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Worksheet

E. coli Transformation and Recovery

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Purpose

This worksheet is designed to guide high school students in the use of a Python protocol for *E. coli* transformation and recovery, written for the Opentrons OT-2 liquid handling robot.

Materials in this worksheet are adapted from Lab Module 10 of the Methods in Modern Plant Biology Course , developed by Dr. Cătălin Voiniciuc, Dr. Moni Qiande, and Abigail Lin at the University of Florida. Instructors can download the full Lab Module 10 lesson plan, including detailed instructions, troubleshooting help, and a pre-lab reading and discussion questions for students.

Background

Bacterial cells, like *Escherichia coli* (*E. coli*) allow scientists to store, maintain, and eventually isolate more copies of DNA. In this lab, you'll *transform* newly assembled plasmids (from a previous lab) into *E. coli* cells.

Transformation is a process by which new DNA, like your assembled plasmids, are introduced into a cell. Most cells are somewhat resistant to new, or 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 commonly ordered from commercial sources for use in the lab. These bacterial cells have undergone treatments to change the *permeability* of the cell membrane and wall, making it easier for the DNA to enter the cell. In addition, two methods are commonly used to "encourage" the cells to take up your DNA:

- **Electroporation:** applying electrical pulses to the competent cells and plasmids with an *electroporator.*
- **Heat shock:** increasing the temperature for a set amount of time.

After transformation, cells need a period of recovery. This typically involves giving cells time to incubate in media at a preferred temperature (like 37 °C).

In this lab, you'll use a Python protocol to automate heat shock on competent cells to *transform* your assembled plasmids into *E. coli* cells on the OT-2. Afterwards, these *E. coli* cells can be tested (to make sure your plasmid is inside the cells) and grown (to get more copies of your plasmid).

Materials

- [ ] Opentrons OT-2 automated liquid handling robot
- [ ] OT-2 P300 Single-Channel GEN2 pipette (in right mount)
- [ ] 2x Temperature Module GEN2 (in deck slots 3 and 4)
- [ ] Opentrons OT-2 96 Tip Rack 300 µL (in deck slot 9)
- [ ] Opentrons OT-2 HEPA Module (optional)
- [ ] 2x Opentrons 96 Well Aluminum Block with NEST Well Plate 100 µL (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 24 Well Aluminum Block with NEST 1.5 mL Snapcap tubes (in deck slot 1)
- [ ] Opentrons *E. coli* Transformation and Recovery Python protocol
- [ ] Bacterial culture equipment (inoculating loops, agar plates, 37 °C shaking incubator)
- [ ] Assembled DNA plasmids (directly from a modular cloning, or MoClo, reaction)

- ☐ Chemically competent *E. coli* cells, such as NEB® 5-alpha competent E. coli (in the 1.5 mL snapcap tubes in the aluminum block in deck slot 1)
- ☐ LB media and agar plates with appropriate antibiotics (in a 50 mL conical tube in deck slot 6)
- ☐ 70% ethanol for cleaning
- ☐ Air-permeable filters for PCR plates such as Breathe-Easy sealing membrane (optional)

E. coli Transformation and Recovery

Before Lab

1. Prepare enough LB media and agar plates with appropriate antibiotics for your experiment.
2. Pre-chill an Opentrons 24-well aluminum block with NEST 1.5 mL snapcap tubes (for keeping competent cells cold on the deck) and an Opentrons 96-well NEST 100 µL plate (for keeping assembled plasmids cold on the deck) in a freezer at -20 °C.

Protocol Preparation

1. Wipe the OT-2 interior with 70% ethanol.

2. Set up an Excel spreadsheet according to the instructions below:
    - First, create a “green table.” This table includes the **initial liquid volumes** you will start with on the OT-2 deck. The table should include well location, initial volumes in the well (in 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_competent_cell	A1	180	competent cell	competent cell	#d9ead3
tube_rack_LB	A4	2000	LB + antibiotics	LB + antibiotics	#d3e6ea
DNA_assembly_Plate	A1	10	DNA_Sample	DNA_Sample	#e9d3ea
DNA_assembly_Plate	B1	10	DNA_Sample	DNA_Sample	#e9d3ea
DNA_assembly_Plate	C1	10	DNA_Sample	DNA_Sample	#e9d3ea
DNA_assembly_Plate	D1	10	DNA_Sample	DNA_Sample	#e9d3ea

    - Next, set up a “blue table.” This table includes the **liquid transfer steps** to mix competent cells with your plasmids (DNA). This table, shown below, should include liquid source (labware and wells the OT-2 will aspirate liquid from) and liquid destination (labware and wells the OT-2 will dispense liquid into), as well as the transfer volume. Here, the `Pick_Up_Tip` data determines where the OT-2 will select a new tip for each step. We recommend entering TRUE to keep your DNA samples “clean.”

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_Volume	Pick_Up_Tip
tube_rack_competent_cell	A1	well_plate	A1	15	TRUE
tube_rack_competent_cell	A1	well_plate	B1	15	TRUE
tube_rack_competent_cell	A1	well_plate	C1	15	TRUE
tube_rack_competent_cell	A1	well_plate	D1	15	TRUE

- Next, set up an “orange table.” This table includes the **LB media transfer steps** to add media to your competent cells + plasmid mixture. The table, as shown below, should include the liquid source (labware and wells the OT-2 will aspirate liquid from) and liquid destination (labware and wells the OT-2 will dispense liquid into), as well as the transfer volume. Again, the `Pick_Up_Tip` data determines whether or not the OT-2 will select a new tip for this step.

	Source_Labware	Source_Well	Destination_Labware	Destination_Well	Transfer_Volume	Pick_Up_Tip
Step 3: Put columns H-L into "csv_transfer_LB_raw". Volume is in uL. This is the table for transferring liquid.	tube_rack_LB	A4	well_plate	A1	20	TRUE
	tube_rack_LB	A4	well_plate	B1	20	TRUE
	tube_rack_LB	A4	well_plate	C1	20	TRUE
	tube_rack_LB	A4	well_plate	D1	20	TRUE

3. Download and open the *E. coli* Transformation and Recovery protocol in a code editing program like Microsoft VSCode.
    - Copy and paste the data from your Excel spreadsheet into the code sections specified (like `csv_transfer_LB_raw`) at the top of the protocol.
    - If you won’t be using a full box of tips in your protocol, update the starting tip for the protocol.

        - Search for the word “start” in your protocol (Ctrl + F).
        - Around line 97 of code, replace ‘A1’ in `s20_pip.starting_tip = tips.well('A1')`. Tips are labeled A1 to H12, just like a 96-well plate.
        - Save your final protocol file and import into the Opentrons App.
4. In the Opentrons App, choose your protocol and click **Start setup.** Follow the instructions to set up your labware and liquids on the OT-2 deck.
    - This protocol uses multiple different liquids, modules, and labware types. Use the attached **Notes** sheet to make your own drawing of how the deck should look at setup. This can help you prepare and prevent temperature-sensitive liquids from staying at room temperature for too long during deck setup.
    - If using the OT-2 HEPA Module, adjust the speed to two-thirds of the maximum level.

Transformation and Recovery

1. Run your protocol.
2. The OT-2 will set the Temperature Module (in deck slot 4) to 4 °C.

3. The pipette will transfer competent cells to the NEST well plate on the Temperature Module (in deck slot 4).
    a. *The OT-2 will repeat this for as many wells as you included in your Excel sheet.*
4. The pipette will transfer DNA samples from the NEST well plate (in deck slot 2) to the NEST well plate on the Temperature Module (in deck slot 4)
    a. *Make sure your DNA samples are sufficiently thawed!*
5. The protocol will pause for 5 minutes.
    a. Then, the protocol will pause so you can move the NEST well plate on the Temperature Module (in deck slot 4) to the Temperature Module (in deck slot 3). *Click in the Opentrons App to resume your protocol.*
6. Your DNA and competent cell mixture incubates for 5 minutes on the Temperature module at 4 °C.
7. The OT-2 will set the second Temperature Module (in deck slot 3) to 42 °C for a 40 second heat shock.
8. The protocol will pause again. Manually move the NEST well plate from the Temperature Module (deck slot 3) back to the Temperature Module (deck slot 4).
9. The OT-2 will set the Temperature Module (deck slot 4) to 4 °C for a 5 minute recovery.
10. The pipette will transfer LB broth from the conical tube to the NEST well plate on the Temperature Module (deck

slot 4); combining transformed and recovered cells with media for recovery.

11. The protocol will pause. If you need to add more LB broth, you can add more manually (up to the maximum volume in the well plate).
    a. (optional) The pause includes a reminder to put an air-permeable filter on the NEST well plate on the Temperature Module (deck slot 4), if you choose to use one. *Click to resume your protocol.*
12. The protocol will pause so you can move your NEST well plate (Temperature Module; deck slot 4) back to the Temperature Module in deck slot 3.
13. The OT-2 will set the Temperature Module to 37 °C for a 1 hour recovery.
14. After 1 hour, the OT-2 will deactivate the Temperature Module and end the protocol.
15. Manually remove the NEST well plate from the Temperature Module and follow your instructor's directions to streak the recovered *E. coli* cells on LB agar plates with appropriate antibiotic added.
16. Incubate your *E. coli* plates at 37 °C.

Notes

Use the back side of this sheet to make your own drawing of how the deck should look at setup. Be sure to label the OT-2's deck slots and add:

- Where each Temperature Module should sit on the deck
- Labware that starts on each Temperature Module and labware that starts on the deck
- Where to load your liquids (be specific with the wells you chose in your Excel sheet!)

Click on your protocol in the Opentrons App. Use the **Timeline** to try making your own list of the steps the OT-2 will perform. You can use the steps in this worksheet's **Transformation and Recovery** section as a guide, but they won't be specific for the wells and volumes you chose. Label steps at key points in the process:

- When do *heat shock* steps take place? What do different temperature settings during the protocol represent?
- When are cells considered *recovered?* When do your assembled plasmids "enter" the *E. coli* cells?
- Why would you put an air-permeable filter on a plate with cells in it?