Opentrons
for Education

Prep Sheet

Introduction to Automation with Protocol Designer

Emily Burghardt, Ph.D.

Opentrons

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
- ☐ Importing protocols to the Opentrons App
- ☐ Test run a protocol on the OT-2

Resources

- OT-2 Manual
- Introducing the New Protocol Library video
- Opentrons Protocol Designer Instruction Manual

For technical support, please check out the 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 the author, Emily Burghardt, at emily.burghardt@opentrons.com.

Opentrons for Education

Educator Guide

Introduction to Automation with Protocol Designer

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Contents

This educator guide includes the following sections:

- Purpose
- Background Knowledge
- Supplies List
- Experimental Duration
- Basic Troubleshooting
- Procedure Guide

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 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

This lesson plan was prepared for high school students with a basic understanding of molecular laboratory methods. *No coding experience 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

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 in this lesson 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

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

Basic Troubleshooting and Tips

- We recommend completing a trial run of a simple protocol from the Opentrons Protocol Library prior to class. In a Protocol Library Search, use the filters to find protocols for your robot (OT-2) and pipette (for example, a P20 Single-Channel GEN2). On an OT-2 robot, a trial run can be a "dry run," completed without using tips or liquid.

- The Opentrons Logo protocol is designed to pipette the Opentrons logo onto a well plate, and uses the same labware linked above. This protocol can be used as a demonstration for students in the lab.

- 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. We recommend performing Labware Position Check 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 when dispensing. When creating a transfer step in a Protocol Designer protocol, you can adjust the aspirate or dispense tip position and enable touch tip (under advanced pipetting settings) to improve this issue.

- 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 different colors of colored water (using food coloring) for the Protocol Designer "drawing."
3. Choose and download a sample protocol from the Protocol Library (like the Opentrons Logo Protocol).
4. 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!) as an introduction. 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 like the OT-2

Instructors should also introduce methods of giving the OT-2 commands:

- *How does the OT-2 know what to do?* The OT-2 must communicate with, and receive directions from a computer in the form of commands. A protocol is a list of commands the OT-2 will complete. Each is a liquid handling task.
  Note: students should understand both uses of the word "protocol:" as a written experimental process for scientists, and instructions for the robot in the form of a protocol file.
- The first method: a ready-made protocol from the Protocol Library (students are familiar with this from the previous module). These protocols can be customized using *parameters* (values chosen by the user and added into the protocol).
- The second method: creating your own Python protocol as a user, either by writing code in a text editor (like Microsoft VSCode) or using the no-code Opentrons Protocol Designer.

Giving the OT-2 commands in a protocol

1. Open a Python protocol file downloaded from the Protocol Library. Have students view a large screen display for comparison. Show students the Python

protocol, pointing out the various parts that detail the protocol and the directions:

   a. *Metadata (robot type, author)-* descriptions of the protocol at the top of the file
   b. `load_labware`- describes to the robot the labware that is present on the deck.
   c. *Tasks for OT-2 to complete:* commands like aspirating and dispensing for a volume transfer

2. Next, import the protocol file into the Opentrons App or [Protocol Designer](). Here, students can compare and contrast the protocol steps in a graphic interface with the code students will also see.

3. Create a simple protocol in Protocol Designer (optional). Show students how to choose pipettes, labware, and modules, and create steps to use in their protocols.

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 Protocol Designer on a large screen display as the instructor creates their pipetting protocol to “draw.”
5. Export the protocol and import into the Opentrons App.
6. We recommend starting with a dry run of the protocol. Demonstrate deck setup to run the protocol and complete the “drawing” on a plate.

Discussion Questions

Direct students to discuss the lab and automation in the laboratory. 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: 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.
- How does the OT-2 know what to do? List the methods discussed in class.
- Explore the Opentrons Protocol Library on your own. What kinds of experiments are automated in these protocols? Give two examples.

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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.

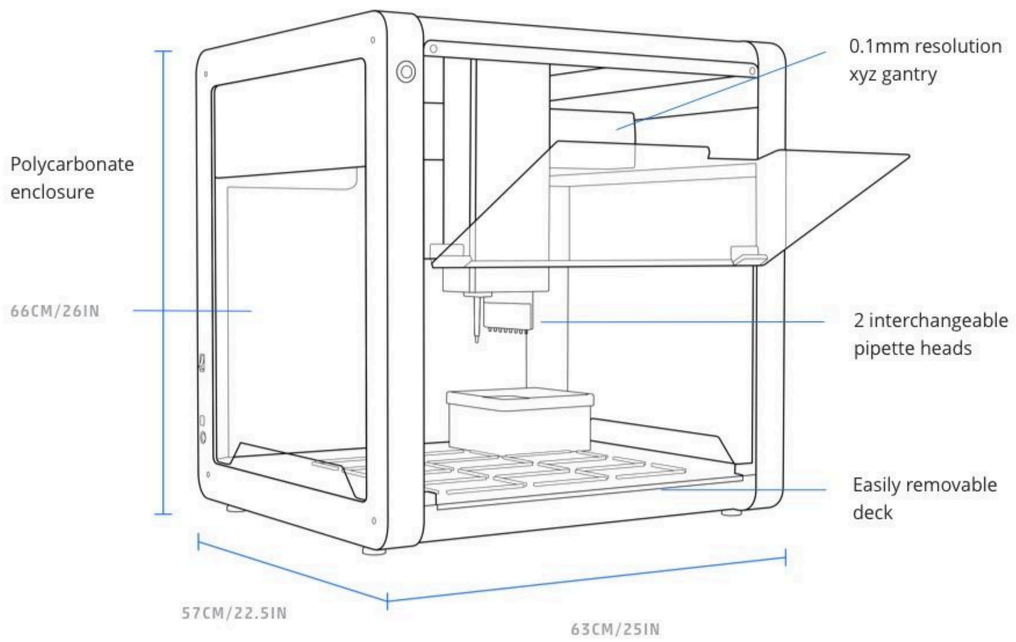
Any scientist will tell you—proper pipetting takes time! Let's consider a polymerase chain reaction (PCR) experiment. This essential reaction combines:

- 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)

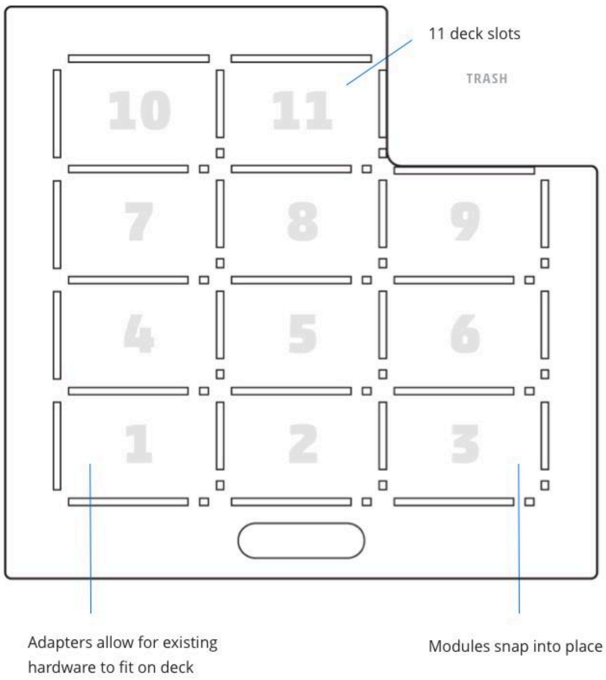
Each of these components represents a liquid that must be pipetted, often in a small amount, into, for example, each well of a 96-well plate. Simple math tells us that these 4 components, multiplied by 96 wells, equals 384 pipetting tasks for a scientist to complete in a single experiment! Perhaps you 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. Today, 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, 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

- ☐ OT-2 automated liquid handling robot
- ☐ OT-2 Single-Channel GEN2 Pipette

Opentrons Protocol and Tools

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Labware

- ☐ Labware supported by Opentrons can be found in the Opentrons Labware Library. Examples of supported labware that can be used in this lesson include:
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  - ☐ NEST 96 Well Plate 100 uL PCR Full Skirt
  - ☐ NEST 12 Well Reservoir 15 mL

Other

- ☐ Printed Downloadable 96 Well Plate Templates
- ☐ Colored water

Procedure Guide

Before Class

1. Complete the pre-lab reading.

Giving the OT-2 commands in a protocol

1. Your instructor will demonstrate the different types of protocols that can be used to provide commands to the OT-2. See the table below, and take your own notes.

Where can I find or create a protocol?	**Notes**
Opentrons Protocol Library	
Opentrons Protocol Designer	
Write your own Python protocol	

Remember that, in automation of lab tasks, the word “protocol” has two meanings: protocols are a written experimental process for scientists to follow, as well as instructions for the robot (as a protocol file).

Protocol Designer "Drawings"

1. Use the printed 96-well plate template to create a simple drawing. The drawing will use colored water 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. Pipettes can be single or multi-channel (for example, to fill 8 pipette tips at the same time!). For this class, you'll choose an OT-2 single-channel pipette and tips you have on hand in the classroom.
   - Next, you'll add *modules*. These are placed on the OT-2 deck and perform different actions, like thermocycling and holding samples at a given temperature. Modules you'll use might include:
     - Temperature Module GEN2
     - Thermocycler Module GEN2
   - Name your protocol and add information to help it stand out from your other protocols.

- In the protocol overview on the next screen, 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 left hand side bar 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 **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 microliters (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 **Add step** in the bottom left to get started.
- Start by adding 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, multiple dispenses 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.
- Click **Export protocol** in the upper right to export and save your protocol.

2. As a group, choose a drawing to be "drawn" on the OT-2: pipetted with colored water into a plate. Your instructor will guide you through creating and running a Protocol Designer on the OT-2 to "draw!"

Discussion Questions

- 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.
- How does the OT-2 know what to do? List the methods discussed in class.
- Explore the Opentrons Protocol Library on your own. What kinds of experiments are automated in these protocols? Give two examples.