Protocol 2: Introduction to Liquid Handling Robotics

Goal: To measure the accuracy of a pipetting using a liquid handling robot (and learn how to use the most common liquid measurement tools in the laboratory)

During this lab you should learn to:

- Setup a liquid handling robot for running a simple protocol
- Design and execute a protocol
- Measure the accuracy and precision of a liquid handling robot
- Perform accuracy and precision analysis on resulting absorbance dat

Introduction:

The advent of automation in laboratory settings has revolutionized the way scientists handle and process samples. Liquid handling robots, in particular, have become indispensable tools in modern research, allowing for precise, efficient, and reproducible transfer of liquids. These robots are equipped with advanced technologies to perform tasks such as pipetting, dispensing, and mixing with high accuracy, minimizing human error and increasing throughput. In this lab, students will gain hands-on experience with a liquid handling robot (Opentrons OT-2), learning to program and operate it to perform various liquid handling tasks. This lab will provide an opportunity to explore the practical aspects of automated liquid handling, from setting up experiments to troubleshooting common issues.

Protocol:

Materials needed:
20 uL filter tips
1000 uL filter tips
Water
Water + food coloring solution
One 96 well microplate
Two OmniTrays

You will design a specified protocol that uses a liquid handler to plate liquid in a particular layout. You will also manually pipette the same layout manually. We will measure the quality of the pipetting steps by measuring the absorbance of light through the generated solution in each well. This is performed on a microplate reader. Microplate readers operate by measuring the intensity of signals produced by the samples in the wells. These signals can be in the form of absorbance, fluorescence, luminescence, or other optical properties. The data collected by the reader is then analyzed to provide quantitative results for the experiment.

Your task:

Design an OT-2 liquid handling protocol to generate a plate with the following layout for rows A and B. The total volume in each well should be 100 microliters. The value is showing the percentage of food coloring mixture. The remaining volume should be water. After running this protocol on the OT-2, one of your group members should pipette rows C and D. All other wells should be left empty.

	1	2	3	4	5	6	7	8	9	10	11	12
A	100.00%	90.00%	80.00%	70.00%	60.00%	50.00%	40.00%	30.00%	20.00%	10.00%	0.00%	
B	100.00%	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.20%	0.10%	
C	100.00%	90.00%	80.00%	70.00%	60.00%	50.00%	40.00%	30.00%	20.00%	10.00%	0.00%	
D	100.00%	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.20%	0.10%	
E												
F												
G												
H												

How to design an OT-2 Protocol:
(IMPORTANT: Steps 1 – 10 have been completed for you in a slightly modified form. To start with step 9, go to the Opentrons Designer app and upload the file called "PC Lab2.json".)

1. Go to https://designer.opentrons.com/. Click "Create New". Select "OT-2". Type an intelligent name for your protocol including your group number in it somewhere.
2. For the right pipette, choose "P1000 Single-Channel Gen 2". Choose the 1000 uL filter tips. For the left pipette, choose "P20 Single-Channel Gen 2". Choose 20 uL filter tips.
   Note: This is the way the robot is configures for you already. The filter tips are chosen to minimize the risk of contamination of the pipette tools themselves.
3. When asked about additional models, select none of them and continue.
4. Select "Continue to Liquids".
5. Click "New Liquid". Under liquid name, type "food coloring" or something similar. Click "Save" and enter a new liquid "Water" and save.
6. Click the "Design" logo on the left hand side of the screen.
7. Click and drag the tips to Deck Position 11. This tells the robot where to find the tip box.
8. Click on Position 8 and click "Add Labware" and add an Axygen 1 well Reservoir. Click "Add Liquids" on your new reservoir. On the liquid drop down select food coloring. Click the center well of the reservoir (large rectangle) to assign the liquid to that position. Type "70000" into the volume box. This tells the robot there is 70 mL of food coloring in position A1 of our reservoir. Complete the same operation for a reservoir of 20 mL of water in deck position 7.
9. To add a pipetting step, return to the deck view and click "+Add Step" and select "Transfer". This menu gives you all of the options for moving liquid using the robot

(which pipette, which pipette tips, what volume, etc.). Choose the most appropriate for your protocol.

10. When I've completed my protocol, I should be able to see the starting state and ending state of the protocol. I can click on each labware and click to see the volume of liquid in each well.
11. Once this all looks good, click "File" and click "Export". This will download a file which can be read and run by the Opentrons robot.

Hint: You may need to use a process called a serial dilution.

A serial dilution protocol involves a stepwise dilution of a substance in solution. Begin by preparing a series of tubes or wells, typically starting with a concentrated stock solution. Add a fixed volume of the stock solution to the first tube, and then add a solvent (usually distilled water) to reach the desired total volume, ensuring thorough mixing. Transfer a fixed volume from the first tube to the next tube containing an equal volume of solvent, mix thoroughly, and repeat this process across the series. Each subsequent dilution reduces the concentration of the solution by a consistent factor, often tenfold, allowing for precise and scalable decreases in concentration. Properly label each tube and ensure consistent volumes and thorough mixing at each step to maintain accuracy. An example is shown in the figure below. You will need to adjust the volumes to get the desired result needed for your protocol.

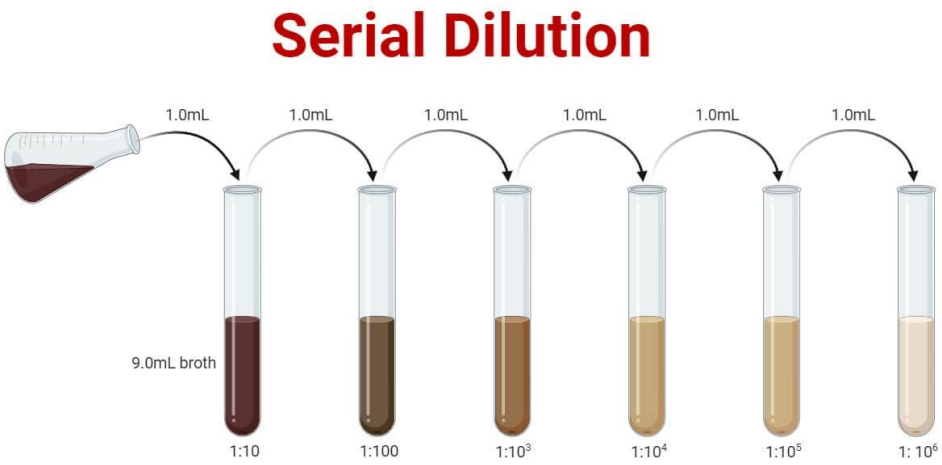

Figure 1: Serial dilution of bacteria.
Source: https://microbenotes.com/wp-content/uploads/2023/09/Serial-Dilution.jpeg

When you have completed your plate, please put a label on the plate and give it to a TA or your instructor. These plates will be read in a different lab while we're on the field trip.

What do you expect in terms of trends in the data generated from your plates? If you were to have pipetted perfectly, how would your data look? If you were terrible at pipetting, how would your data look?