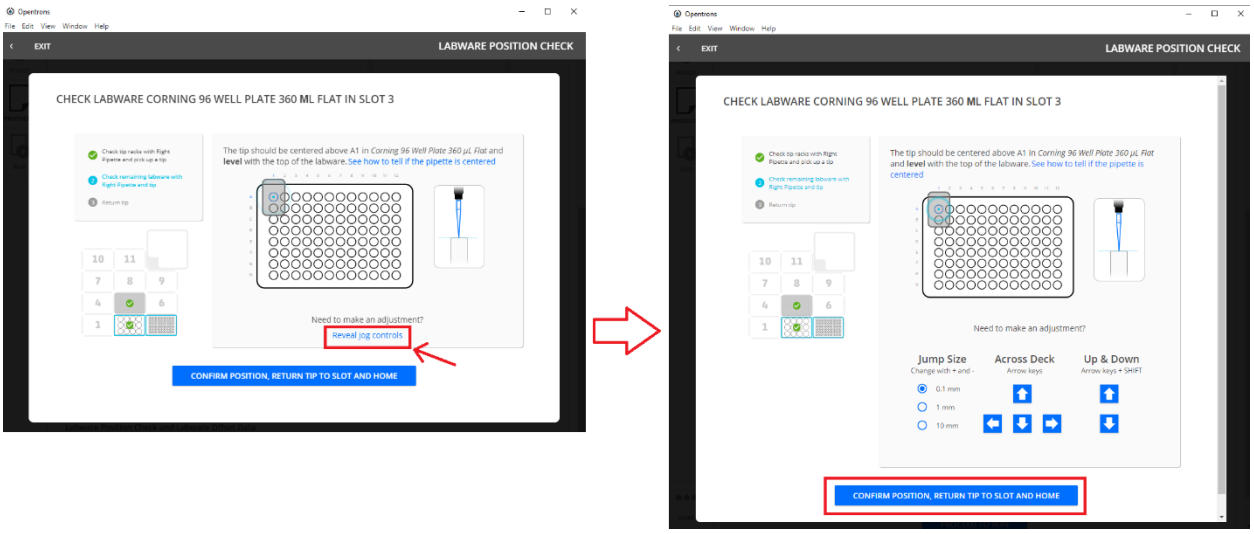


d. Once clicked *confirm and pick up tip,* the head performs its regular motion while picking up any tips (2-3 times downward motion). You will see a message regarding whether the tip is successfully picked up or not. **If you see the pipette picked up the inoculation loop properly, do not immediately confirm to move it to the next slot. Since the robot thinks it picked up the regular 10 uL pipette tip, it'll not recognize the physical difference between the inoculation loop and the regular tip. That will cause crashing the loop to the second labware and bends of the inoculation loop. Hence, it is recommended to detach the inoculation loop from the pipette so that you can avoid crashing the loop into the well-plate.** Once detached the loop from the head, you can click on *Confirm Position and Move to Slot X.*

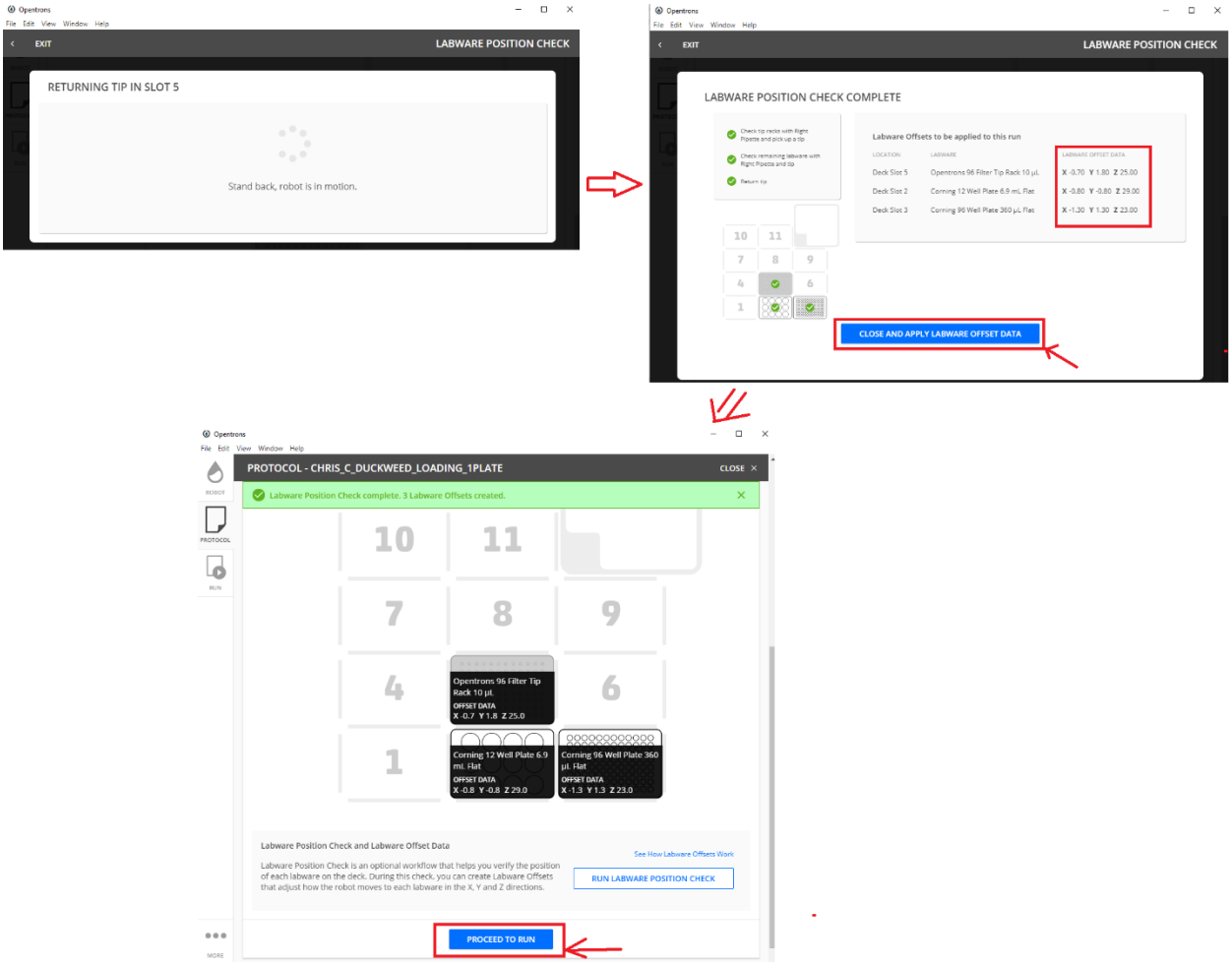
The similar alignment procedure will be followed for the second labware which is the 12-well-plate in this protocol example. To align the inoculation loop and the A1 well based on the guidelines shown in the software, you need to elevate the head again around 4 cm in order to attach the inoculation loop again. After attaching the loop, align it so that it would stay on the center of the well and at the same height of the top surface of the well-plate.



e. **When satisfied the alignment of the inoculation loop and A1 well, you should detach the inoculation loop once again in order to avoid crashing the loop to the next labware**, which is the 96-wellplate in this example protocol. Once the loop is detached, you can press *Confirm Position, Move to Slot X,* which is Slot 3 in this example. After the robot head moves to the next labware (over well A1 again), you can elevate the head in order to attach the inoculation loop to the pipette head. Once attached, you can align the inoculation loop and the A1 well using jog control buttons on the software. **The centering of the tip of the inoculation loop and the height of the inoculation loop is critical to execute the protocol efficiently.**

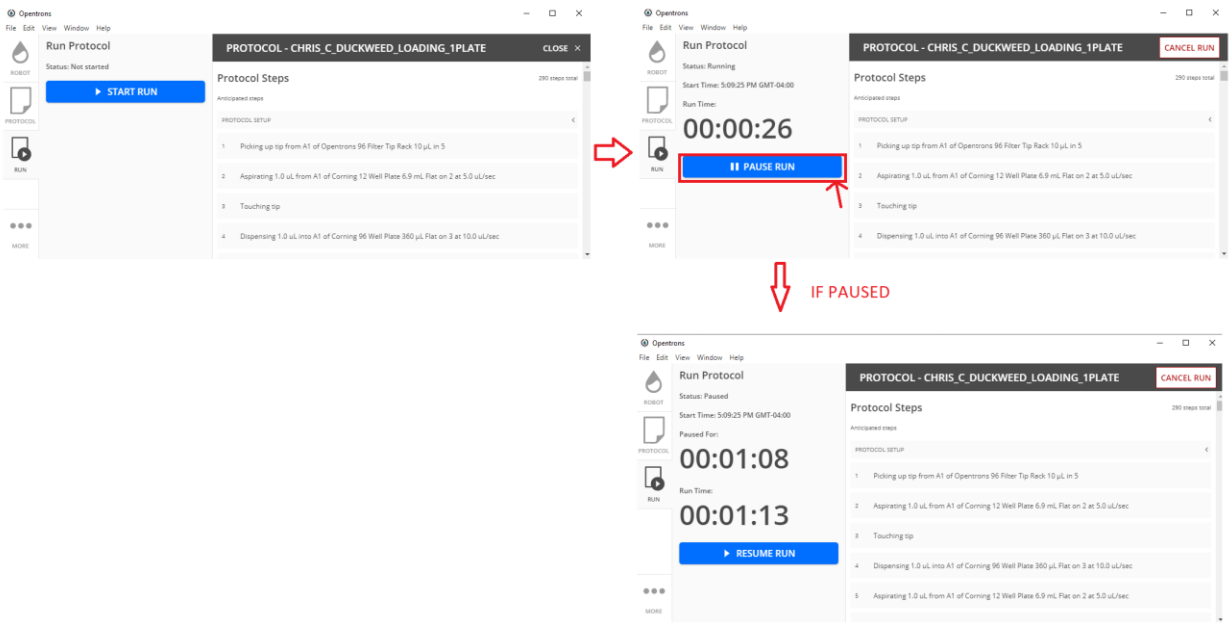


f. When the final position alignment is done, you can click *Confirm Position, Return Tip to Slot and Home.* This would make the head return to the Tip Rack (A1 position) and drop the tip and after that, it would go to its Home position.
When the head returns to the Home position, you will see the adjustments made on the screen as “Labware Offset Data”. These values can be recorded, and applied in your next position calibration, which means you don’t really have to use the inoculation loops in the calibration process and avoid detaching/attaching aligning manually every time. This would work under the assumption that no other labware position have been changed and the inoculation loops are identical in terms of their physical properties.



4- Running the protocol

After position calibration of the robot, you can now run the protocol by clicking on *Proceed to Run* button. That would bring you to the Run Protocol Tab. Clicking on Start Run button will commence your protocol and the robot will follow your protocol from the beginning till the end. In case of any unexpected motion, crash or anything weird, you can stop the robot by pressing Pause Run. After your interference, you can either Resume Run or if you think you need to change the protocol or recalibrate the positions or start over somehow, you can press Cancel Run on the top right corner to reset the protocol.



5- Final touches and closing the well-plates for autonomous imaging system

After the protocol of the duckweed operation is run, we expect to have 70-90% of the wells filled with one or more duckweed in your 96 wellplate. Don't forget to inspect your destination wellplate. You can fill the empty wells manually before coming to the packaging.

Packaging means closing the well-plates with membranes. If these well-plates were to be placed inside the growth chamber for the autonomous imaging, you need to use two types of membranes (Breathe Easy -transparent- and Breathe Easier -opaque-). First, use the Breathe Easier and on top it add the Breathe Easy. Use of two membranes eliminate the condensation and avoid formation of bubbles between the wells, which would affect the feature extraction.