Dispensing Liquid with the Opentrons **(One Volume Across Plates)**

1. Download the Opentrons app (**version 6.2.0**) on your computer from their website:
   https://github.com/Opentrons/opentrons/releases/download/v6.2.0/ot2-system.zip
   Follow these instructions to downgrade your app to the 6.2.0:
   https://support.opentrons.com/s/article/Downgrade-to-an-older-software-version
2. Turn on the Opentrons by flipping the switch on the back of the left side of the machine.
3. Wait ~30 seconds to a minute for the blue circular light on the front to change from blinking to solid.
4. Plug the Opentrons into your computer.
5. On your computer, download *generateSingleDispenseList.py* and *SingleDispense.py* from:
   https://github.com/madisonwahlsten/Immunodynamics_OT-2/tree/main/scripts
   and move them to an easily accessible place, such as an "Opentrons" folder on your desktop.
6. Open a Terminal window and navigate to the directory with your scripts using the "cd" command, for example:

   ```
   cd ~/Desktop/Opentrons/ [enter]
   ```

7. To run the script to determine which plates to dispense to, type:

   ```
   python generateSingleDispenseList.py [enter]
   ```

8. Follow the prompts to answer where you will dispense from, how many + what types of plates you will dispense in, and your dispensing volume (uL). The options you can type should appear in square brackets following the question. **Please type your answer exactly as the prompt option appears.** If you make a typo, the prompt will ask you again for an accepted answer.
   If you want to dispense the same volume to multiple plates, when the script asks:

   ```
   Would you like to fill a deck slot with a plate to dispense
   to? [y,n]:
   ```

   Answer **y** to repeat the prompts until you have filled out all desired plates. Otherwise, answer **n**.
9. After the script finishes running, it should generate a file called *BenchItems.pkl* in the same folder as your scripts.

10. From the taskbar on the bottom of the screen, open the Opentrons app. Click to the “Devices” tab on the left. **If a pop-up about an update appears, click “Not now.”**
11. When the robot connects, the pipettes and previous runs will populate the screen. At this point, click the 3 dots on the top right and go to robot settings.
12. Click to “Advanced” settings and launch Jupyter Notebooks; it should open in your web browser.
13. Upload *BenchItems.pkl* to this location.
14. Open the notebook titled *UpdateSingleDispenseList.ipynb* and click “Cell” then “Run All” at the top to run all cells of the notebook.
15. Navigate back to the Opentrons app and open the “Protocols” tab.
16. If you haven’t used this protocol before, import *SingleDispense.py* using the button in the top right corner of the app window.
17. Find the “Single Volume Dispense Protocol” on the list of Protocols. Click the 3 dots on the top right and then “Run."
18. Make sure the device “ImmunodynamicsOT2” appears and click “Proceed to setup.”
19. The robot should now analyze the protocol on the robot. When it finishes loading, expand the “Labware Setup” section. If an option comes up to apply stored Labware Offset data, click “Apply stored data.”
20. In the Labware Setup section, you should see the reservoir + plates you picked in the correct slots of the deck. If not, “Cancel run” and start again from step 7.
21. Place your filled reservoir and plates onto the proper deck slots. Close the robot’s door.
22. If you are satisfied with the deck position, you can run a Labware Position Check if you want to double check labware offsets. Otherwise, proceed.
23. Click Start run/Proceed to run! The Opentrons should now dispense your desired volume to all your plates.