

Prep Sheet

Learn Python with Protocol Designer

OT-2 Edition

Authored by Emily Burghardt, Ph.D.

Opentrons

July 2026

Getting Started

In this lesson plan, students use Opentrons Protocol Designer to create a PCR protocol for the Opentrons OT-2 liquid handling robot. In the next lab module, students use an attached worksheet to program the same Protocol Designer steps into their first Python protocol.

Additional Support and Resources

[Opentrons Protocol Designer Instruction Manual](#)

[Opentrons OT-2 Instruction Manual](#)

[Protocol visualization](#) and the [Opentrons OT-2 App](#)

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

Learn Python with Protocol Designer

OT-2 Edition

Authored by Emily Burghardt, Ph.D.

Opentrons

Contents

This educator guide includes the following sections:

- Purpose
- Background Knowledge
- Supplies
- Experimental Duration
- Basic Troubleshooting and Tips
- Procedure Guide
- Discussion Questions

Purpose

In this lab, students will learn about and design a polymerase chain reaction (PCR) protocol. They'll use the no-code [Opentrons Protocol Designer](#) to automate a PCR experiment appropriate for your classroom. This can be as simple as pipetting reagents together, or as complex as running PCR on the robot's deck with the [OT-2 Thermocycler Module](#).

Students will gain experience with:

- Automating basic pipetting tasks
- Experimental design in Protocol Designer

Core Competencies

Laboratory Skills:

- Experimental design for PCR
- Reagent preparation

Automation Skills:

- Translating basic lab experiments for automation
- Using an OT-2 liquid handling robot

Background Knowledge

This lesson plan was developed for students with an understanding of basic lab tasks, like reagent use and pipetting. Although some basic Python experience may be useful for the accompanying second lab module, *no coding experience is required for this lab.*

The **Student Guide** contains a pre-lab reading with a brief overview of PCR.

Supplies

Opentrons Equipment

- ☐ Opentrons OT-2 liquid handling robot
- ☐ OT-2 [Single-Channel GEN2 pipette](#)
- ☐ [OT-2 Thermocycler Module](#) (*optional*)
- ☐ [OT-2 Temperature Module](#) (*optional*)

Opentrons Tools

- ☐ Opentrons [Protocol Designer](#) (must be used in Google Chrome)
- ☐ [Learn Python with Protocol Designer](#) protocol

Non-Opentrons Equipment

- ☐ Benchtop thermocycler (*optional*)
- ☐ Benchtop microcentrifuge or vortex (*optional*)

Labware

- ☐ Opentrons pipette tips compatible with your chosen pipette
- ☐ PCR well plates, like [Opentrons Tough 96 Well Plate 200 \$\mu\$ L PCR Full Skirt](#)
- ☐ Snapcap tubes (like [Opentrons 24 Well Aluminum Block with NEST 2 mL Snapcap](#)) or generic PCR strip tubes (like [Opentrons 96 Well Aluminum Block with Generic PCR Strip 200 \$\mu\$ L](#))

Reagents and Other Materials

PCR reagents of your choice, including:

- ☐ Master mix (or individual polymerase, buffers, dNTPs mix, and cofactor like MgCl_2)
- ☐ DNA templates for amplification
- ☐ Forward and reverse primers
- ☐ Molecular grade water

Experimental Duration

This lesson plan was prepared for a traditional laboratory class time of 80–90 minutes. Instructors can choose to split this lesson plan in two to save time in class:

- Students plan, create, and edit using Protocol Designer.
- Students run their PCR protocol on the OT-2.

Basic Troubleshooting and Tips

- Protocols can be tested using a *dry run* on the OT-2. Any protocol can be run without tips or liquids on the robot deck to check for expected movements and behavior. Instructors can use this lesson plan's [example protocol](#) or a student protocol.
- Before running any new protocol on the OT-2, we recommend creating [labware offsets](#) to confirm proper combinations of deck slots and labware definitions.
- Instructors can choose the best fit for their lab's needs from the full list of Opentrons supported labware. Check our [Labware Library](#) to see compatible well plates,

reservoirs, and more. As your students work in Protocol Designer, they'll also be able to see labware compatible with the modules used in lab.

- In this lab, students should use [Protocol Designer](#) to create a PCR protocol. Here's a few tips to help your students with their protocol design:
 - For best results, Protocol Designer should be used in Google Chrome.
 - You or your students can compare with this lesson plan's [example protocol](#) from the Opentrons Protocol Library.
 - The example protocol uses the OT-2 Temperature Module to hold samples at a target temperature. If you want to keep samples cold for longer on the OT-2's deck without using a module, you can pre-chill an Opentrons [aluminum block](#) and labware.
 - The example protocol uses an OT-2 single-channel pipette. If your lab has both a single- and 8-channel pipette, this is an important automation consideration for your students—the 8-channel can complete the same protocol much more quickly!
 - In Protocol Designer, you can choose to run other protocol steps [at the same time](#) as a Thermocycler Module profile. Protocol Designer will ask if you'd like



to choose this option when creating a new Thermocycler step.

- Students can click the three dots on the right side of each protocol step and choose **Duplicate step** to save time during protocol creation.
- Protocol Designer generates Python protocols that can be run on the OT-2. You can open these files in a code editor to view them, but files you edit outside of Protocol Designer *cannot* be reimported and edited again.

You can rename steps and add custom descriptions in Protocol Designer. This plain text is exported and will appear alongside your Python commands, and can make it easier for your students to read and understand the final protocol. Click **Rename** in each step's menu to add a custom name and description. For more details, see [Editing Steps](#) in the Protocol Designer Instruction Manual.

- Thermocycler Module profiles you create in Protocol Designer end without an infinite temperature hold like many benchtop thermocyclers use. You can add an extra [Thermocycler Module step](#) after the profile to set the lid



and block temperature. Unless you've turned the Thermocycler Module's block or lid temperature off, the module won't deactivate on its own when the protocol ends, and will continue to keep samples at your chosen temperature.

- In the [example protocol](#), the OT-2 will pipette to combine buffers, dNTPs, and cofactor in the master mix, then combines your DNA template, primers, and water with the master mix. You and your students can decide which components should be assembled on- and off-deck.
- An attached worksheet in the **Student Guide** provides space for students to plan their experiment, from master mix assembly to protocol steps they'll add in Protocol Designer. The worksheet does not include example volumes or thermocycler profiles—students can complete independent research on PCR reaction conditions and/or work with a protocol tested in your classroom.
- Want to start your students with something even simpler to plan and program? The attached worksheet in the

Student Guide can be used to guide students through a step as simple as resuspending and diluting dry oligos.

Procedure Guide

Lab Introduction and Experimental Planning

1. Instructors may want to review PCR reaction basics with students.
2. Students can use the attached worksheet to plan automating their PCR protocol, including reagents, protocol steps, and thermocycler profiles. Students should write out all the steps needed to prepare for and run their PCR reaction, then decide which steps to automate and which to perform manually, if any.

Protocol Writing: Protocol Designer and Testing

1. Students should use the provided worksheet in the **Student Guide** to create their protocol.
2. Finished protocols can be exported and imported into the Opentrons OT-2 App. The app will automatically run analysis and check for errors.
3. Students can use [protocol visualization](#) in the Opentrons OT-2 App to view and troubleshoot their protocol before running it on the OT-2. They'll be able to see labware and

liquid changes for each protocol step, and any errors that they'll need to fix.

4. Protocols can be reimported into Protocol Designer to fix any errors. Repeat the process as needed. For extra help, see our [Protocol Designer Instruction Manual](#).
5. Before running any new protocol on the OT-2, students should create and confirm [labware offsets](#) with a labware position check. This process is required before running a new protocol and confirms that labware and pipette tip positions are where the OT-2 expects them to be.
6. Final protocols can be first run on the OT-2 as a *dry run*, without tips or liquids, to check for expected behavior.

PCR Reaction

1. A finished protocol can be used to run the PCR reaction on the OT-2.

Discussion Questions

Direct students to discuss the lab activities with one another. Example prompts might include:

- Describe in your own words what took place in the PCR reaction you ran or designed in class today.
- Which steps were more difficult to automate? Did you find any errors or have to make extra considerations to automate them?
- What components are included in a master mix? What are some reasons a scientist might choose to use a master mix?

Student Guide

Learn Python with Protocol Designer

OT-2 Edition

Authored by Emily Burghardt, Ph.D.

Opentrons

Pre-Lab Reading

Today in lab, you'll *automate* steps of a polymerase chain reaction (PCR). The PCR reaction was a major scientific breakthrough in the mid-1980s. This reaction amplifies a specific segment of DNA to produce millions (or even billions!) of copies of your specific gene or region of interest.

Each PCR reaction consists of the following:

- **DNA template:** contains your gene or region of interest.
- **DNA polymerase:** an enzyme that assembles new pieces of DNA.
- **dNTPs**, or *deoxynucleotide triphosphates*: single bases (dATP, dCTP, dGTP, or dTTP) used to assemble new DNA.
- **Forward and reverse primers:** templates for the polymerase to use to construct new copies of each strand of DNA.

These reagents are generally mixed with a buffer and water. Scientists often order a *master mix*, which contains some of the above ingredients pre-mixed together. Your instructor will help you choose the reagents you'll use in lab today.



You'll use [Opentrons Protocol Designer](#) to create an automated protocol to run your PCR experiment on the OT-2 liquid handling robot.

Protocol Designer is an online tool that lets you design protocols without writing code. You'll be able to see your labware, like well plates, and liquids on the OT-2's deck, and click to add steps, like transferring, or pipetting, liquid. You can check out the [Protocol Designer Instruction Manual](#) before class to get used to the different steps and features in Protocol Designer.

Purpose

In this lab, you'll learn about and design a PCR protocol to complete on the OT-2 liquid handling robot. You'll use the attached worksheet to plan your experiment, including available reagents, steps, and automation considerations.

Learning Outcomes

- Design an experiment in [Opentrons Protocol Designer](#), considering differences between a manual and automated experiment
- Plan and automate a PCR experiment

Supplies

Opentrons Equipment

- ☐ Opentrons OT-2 liquid handling robot
- ☐ OT-2 [Single-Channel GEN2 pipette](#)
- ☐ [OT-2 Thermocycler Module](#) (*optional*)
- ☐ [OT-2 Temperature Module](#) (*optional*)

Opentrons Tools

- ☐ Opentrons [Protocol Designer](#) (must be used in Google Chrome)
- ☐ [Learn Python with Protocol Designer](#) protocol

Non-Opentrons Equipment

- ☐ Benchtop thermocycler (*optional*)
- ☐ Benchtop microcentrifuge or vortex (*optional*)

Labware

- ☐ Opentrons pipette tips compatible with your chosen pipette
- ☐ PCR well plates, like [Opentrons Tough 96 Well Plate 200 \$\mu\$ L PCR Full Skirt](#)
- ☐ Snapcap tubes (like [Opentrons 24 Well Aluminum Block with NEST 2 mL Snapcap](#)) or generic PCR strip tubes (like [Opentrons 96 Well Aluminum Block with Generic PCR Strip 200 \$\mu\$ L](#))

Reagents and Other Materials

PCR reagents of your choice, including:

- ☐ Master mix (or individual polymerase, buffers, dNTPs mix, and cofactor like MgCl_2)
- ☐ DNA templates for amplification
- ☐ Forward and reverse primers
- ☐ Molecular grade water



Procedure Guide

Before Class

1. Complete the pre-lab reading.

Experimental Planning

1. Use the attached worksheet to plan automating your PCR protocol, including reagents, protocol steps, and thermocycler profiles.

Protocol Creation with Protocol Designer

1. Open [Protocol Designer](#) in Google Chrome to get started.
2. Start by choosing your robot, pipette(s), and tips.
3. Next, add any modules you'll use in your protocol, like a Temperature or Thermocycler Module, and add details like a protocol name and description.
4. Click **Edit protocol** in the upper right to customize the OT-2's deck and add protocol steps.
5. On the left hand side of the screen, click to edit:
 - a. Deck hardware:** remove or add additional hardware modules.
 - b. Liquids:** define liquids, like master mix or DNA, before you add them to labware on the deck.
 - c. Starting deck:** Click open slots to add labware, like well plates, to the deck. You can also add compatible labware directly to modules.
 - d. Add step:** Click to add transfer, mix, move, pause, or module-specific steps to your protocol. Adding each kind of step opens a menu on the right side of the screen with advanced options, especially for transfer steps.
6. Click **Export** in the upper right when you're finished with your protocol.



Here are some Protocol Designer tips to help you create your PCR protocol:

- You can choose custom colors for each liquid you add to the deck, like primers and your DNA, to help you tell them apart on the birds-eye view in the middle of the screen.
- Protocol Designer includes warnings and errors to help you create a protocol that can run safely on the OT-2. These can appear in red in your protocol timeline, on the left, or on the right when adding a new step. We recommend resolving all warnings and errors before you export your finished protocol.
- If your OT-2 deck includes modules like a Thermocycler, Protocol Designer might block you from adding labware or other modules to the slots nearby. This helps prevent collisions with the pipette as the OT-2 raises and lowers it to move liquid.
- When adding a new step, click **Rename** in the upper right corner to give your steps a custom name. This can help you easily keep track of multiple steps of the same type, and any custom names and descriptions you add will be exported in your finished protocol.

Protocol Testing

Now that your protocol is ready, let's make sure it can run successfully on the OT-2! You should always check new protocols for errors before you run them on the robot. This includes:

- **Analyze for errors:** Import your finished protocol into the Opentrons OT-2 App. The app automatically runs protocol analysis to check for errors.

If the app finds an error, click to view error details. Return to Protocol Designer as needed to make edits.

- **Protocol visualization:** In the Opentrons OT-2 App, click **Visualize** to [“play” your protocol](#) and check for expected behavior.
- **Create labware offsets:** Before you run a protocol on the OT-2, you'll need to set and confirm [labware offsets](#) with a labware position check. The OT-2 takes data from your protocol about where to expect labware on the deck. Creating labware offsets is an extra step that ensures that those expected labware and pipette tip positions are correct. The app will guide you through this process before starting your protocol run.
- **Dry run:** Your instructor can help you complete a protocol *dry run* on the OT-2—in other words, run your



protocol without any tips or liquids to make sure you see the robot behavior you expect.

PCR Reaction

Work with your instructor to run your PCR protocol on the OT-2!

Discussion Questions

Follow your instructors directions to discuss the following prompts, either with a neighbor or in a lab report:

- Describe in your own words what took place in the PCR reaction you ran or designed in class today.
- Which steps were more difficult to automate? Did you find any errors or have to make extra considerations to automate them?
- What components are included in a master mix? What are some reasons a scientist might choose to use a master mix?



PCR Design and Automation Worksheet

Section A: PCR Experimental Design

Resuspending Dry Oligos

It's common for forward and reverse primers to arrive at the lab as dry oligos. These often look like dry pellets or powders, and can be reconstituted in molecular grade water or a buffer of your choice. A typical stock solution concentration is 100 μM , which can then be diluted to a working concentration (often 10 μM) to use in your PCR reaction.

Use the table and the space provided below to calculate dilutions to make a stock and working concentration of your primers, if needed.



	Forward primer	Reverse primer
List any included directions from the manufacturer here.		
Stock concentration		
Working concentration		

Dilution calculations:

Master Mix Assembly

Check with your instructor to see if your master mix arrived in the lab pre-assembled, or if you'll need to pipette its components together. Use the table and the space provided below to plan assembling your master mix, if needed.

Reagent	Concentration	Volume (for 1 reaction)	Total number of reactions	Total volume needed
Polymerase				
Buffer(s)				
dNTPs				
Cofactor				

Other				
-------	--	--	--	--

Dilution calculations:

Reagent Assembly

Next, you'll need to assemble your PCR reaction. Use the table below to add volumes for each reagent.

Reagent	Concentration	Volume (for 1 reaction)	Total number of reactions	Total volume needed
Master mix	—			
Forward primer				
Reverse primer				
Template DNA				
Water				

Total volume	—		—	
---------------------	---	--	---	--

Dilution calculations (for any component you might need to dilute further, like template DNA):

Thermocycler Profiles

You can run your PCR experiment in the OT-2's Thermocycler Module or in a benchtop thermocycler. Either way, you can use the table below to plan your thermocycler *profile*, including timed temperature holds for steps like denaturation, extension, and annealing of your DNA.

Step	Temperature (°C)	# of Repeats
-------------	-------------------------	---------------------

Initial denaturation		
Denaturation		
Extension		
Annealing		
Temperature hold		

What temperature will you need to set the thermocycler's lid to? A heated lid helps to prevent evaporation or condensation of your PCR samples.

Section B: Automating PCR

Next, use the space below to make a list of the steps you'll need to automate to run PCR on the OT-2.

Here are some things you should consider when creating your protocol:

- You'll always need to tell the OT-2 which pipette and tips to use for moving liquid. If you have more than one pipette, include this information for each step.
- Be sure to include any steps you would do while manually pipetting the liquids. Where would you mix your samples?



Add this information to your protocol steps and see how much of it you can automate in Protocol Designer.

- In an OT-2 protocol, you need to tell the robot when to pause so you can manually move labware on the deck. For example, if you'll need to move a well plate to or from a module like the Thermocycler Module, include a step in your list.
- If you're using the OT-2's Thermocycler Module, add steps for every action the robot will need to take, including opening and closing the lid!
- You can use the image below to plan where your labware and hardware, like modules, should be on the OT-2's deck:



10	11	TRASH
7	8	9
4	5	6
1	2	3

—

—