Opentrons for Education

Prep Sheet
Lab Module 1: Introduction to Automation

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University of Florida

Getting Started

Follow the provided tutorials to set up your OT-2 prior to class.

- ☐ Unboxing the OT-2
- ☐ Unlocking the OT-2
- ☐ Setting up the Opentrons App
- ☐ Attaching pipettes to the OT-2
- ☐ Deck calibration on the OT-2
- ☐ Tip length and pipette offset calibration
- ☐ Running a protocol on the OT-2

Additional Support and Resources

OT-2 Instruction Manual

Introducing the New Protocol Library video

Opentrons Protocol Designer Instruction Manual

For technical support, check our Opentrons Help Center for relevant articles. If you need further support, please contact support@opentrons.com. Inform them that you are a part of the Opentrons for Education program and provide the date of your next laboratory class.

If you have questions related to the lesson plan, please reach out to Dr. Cătălin Voiniciuc at cvoiniciuc@ufl.edu.

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Educator Guide
Lab Module 1: Introduction to Automation

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Contents

This educator guide includes the following sections:

Purpose

This lab introduces automation in the laboratory with the OT-2 liquid handling robot. Students will explore and learn to use the OT-2 and related tools.

During this lab class, students will learn about and gain experience with:

- Using the OT-2 to automate pipetting tasks
- Opentrons Protocol Library and Protocol Designer tools
- Comparison of manual and automated pipetting tasks

Core Competencies

Automation skills:

- Experimental design in protocols for the OT-2
- Automation of pipetting tasks
- Use of the Opentrons OT-2 and no-code protocol tools

Background Knowledge

Students should start this lab with an understanding of common molecular laboratory methods and experimental design in order to explore the Protocol Library and Protocol

Designer. *No coding is required for this lab.* A pre-lab reading is included in the Student Guide to introduce students to the OT-2 prior to class.

Supplies

Opentrons Equipment

- ☐ OT-2 automated liquid handling robot
- ☐ OT-2 Single-Channel GEN2 Pipette (P20, P50, P300, or P1000 GEN2)

Opentrons Protocol and Tools

- ☐ Opentrons Protocol Library
- ☐ Opentrons Protocol Designer (must be used in Google Chrome browser)

Labware

- ☐ Labware supported by Opentrons can be found in the Opentrons Labware Library. Examples of supported labware that can be used for this protocol include:
  - Opentrons OT-2 96 Filter Tip Rack 200 uL
  - NEST 96 Well Plate 100 uL PCR Full Skirt
  - NEST 12 Well Reservoir 15 mL

Other

- ☐ Printed Downloadable 96 Well Plate Templates
- ☐ Food coloring (1-2 colors)

Experimental Duration

Required Class Sessions

1

Lab Run Time

This lesson plan was prepared for a traditional laboratory class time of 80-90 minutes. Students arrive to class with the OT-2 and the Opentrons App set up and ready for use.

Basic Troubleshooting

- We recommend completing a trial run of a simple protocol from the Opentrons Protocol Library prior to class. The Opentrons Logo Protocol is designed to pipette the Opentrons logo onto a well plate, and uses the same labware linked above. On the OT-2 robot, this trial run can be completed with or without tips.

- Issues with tips striking plates are almost always due to using alternate labware or robot calibration issues. If you experience this issue, first confirm that the correct labware specified in the protocol is in use; then, re-calibrate the robot. A Labware Position Check is also recommended after importing a protocol and before you run it to confirm the combination of deck slot and labware definition on the OT-2.

- Droplets can stick to the outside of the pipette tip when the OT-2 aspirates liquid. This is usually due to the pipette tip submerging more than is necessary when aspirating, or incomplete droplet release depending on the volume dispersed. Ensuring that the OT-2 pipettes a volume large enough to be released cleanly from the pipette (for example, 50-100 µL in our example Logo Protocol, can improve this issue. If writing or editing Python protocols, complex liquid handling parameters such as the touch_tip parameter can be used to customize tip handling by the OT-2.

- In this lab, students use Opentrons Protocol Designer to design a “drawing” with colored water on a 96-well plate. Protocol Designer is only supported in Google Chrome. A brief set of instructions are included in the Student Guide. For more, see the Protocol Designer Instruction Manual.

Procedure Guide

Before Class

1. Set up the OT-2 robot and the Opentrons App by following the tutorials listed in **Getting Started.**

2. Prepare 1-2 colors of colored water (using food coloring) for the Protocol Designer "drawing."
3. Choose and download a sample protocol from the Protocol Library (we suggest the Logo Protocol).

Create and run a sample protocol in Protocol Designer to prepare for demonstration to students.

Lab Introduction

Instructors may wish to define *automation* and cover examples of automation in our everyday lives (and in science) in an introductory lecture. Examples include:

- Alarm clocks and calendar reminders
- Content recommendations, or autoplay, on social media, Youtube, Netflix, etc.
- Simple automation in science: thermocyclers (run a program where temperatures change after a given amount of time), tube/sample inverters (mix your samples at a given speed and change direction after a given amount of time)
- More complex automation in science: liquid handling robots such as the OT-2, and others

Exploring Protocol Designer

1. Students will use personal computers to explore the Opentrons Protocol Library (15 minutes)

2. As a group, students will choose a protocol they are interested in.
3. Configure and download the chosen protocol (for our example Logo Protocol, this includes choosing labware and pipette location).
4. Import the chosen protocol into the Opentrons App.
5. Have students view the app on a large screen display to demonstrate the chosen protocol, hardware, labware, and liquids.
6. Demonstrate deck setup, including placing labware in the specified deck location. For our example Logo Protocol, the setup would look like this:
    a. Opentrons Single-Channel GEN2 pipette (select your size and left/right mount in the “configure and download” menu)
    b. Chosen destination plate labware in deck slot 1
    c. Chosen dye source labware in deck slot 2
        i. If using a 12-row trough reservoir, store two solutions (differently colored using food dye) in column 1 and column 2.
        ii. If using a tube rack, dye 1 should be stored in ‘A1’ and ‘B1’ and dye 2 should be stored in ‘C1’ and ‘D1.’ Be sure to choose your desired labware in the “configure and download” menu.
    d. Opentrons 96 Tip Rack 300 µL in deck slot 3
7. Run a “dry run” of the chosen protocol without tips.

Protocol Designer "Drawings"

1. Students can create a simple "drawing" on a printed 96 well plate template using 1-2 colors.
2. Students that finish early can use personal computers to explore the Opentrons Protocol Designer (must be used in Google Chrome).
3. Students will vote on their favorite "drawing" to be pipetted onto the OT-2.
4. Have students view the Protocol Designer on a large screen display as the instructor creates their pipetting protocol to "draw."
5. Import the .json file into the Opentrons App to run the protocol and complete the "drawing" on a plate.

Discussion Questions

Direct students to discuss the lab, and automation in the laboratory, with their labmates. Example prompts might include:

- What are some examples of automation in your everyday life, besides those we discussed in class? Give at least two examples.
    - Potential answers might include: alarm clocks, calendar reminders, email sorting, software updates, password autofill and password managers, and smart devices like Alexa, Google Home, or a doorbell camera.

- Can you think of two experiments where the use of a robot, like the OT-2, might be helpful? Give a reason for each.
- What kinds of protocols did you notice in the Protocol Library? Did the protocols you looked at include steps where scientists still need to move tubes, samples, etc. *manually* (with their hands), or were they *fully automated*? Give at least one example.

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Student Guide

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Pre-lab Reading

Molecular biology is the study of small things- cells and the molecules they contain, like proteins and nucleic acids- that are the building blocks of life. When scientists work with these molecules, they really work with liquids. Cells are grown in and suspended in media, a liquid which contains nutrients required for cells' growth. DNA or RNA samples might be suspended in molecular grade water and frozen for long-term storage, and buffers are liquids that might be added to a tube for a given experiment or reaction.

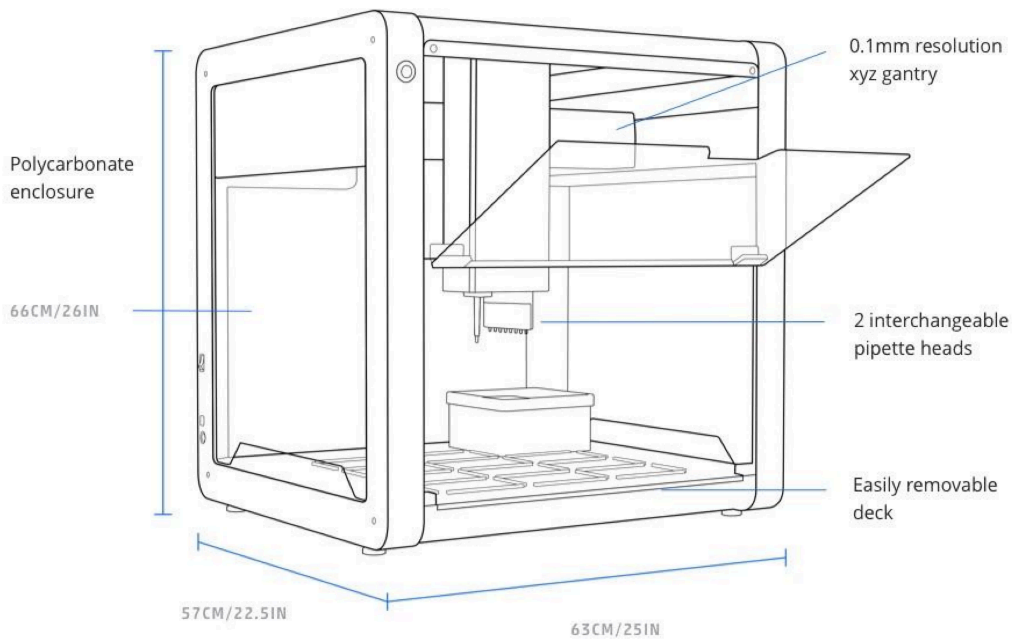
Any scientist will tell you- proper pipetting takes time! Let's consider a polymerase chain reaction (PCR) experiment. This essential reaction combines:

- A piece of **DNA**, which the scientist needs more of, with:
- **DNA polymerase**, an enzyme that can assemble new pieces of DNA
- **Nucleotides**, the building blocks that will be assembled into new pieces of DNA
- **Primers**- a template, or instructions, for how the nucleotides will be assembled into DNA (and what order they will be assembled in)

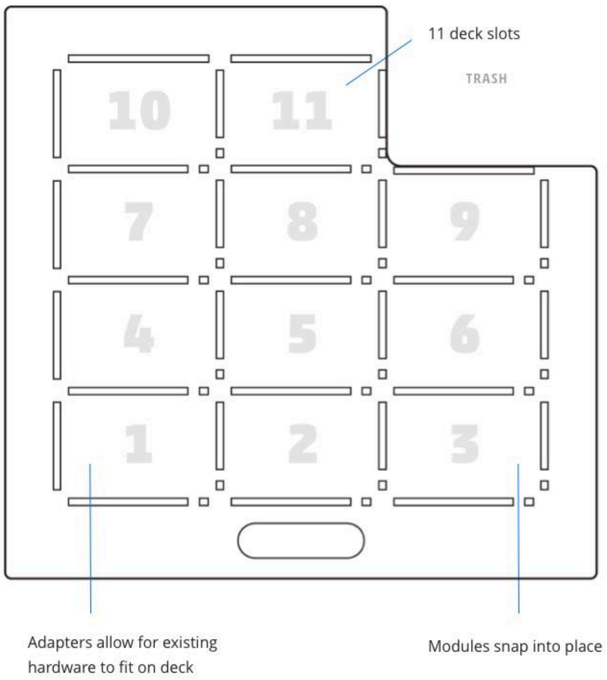
Each of these components represents a liquid that must be pipetted, often in a small amount, into, for example, each well of a 96-well plate. Simple math tells us that these 4 components, multiplied by 96 wells, equals 384 pipetting tasks for a scientist to complete in a single experiment! Perhaps you can see why this is a task that might make sense to *automate*.

Automation is the application of technology, such as robotics, to reach the same goal with less hands-on, human input, like the pipetting tasks a scientist needs to complete. Opentrons robots, like the OT-2 you'll work with in this course, are liquid handling robots, specifically designed to complete pipetting tasks in the lab.

The OT-2, shown here, fits on the lab bench. It has two interchangeable *pipette heads*, where different pipettes can be attached to pick up and dispense different volumes of liquid. The robot moves the pipette by moving the *gantry*. If the pipette is the hand of the robot, the gantry is the arm.



The pipette will move liquids between pieces of *labware*, like tube racks and well plates, using **tips**. Each of these sits on the *deck*. The deck has 11 *slots* (labeled 1-11 in the image below) that labware can be placed in.



So, how does the OT-2 know what pipetting tasks to perform? You, the scientist, will tell the OT-2 exactly what it should do! This is accomplished using a *protocol*, or a series of commands the robot will follow. The protocol is imported into the Opentrons App on the computer the robot is connected to. In your first lab class, we'll be exploring the different ways you can direct the OT-2 to complete pipetting tasks, and we'll even see some pipetting in action!

To see an example of the OT-2 running a PCR experiment, like the one discussed in the pre-lab reading, you can watch the following video: Automating PCR Prep with OT 2 Pipetting Robot.

Purpose

In this lab, you'll explore *automation* in the laboratory using the OT-2, a liquid handling robot designed by Opentrons. Your instructor will guide you through the tools you can use to tell the OT-2 what pipetting tasks you'd like it to complete. Finally, you'll work with your class to build a *protocol.*

Learning Outcomes

- Understand laboratory automation and types of lab pipetting tasks that can be automated
- Understand how the OT-2 receives directions in the form of a protocol
- Be able to develop a simple protocol using Protocol Designer

Supplies

Opentrons Equipment

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Opentrons Protocol and Tools

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  - NEST 12 Well Reservoir 15 mL

Other

- ☐ Printed Downloadable 96 Well Plate Templates
- ☐ Food coloring (1-2 colors)

Procedure Guide

Before Class

1. Complete the pre-lab reading.

Exploring Protocol Designer

1. Use your personal computer to explore the Opentrons Protocol Library.

   What kinds of protocols do you see? In other words, what kinds of experiments can scientists use the OT-2 to automate?

   Try the filters on the left-hand side in the "search" feature of the Protocol Library. Under "Robot model," try sorting protocols by robot type (OT-2, which you'll use in this class, or the Flex). What are the different capabilities of the two robots? Are there any experiments you can do on one, and not the other?

   Choose a favorite protocol and discuss with your labmates. You and the rest of the class will vote on a favorite to see in action on the OT-2!

Protocol Designer "Drawings"

1. Use the printed 96-well plate template to create a simple drawing. The drawing should use 1-2 colors in the different wells of the plate.

   If you have extra time, use your personal computer to explore the Opentrons Protocol Designer (must be used in Google Chrome).

- Begin by choosing your robot type (OT-2), pipettes, and tip racks. A few common pipettes used in the course are:
    - GEN2 P20 Single-Channel pipette
    - GEN2 P300 Single-Channel pipette
- Next, you'll add modules. Here are a few modules used in the course:
    - Heater-Shaker Module GEN1
    - Temperature Module GEN2
- Name your protocol and add information to help it stand out from your other protocols.
- In the protocol overview, you can see the information you've just added, like protocol metadata and instruments. Click **Edit protocol** to add labware, liquids, and protocol steps.
- Click **Liquid** in the upper right to add liquids. Choose your liquids as if you were pipetting your drawing onto a plate on the OT-2- for example, create a liquid "blue dye" and choose a color.
- After adding liquids, you'll add labware to the deck. Be sure you have selected **Protocol starting deck** in the upper left. Click any slot to add a well plate, like the Bio-Rad 96 Well Plate 200 µL PCR plate. Click on your plate to name it, but don't add any liquids for now. This is where your drawing will be pipetted.

- Next, add a reservoir, like the NEST 12 Well Reservoir 15 mL. Click on the reservoir to add your liquids, or water colored with food dyes, to the wells. Don't forget to specify your initial volume (in µL). Now, your liquids should be clearly seen on your deck map.
- To create your drawing, you'll now add steps to your protocol timeline, like transfer steps. Click **Protocol steps** in the upper left to get started.
- Click **Add step** to add a transfer step to your protocol. Here, you'll have several options to customize how the OT-2 will transfer colored water from the reservoir to your 96-well plate to "draw." You'll need to specify how much liquid (in µL) will be added to each well, as well as the source and destination. You can also decide how many tips the OT-2 will use to create your drawing and the pipette's *path of motion* (single transfers, multi-dispense across wells, etc.).
- When you're finishing designing your protocol, hover over "Ending deck" in your protocol steps to see the final result. Is everything set up correctly to complete your "drawing?"
- Click **Done** in the upper right to return to the protocol overview. Now, you'll see a summary of the liquids and protocol steps added on the left hand side.

- To export and save your protocol to be imported into the Opentrons App, click **Export protocol** in the upper right.

2. As a class, you and your labmates will choose a drawing to be “drawn” on the OT-2: pipetted with colored water into a plate. Your instructor will guide you and the rest of the class through creating a protocol in Protocol Designer to instruct the OT-2 to do this.

Discussion Questions

Turn to a neighbor and discuss the following prompts.

- What are some examples of automation in your everyday life, besides those we discussed in class? Give at least two examples.
- Can you think of two experiments where the use of a robot, like the OT-2, might be helpful? Give a reason for each.
- What kinds of protocols did you notice in the Protocol Library? Did the protocols you looked at include steps where scientists still need to move tubes, samples, etc. *manually* (with their hands), or were they *fully automated*? Give at least one example.