Opentrons
for Education

Prep Sheet

Lab Module 10: *E. coli* Transformation and Recovery

Dr. Cătălin Voiniciuc, Dr. Moni Qiande, and Abigail Lin

University of Florida

Getting Started

This lesson plan uses values from an Excel template to customize the *E. coli* Transformation and Recovery protocol . Directions are included in this lesson plan to create and use the Excel template. Values from the template are copied and pasted into labeled sections of the Python protocol in a code editing program. Questions about using the Excel template for protocol customization can be directed to cvoiniciuc@ufl.edu.

This course does not require previous coding experience. For guidance working with Python code in this Opentrons protocol, you can refer to the following resources:

- ☐ Python Protocol API Tutorial
- ☐ Python Protocol API - Labware
- ☐ Python Protocol API- Temperature Module
- ☐ Python Protocol API- Loading Labware on Adapters

Additional Support and Resources

OT-2 Manual
Temperature Module video
Running a protocol on the OT-2

For technical support, please 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

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Contents

Included in this document are the following sections:

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

Purpose

In this lab, students transform their assembled plasmids directly into *E. coli* cells. With the HEPA Module on, the OT-2 automates sterile handling, DNA mixing, and incubation of chemically competent *E. coli* cells.

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

- *E. coli* culture, including transformation and recovery
- Sterile laboratory work
- Automation of sterile handling pipetting tasks

Core Competencies

Laboratory Skills:

- *E. coli* transformation and recovery
- Sterile laboratory techniques

Automation Skills:

- Automation of sterile liquid handling tasks

Background Knowledge

Students should begin this lab with an understanding of the cloning workflow and the results of their “one-pot” digestion and ligation. An included pre-lab reading introduces the transformation and recovery process students will complete in this lab. *No coding experience is required for this lab,* but students and/or instructors will need to edit a Python protocol file.

Supplies

Opentrons Equipment

- [ ] OT-2 automated liquid handling robot
- [ ] 2x Temperature Module (in deck slots 3 and 4)
- [ ] OT-2 P300 Single-Channel GEN2 pipette (in right mount)
- [ ] OT-2 HEPA Module

Opentrons Protocol

- [ ] *E. coli* Transformation and Recovery protocol

Non-Opentrons Equipment

- [ ] Bacterial culture equipment (inoculating loops, 37 °C shaking incubator)

Labware

- [ ] Opentrons 24 Well Aluminum Block with NEST 1.5 mL Snapcap tubes in deck slot 1
- [ ] 2x Opentrons 96 Well Aluminum Block with NEST Well Plate 100 uL (one on the Temperature Module in deck slot 4, one in deck slot 2)

- ☐ Opentrons 10 Tube Rack with Falcon 4x50 mL, 6x15 mL Conical tubes in deck slot 6
- ☐ Opentrons OT-2 96 Tip Rack 300 µL in deck slot 9

Reagents and Other Materials

- ☐ Assembled DNA plasmids (in the 96-well “post-reaction plate” directly from MoClo reaction)
- ☐ Chemically competent *E. coli* cells, such as NEB® 5-alpha Competent E. coli (in the 1.5 mL snapcap tubes on the aluminum block in deck slot 1)
- ☐ LsLB media with appropriate antibiotics (in a 4x50 mL Conical in deck slot 6)
- ☐ 70% ethanol for cleaning
- ☐ Air-permeable filters for PCR plates such as Breathe-Easy sealing membrane
- ☐ LB agar plates with appropriate antibiotics

Experimental Duration

Required Class Sessions

1

Lab Run Time

This lesson plan was prepared for a traditional laboratory class time of 80-90 minutes. Recovered *E. coli* cells containing transformed plasmids should be incubated at 37 °C until the following class. To save time in class and ensure smooth protocol editing, instructors may wish to create an Excel template according to the directions included in this lesson plan before class. See the **Protocol Prep** section in the **Procedure Guide** for detailed instructions.

Basic Troubleshooting and Tips

- We recommend completing a trial run of the protocol required for this lesson plan prior to class. On the OT-2 robot, this trial run can be completed with or without tips.

- When using this protocol to aspirate and dispense LB medium, you may need to adjust the aspirate height.

- Competent cells are extremely sensitive to temperature fluctuations. Be sure to check the manufacturer temperature recommendations for your competent cells of choice.

- If time is a concern, the heat shock process can be sped up by swapping the pre-chilled aluminum block with one that is at room temperature. With this option, that aluminum block will need to be swapped again afterwards to help the Temperature Module quickly reach 4 °C again. However, this protocol works without any swapping of the aluminum blocks.

Procedure Guide

Before Class

1. Prepare a sufficient amount of LsLB media (for cell recovery on the OT-2 deck) and sufficient LB agar plates with appropriate antibiotic (for manually plating recovered cells).
2. *Optional:* Prepare an Excel template for students to use for protocol customization according to the **Protocol Prep** instructions below.

3. Pre-chill an Opentrons 24-well aluminum block with NEST 1.5 mL snapcap tubes (for holding competent cells on the deck) and an Opentrons 96-well NEST 100 µL plate at -20 °C (for holding assembled plasmids on the deck).

Protocol Prep

1. Turn the HEPA Module on.
2. Direct a student to wipe the interior of the robot with 70% ethanol.
3. Keep the HEPA Module on for 30 minutes at maximum level.
4. While the HEPA Module runs, direct students to set up an Excel spreadsheet with the following tables, shown in both screenshots and written descriptions. *Note:* this spreadsheet differs from your previous Excel template in that additional tables will be created for specific volume transfers in the protocol.
    a. First, create a “green table.” This table includes the **initial volumes** you will put into wells. The table, as shown below, should include the labware name, initial well location, initial volume in the well (500 µL), and a name, description, and color for each liquid.

Step 1: Put columns B-F into "csv_volume_data_raw" This is how you tell the robot how much liquid is initially in the wells.

Labware	Initial_Wells	Initial_Volume	Liquid_Name	Description	Color
tube_rack	A4	0.5	LsLB	LsLB for recovery	#FFD966
tube_rack2	A1	0.5	Competent cell	Competent cell	#FDF1BC
temp_PCR_tube	A1	0.0025	Assembled plasmid product	DNA sample	#00FF00
temp_PCR_tube	B1	0.0025	Assembled plasmid product	DNA sample	#00FF00

b. Next, set up a “blue table.” This table includes the **liquid transfer steps** to mix competent cells with the assembled plasmids (MoClo products). The table, as shown below, should include the liquid source (labware and wells, where the OT-2 will aspirate liquid from) and liquid destination (labware and wells, where the OT-2 will dispense liquid into), as well as the transfer volume. Here, the Pick_Up_Tip data determines whether or not the OT-2 will select a new tip for this step.

Step 2: Put columns H-L into "csv_transfer_competent_cell_raw". Volume is in uL. This is the table for transferring liquid.

Source_Labware	Source_Well	Destination_Labware	Destination_Well	Transfer_Volum	Pick_Up_Tip
tube_rack2	A1	temp_PCR_tube	A1	15	TRUE
tube_rack2	A1	temp_PCR_tube	B1	15	TRUE

c. Next, set up an “orange table.” This table includes the **LsLB media transfer steps** to add media to the competent cells + assembled plasmid mixture. The table, as shown below, should include the liquid

source (labware and wells, where the OT-2 will aspirate liquid from) and liquid destination (labware and wells, where the OT-2 will dispense liquid into), as well as the transfer volume. Here, the Pick_Up_Tip data determines whether or not the OT-2 will select a new tip for this step.

Step 3: Put columns H-L into "csv_transfer_LB_raw". Volume is in uL. This is the table for transferring liquid.	Source_Labware	Source_Well	Destination_Labware	Destination_Well	Transfer_Volum	Pick_Up_Tip
	tube_rack	A4	temp_PCR_tube	A1	150	TRUE
	tube_rack	A4	temp_PCR_tube	B1	150	TRUE

5. Download and open the *E. coli* Transformation and Recovery protocol in a code editing program.
6. Copy and paste the data from your Excel spreadsheet into the code sections specified to modify the protocol for your experiment.
    a. As needed, update the starting tip for the protocol (the first available tip the OT-2 should pick up in the tip box; around line 80).
7. Save your final protocol file and import into the Opentrons App.
8. Adjust the HEPA Module speed to two-thirds of the maximum level.
9. Set up your labware and liquids:

b. Opentrons 96 Tip Rack 300 µL in deck slot 9
c. Opentrons 10 Tube Rack with Falcon 4x50 mL in deck slot 6
d. Wipe down your Falcon tube with LsLB media and place it in the correct position in the tube rack in deck slot 6. Open the lid.
e. Remove the Opentrons 24-well aluminum block with 24-well NEST 1.5 mL snapcap tubes from storage at -20 °C. Load your required volume of competent *E. coli* cells into the specified wells and place the block onto the deck in slot 1.
f. Load the Temperature Modules in slots 3 and 4.
g. Remove the NEST 96-well "post-reaction plate" (assembled plasmids directly from MoClo) from 12 °C storage and the pre-chilled Opentrons 96 Well Aluminum Block with NEST Well Plate 100 µL from storage at -20 °C.
h. At least 2.5 µL of assembled plasmids should be in wells of the pre-chilled NEST well plate in deck slot 2. Remind students to take notes on which well their assembled product is in, including a class "plate map."
i. Place the pre-chilled, empty NEST well plate and aluminum block onto the Temperature Module in deck slot 4.

Transformation and Recovery

1. Save your modified *E. coli* Transformation and Recovery protocol. Import the protocol file into the Opentrons App.
    a. Check the setup instructions to confirm hardware, labware, and liquids in the protocol.
2. Run the protocol.
3. The OT-2 will set the Temperature Module in slot 4 to 4 °C.
4. The OT-2 will aspirate 15 µL from the NEST 1.5 mL snapcap tube holding competent cells and dispense into well A1 (or other specified well) of the NEST well plate on the Temperature Module in deck slot 4.
    a. The OT-2 will repeat this for well B1, and any other specified wells.
5. The OT-2 will aspirate 2.5 uL of DNA samples from the NEST well plate in deck slot 2 and dispense into the same wells of the NEST well plate on the Temperature Module in deck slot 4, combining competent cells and plasmid DNA samples.
6. The OT-2 will pause for 5 minutes (remaining at 4 °C).
7. The protocol will pause. Manually move the NEST plate on the Temperature Module in deck slot 4 to the Temperature Module in deck slot 3.
8. The OT-2 will set the second Temperature Module in deck slot 3 to 42 °C for a 40 second heat shock.

9. The protocol will pause. Manually move the NEST plate from the Temperature Module in deck slot 3 back to the Temperature Module in deck slot 4.
10. The OT-2 will set the Temperature Module in deck slot 4 to 4 °C for the 5 minute recovery.
11. The OT-2 will aspirate 150 µL from the Falcon tube with LsLB media and dispense into well A1 (or other specified well) of the 96-well plate on the Temperature Module in deck slot 4, combining transformed/recovered cells with media for recovery.
12. The OT-2 will pause with a reminder to put an air-permeable filter on the NEST well plate on the Temperature Module in deck slot 4.
13. Manually move the NEST well plate in the Temperature Module in deck slot 4 to the second Temperature Module in deck slot 3.
14. The OT-2 will set the temperature to 37 °C for the 1 hour recovery.
15. The OT-2 will deactivate the Temperature Module and end the protocol.
16. Students should manually remove the NEST well plate from the Temperature Module and streak their recovered *E.coli* cells with assembled plasmids on LB agar plates with appropriate antibiotic added.
17. Incubate recovered *E. coli* culture at 37 °C.

Discussion Questions

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

- In your own words, what happens during a *transformation* like the OT-2 automated in class today?
- Why do cells need to recover after a transformation?
- If you had more (or less) different kinds of plasmids to transform today, could you modify the protocol to automate this?

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Student Guide

Lab Module 10: *E. coli* Transformation and Recovery

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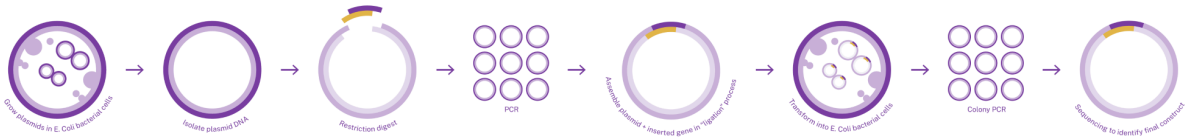
Pre-Lab Reading

At the beginning of the semester, you isolated your plasmid DNA from *E. coli* bacterial cultures. As you learned, *E. coli* culture allows you to store, maintain, and eventually obtain more copies of your plasmid through growth and isolation. Today, you'll work in the opposite direction. Let's take a look at the cloning workflow:



After the last class, you now have assembled plasmid products- your plasmid backbone and your now-inserted gene of interest! Now, we will *transform* those assembled plasmids into *E. coli. Transformation* is a process by which foreign DNA, such as your plasmid, is introduced into a cell. However, most cells are somewhat resistant to taking up foreign DNA- this helps to protect them from dangers like infection. Therefore, special cells, known as *competent cells,* are used in transformation.

Competent cells are most commonly ordered from commercial sources for use in the lab. These cells have undergone treatments to change the permeability of the cell membrane and wall, making it easier for the DNA to enter the

cell. Non-competent cells will not “take up” plasmids- in other words, will not allow them to enter the cell. Once these competent cells are recieved, two common methods are used for transformation: *electroporation* or *heat shock.* Electroporation involves applying electrical pulses to the competent cells and plasmids with a special piece of equipment called an *electroporator.* In a heat shock, the temperature is increased for a set amount of time. Either of these methods cause changes to the *membrane potential* of a cell, or the differences in electrical potential between the inside of a cell and the outside environment. When the membrane potential is changed, it makes it easier for a charged molecule, such as a DNA molecule, to move inside the cell.

After transformation, cells need a period of recovery. Recovery typically involves giving cells time to incubate in media, at a temperature cells prefer (such as 37°C). The recovery period also closes pores in the cell wall created by competency treatments and heat shock (or electroporation). Now, the foreign plasmid DNA is sealed inside the cell, and the cell is recovered.

In this class, you’ll use a heat shock on competent cells to *transform* your assembled plasmids into *E. coli.* Allowing those *E. coli* cells to grow will generate more copies of your plasmid in colonies, and set you up for *colony PCR* in the next class.

To read more about competent cells and transformation methods, you can read this GoldBio article: [Understanding Competent Cells for Bacterial Transformation | GoldBio](#).

Purpose

In this lab, you'll transform assembled plasmids (from Lab Module 9) directly into *chemically competent E. coli cells.* With the HEPA Module on, the OT-2 automates sterile handling, DNA mixing, and incubation of your cells for transformation and recovery.

Learning Outcomes

- Gain experience with *E. coli* culture, including transformation and recovery
- Understand considerations for sterile laboratory work
- Automate sterile handling pipetting tasks

Supplies

Opentrons Equipment

- [ ] OT-2 automated liquid handling robot
- [ ] 2x Temperature Module (in deck slots 3 and 4)
- [ ] OT-2 P300 Single-Channel GEN2 pipette (in right mount)
- [ ] OT-2 HEPA Module

Opentrons Protocol

- [ ] *E. coli* Transformation and Recovery protocol

Non-Opentrons Equipment

- [ ] Bacterial culture equipment (inoculating loops, 37 °C shaking incubator)

Labware

- [ ] Opentrons 24 Well Aluminum Block with NEST 1.5 mL Snapcap tubes in deck slot 1
- [ ] 2x Opentrons 96 Well Aluminum Block with NEST Well Plate 100 uL (one on the Temperature Module in deck slot 4, one in deck slot 2)

- ☐ Opentrons 10 Tube Rack with Falcon 4x50 mL, 6x15 mL Conical tubes in deck slot 6
- ☐ Opentrons OT-2 96 Tip Rack 300 µL in deck slot 9

Reagents and Other Materials

- ☐ Assembled DNA plasmids (in the 96-well "post-reaction plate" directly from MoClo reaction)
- ☐ Chemically competent *E. coli* cells, such as NEB® 5-alpha Competent E. coli (in the 1.5 mL snapcap tubes on the aluminum block in deck slot 1)
- ☐ LsLB media with appropriate antibiotics (in a 4x50 mL Conical in deck slot 6)
- ☐ 70% ethanol for cleaning
- ☐ Air-permeable filters for PCR plates such as Breathe-Easy sealing membrane
- ☐ LB agar plates with appropriate antibiotics

Procedure Guide

Before Class

1. You or your instructor should prepare a sufficient amount of LsLB media with appropriate antibiotics.

2. Pre-chill an Opentrons 24-well aluminum block with NEST 1.5 mL snapcap tubes (for holding competent cells on the deck) and an Opentrons 96-well NEST 100 µL plate at -20 °C (for holding assembled plasmids on the deck).

Protocol Prep

1. You or your instructor should turn the HEPA Module on. The HEPA Module should stay on for 30 minutes at maximum level.
2. A student will wipe the interior of the robot with 70% ethanol.
3. While the HEPA Module runs, set up an Excel spreadsheet with the following tables, shown in both screenshots and written descriptions. If your instructor has already set up an Excel spreadsheet for the class, follow the directions to determine the information that you'll enter into your spreadsheet.
    a. First, create a "green table." This table includes the **initial volumes** you will put into wells. The table, as shown below, should include the labware name, initial well location, initial volume in the well (500 uL), and a name, description, and color for each liquid.

Step 1: Put columns B-F into "csv_volume_data_raw" This is how you tell the robot how much liquid is initially in the wells.

Labware	Initial_Wells	Initial_Wells	Liquid_Name	Description	Color
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tube_rack2	A1	0.5	Competent cell	Competent cell	#FDF1BC

b. Next, set up a "blue table." This table includes the **liquid transfer steps** to mix competent cells with the assembled plasmids (MoClo products). The table, as shown below, should include the liquid source (labware and wells, where the OT-2 will aspirate liquid from) and liquid destination (labware and wells, where the OT-2 will dispense liquid into), as well as the transfer volume. Here, the Pick_Up_Tip data determines whether or not the OT-2 will select a new tip for this step.

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6. Save your final protocol file and import into the Opentrons App.
7. You or your instructor should adjust the HEPA Module speed to two-thirds of the maximum level.
8. Set up your labware and liquids:
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c. Opentrons 10 Tube Rack with Falcon 4x50 mL in deck slot 6
d. Wipe down your Falcon tube with LsLB media and place it in the correct position in the tube rack in deck slot 6. Open the lid.
e. Remove the Opentrons 24-well aluminum block with 24-well NEST 1.5 mL snapcap tubes from storage at -20 °C. Load your required volume of competent *E. coli* cells into the specified wells and place the block onto the deck in slot 1.
f. Load the Temperature Modules in slots 3 and 4.
g. Remove the NEST 96-well “post-reaction plate” (assembled plasmids directly from MoClo) from 12 °C storage and the pre-chilled Opentrons 96 Well Aluminum Block with NEST Well Plate 100 µL from storage at -20 °C.
h. At least 2.5 µL of assembled plasmids should be in wells of the pre-chilled NEST well plate in deck slot 2. Remind students to take notes on which well their assembled product is in, including a class “plate map.”
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17. Incubate recovered *E. coli* culture at 37 °C.

Discussion Questions

Discuss the lab activities with a neighbor.

- In your own words, what happens during a *transformation* like the OT-2 automated in class today?
- Why do cells need to recover after a transformation?
- If you had more (or less) different kinds of plasmids to transform today, could you modify the protocol to automate this?