Opentrons
for Education

Worksheet

DNA Assembly

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Opentrons

Purpose

This worksheet is designed to guide high school students in the use of a Python protocol to automate DNA assembly on the Opentrons OT-2 liquid handling robot. The protocol relies on the popular Golden Gate modular cloning (MoClo) assembly method.

Material in this worksheet has been modified based on Lab Module 9 of the Methods in Modern Plant Biology Course, developed by Dr. Cătălin Voiniciuc, Dr. Moni Qiande, and Abigail Lin for undergraduate students at the University of Florida. Instructors can download the full Lab Module 9 lesson plan, including detailed instructions, troubleshooting help, and a pre-lab reading and discussion questions for students.

Background

In today's lab, you'll perform a Golden Gate assembly. The *Golden Gate* and *modular cloning (MoClo)* methods are a particular type of cloning known as *hierarchical assembly,* in which you can complete multiple steps of cloning to build larger and larger molecular constructs.

During each assembly step, you can use a “one-pot” digestion and ligation to combine two steps of the assembly process into one:

- **Digestion:** cutting DNA pieces with a specific restriction enzyme. Both your plasmid backbone (destination vector) and gene of interest to be inserted need to contain compatible restriction enzyme sites- so the enzymes know where to “cut” the DNA.
- **Ligation:** sealing together cut pieces of DNA into a single assembled plasmid.

You’ll use a Python protocol to 1) combine the “parts” of your reaction on the OT-2 and 2) complete your “one-pot” reaction in a thermocycler.

To learn more about MoClo and Golden Gate cloning, you can read the following articles:
Modular Cloning (MoClo) Guide
A User's Guide to Golden Gate Cloning Methods and Standards

Materials

- ☐ OT-2 automated liquid handling robot
- ☐ OT-2 P20 Single-Channel GEN2 pipette (in left mount)

- ☐ Temperature Module GEN2 (in deck slot 3)
- ☐ Opentrons OT-2 96 Filter Tip Rack 20 µL (in deck slot 1)
- ☐ 96-well Aluminum Block with PCR Strip 200 µL (in the Temperature Module in deck slot 3)
- ☐ Opentrons 24 Tube Rack with NEST 1.5 mL Snapcap tubes (in deck slot 5)
- ☐ Opentrons MoClo Assembly Preparation protocol
- ☐ Benchtop thermocycler
- ☐ Amplified plasmid backbone and DNA insert
- ☐ Restriction enzymes
- ☐ DNA ligase and ligase buffer
- ☐ Molecular grade water
- ☐ Other reagents, like ATPs, as needed

DNA Assembly

Reaction Component Assembly

1. Follow your instructor's directions to manually assemble your master mix with enzyme(s), DNA ligase, buffer(s), and molecular grade water.

Protocol Preparation

1. Set up an Excel spreadsheet according to the instructions below:
    a. 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.

	Labware	Initial_Wells	Initial_Volume	Liquid_Name	Description	Color
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.	tube_rack	A1	120	master mix	enzyme, buffer, gene insert, etc.	#00FF00
	tube_rack	A2	10	Promoter 1	Promoter 1	#d3e6ea
	tube_rack	A3	10	Promoter 2	Promoter 2	#d3e6ea
	tube_rack	A4	10	Promoter 3	Promoter 3	#d3e6ea
	tube_rack	A5	10	Promoter 4	Promoter 4	#d3e6ea
	tube_rack	A6	10	Promoter 5	Promoter 5	#d3e6ea
	tube_rack	B1	10	Promoter 6	Promoter 6	#d3e6ea
	tube_rack	B2	10	Promoter 7	Promoter 7	#d3e6ea
	tube_rack	B3	10	Promoter 8	Promoter 8	#d3e6ea
	tube_rack	B4	10	Promoter 9	Promoter 9	#d3e6ea
	tube_rack	B5	10	Promoter 10	Promoter 10	#d3e6ea
	tube_rack	B6	10	Promoter 11	Promoter 11	#d3e6ea

    b. 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_data_raw". Volume is in uL. This is the table for transferring liquid.

Source_Labware	Source_Well	Destination_Lab	Destination_Well	Transfer_Volume	Pick_Up_Tip
tube_rack	A1	well_plate	A1	9	TRUE
tube_rack	A1	well_plate	B1	9	FALSE
tube_rack	A1	well_plate	C1	9	FALSE
tube_rack	A1	well_plate	D1	9	FALSE
tube_rack	A1	well_plate	E1	9	FALSE
tube_rack	A1	well_plate	F1	9	FALSE
tube_rack	A1	well_plate	G1	9	FALSE
tube_rack	A1	well_plate	H1	9	FALSE
tube_rack	A1	well_plate	A2	9	FALSE
tube_rack	A1	well_plate	B2	9	FALSE
tube_rack	A1	well_plate	C2	9	FALSE
tube_rack	A1	well_plate	D2	1	TRUE
tube_rack	A2	well_plate	A1	1	TRUE
tube_rack	A3	well_plate	B1	1	TRUE
tube_rack	A4	well_plate	C1	1	TRUE
tube_rack	A5	well_plate	D1	1	TRUE
tube_rack	A6	well_plate	E1	1	TRUE
tube_rack	B1	well_plate	F1	1	TRUE
tube_rack	B2	well_plate	G1	1	TRUE
tube_rack	B3	well_plate	H1	1	TRUE
tube_rack	B4	well_plate	A2	1	TRUE
tube_rack	B5	well_plate	B2	1	TRUE
tube_rack	B6	well_plate	C2	1	TRUE

2. Download and open your Python protocol (MoClo Assembly Preparation) in a code editing program like Microsoft VSCode.

- Copy and paste the data from your Excel spreadsheet into the code sections specified (like `csv_volume_data_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 140 of code, replace 'A1' in `s20_pip.starting_tip = tips.well('A1')`. Tips are labeled A1 to H12, just like a 96-well plate.
- It's easy to customize your protocol for your own temperatures! Around line 100 of code, you can change the temperature (in °C) that the Temperature Module will hold your samples at during pipetting.
  - For more details, see the Temperature Module section of Opentrons' Python API documentation.
- Save your final protocol and import it into the Opentrons App.

3. 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:

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.

DNA Assembly

1. Run your protocol.
2. The P20 pipette will transfer master mix and DNA to the well plate on the Temperature Module.
3. When the protocol is complete, manually remove the PCR strip tubes from the Temperature Module.

“One-Pot” Digestion and Ligation

1. Gently mix your PCR tubes by flicking the bottom of each.
2. Place the PCR strip tubes in the benchtop thermocycler and follow your instructor’s directions to set up your reaction. You can fill in the table below with your reaction conditions to help you keep track (we’ve included a few hints for you):

Temperature	Time (min)	# of Cycles	Notes
			Final digest
			Inactivation
			Sample storage

3. Store your final products at 4 °C (or -20 °C for longer storage).

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 labware should be placed 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. Label steps at key points in the process:

- What temperature is the Temperature Module set at? Why?
- Which liquids are being combined?