Opentrons
for Education

Introduction to the Course
Methods in Modern Plant Biology

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Welcome to the Methods in Modern Plant Biology course, developed by Cătălin Voiniciuc, Dr. Moni Qiande, and Abigail Lin for undergraduate students at the University of Florida.

At a glance

Level	Undergraduate (sophomore - senior)
Class time	1 semester (13 weeks)
Number of students	12
Student : robot ratio	12:1
Content	12 plant molecular biology labs
Protocol types	Protocol Designer, Python protocols (no coding experience required)

Course overview

This laboratory course builds student knowledge and confidence in both modern molecular biology techniques and the use of automation in a laboratory setting.

Students can work in lab pairs or individually to complete activities covering a wide range of molecular techniques, including work with plasmids, modular cloning, *E. coli* work and transformation, and DNA purification and preparation techniques. The experiments and activities in this course are designed to guide student learning in advanced topics, including primer and experiment design, cloning, troubleshooting, and analyzing results. Most lesson plans include options for customization in your classroom, allowing for differences in available equipment, class time, and desired student learning outcomes. For example, cloning in this course incorporates selection markers into a set of suggested plasmid backbones; however, instructors can create their own workflow to include more or less genetic components, or specific genes of interest. A wide variety of kits for cloning, including the Golden Gate "MoClo" workflow used in this course, are also available from Addgene and others.

During each lab, students will use protocols developed for use on the OT-2 robot by Dr. Voiniciuc and the Opentrons team. Here, automation not only reduces the amount of pipetting work students do, but also increases accuracy and reproducibility. Reduced hands-on time during pipetting tasks allows students to develop additional laboratory skills, including experimental design and troubleshooting.

Despite the use of Python protocols in the course, *absolutely no coding experience* is required from students. Early laboratory activities will demonstrate viewing and running Python protocols on the OT-2, with comparison to protocols developed in the no-code, user-friendly Opentrons Protocol Designer.

Lab Module	**Description**
Module 1: Introduction to Automation	Introduction to the OT-2, Opentrons Protocol Library, and Opentrons Protocol Designer
Module 2: Automating Laboratory Work with the OT-2	Compare Python and Protocol Designer protocols; complete a Bradford BSA assay (side-by-side manual and OT-2 pipetting)
Module 3: Assembling Your Molecular Toolkit	Isolate plasmid DNA in a spin column purification; explore Agrobacterium selection markers with the OT-2
Module 4: DNA Normalization	Prepare for cloning assembly with the OT-2 (DNA normalization)

Module 5: Restriction Enzyme Digest	Plan a restriction digest and anticipate results while the OT-2 pipettes
Module 6: Restriction Digest Analysis	Analyze digest results and “draw” using the OT-2
Module 7: Primer Design for PCR and Cloning	Design and prepare primer stocks for upcoming experiments
Module 8: DNA Amplification with PCR	Plan and execute a PCR experiment using the OT-2
Module 9: DNA Assembly Methods	Modular cloning assembly of plasmids with genes of interest on the OT-2
Module 10: *E. coli* Transformation and Recovery	The OT-2 allows for sterile handling and transformation of plasmids into *E. coli*
Module 11: Genotyping Bacteria with Colony PCR	Colony selection followed by plasmid amplification via the OT-2
Module 12: Sanger Sequencing and Multiple Sequence Alignment	Plasmids and PCR fragments are prepared for sequencing using the OT-2
Module 13: Lab Report	Final lab report

Course materials

This section provides an overview of the equipment, labware, and reagents used in this course. Please refer to individual lab modules for additional details on general reagents and other required materials. An asterisk denotes labware used with a labware library definition.

Opentrons Equipment

- ☐ OT-2 automated liquid handling robot
- ☐ OT-2 P20 single-channel pipette
- ☐ OT-2 P300 single-channel pipette
- ☐ OT-2 P1000 single-channel pipette
- ☐ Temperature Module GEN2
- ☐ Heater-Shaker Module GEN1
- ☐ OT-2 HEPA Module

Opentrons Protocols and Tools

- ☐ Opentrons Protocol Library
- ☐ Opentrons Protocol Designer
- ☐ Pierce Bradford Protein Assay protocol
- ☐ LB Distribution protocol
- ☐ DNA Sample Normalization protocol
- ☐ Restriction Enzyme Digest protocol
- ☐ Primer Dilution protocol
- ☐ PCR Preparation protocol
- ☐ MoClo Assembly Preparation protocol
- ☐ E. coli Transformation and Recovery protocol
- ☐ Colony PCR Preparation protocol
- ☐ Sanger Sequencing Preparation protocol

Non-Opentrons Equipment

- ☐ NanoDrop spectrophotometer
- ☐ Bacterial culture equipment (37 °C shaking incubator, inoculating loops, culture tubes and microwaveable flasks)
- ☐ Tabletop microcentrifuge
- ☐ Benchtop thermocycler
- ☐ Gel electrophoresis equipment (casting tray, well combs, voltage source, gel box, microwave, gel imager)
- ☐ Imaging equipment (for visualizing *Agrobacterium* reporter strains of choice)
- ☐ Plate reader

Labware

- ☐ Opentrons Universal Flat Adapter for Heater-Shaker Module*
- ☐ Opentrons Aluminum Block Set*
- ☐ Opentrons 4-in-1 Tube Rack Set*
- ☐ OT-2 Filter Tip Racks (20 µL, 200 µL, 1000 µL)*
- ☐ OT-2 Tip Racks (300 µL)*
- ☐ Corning 24-well deep well plate (10,000 µL)*
- ☐ Corning 96-well plate (360 µL; flat)*
- ☐ NEST 96-well PCRplate (100 µL; full skirt)*
- ☐ Axygen 1-well reservoir (90 mL)*

- ☐ NEST 12-well reservoir (15 mL)*
- ☐ Generic PCR strip tubes (200 µL)*
- ☐ NEST snapcap tubes (1.5 mL and 2 mL)*
- ☐ NEST screwcap tubes (1.5 mL and 2 mL)*
- ☐ Generic screwcap tubes (2 mL)*
- ☐ Falcon conical tubes (15 mL, 50 mL)*
- ☐ DNAse- and RNAse-free snapcap tubes appropriate for classroom microcentrifuge (1.5 mL, 2 mL)

Reagents and Other Materials

- ☐ Bacterial culture materials (plates, LB media, agarose powder, appropriate antibiotics for selection)
- ☐ *Agrobacterium* reporter strains and chemically competent cells from GoldBio
- ☐ Plant tissues, leaf punches, micropestles, and liquid nitrogen (*optional*)
- ☐ Food coloring
- ☐ Empty vector plasmid backbones from Addgene
- ☐ Plasmids or DNA fragments for insertion into backbones
- ☐ Rainbow Chromprotein Plasmid Set or other preferred colorimetric plasmids
- ☐ QIAprep Spin Miniprep kit or SydLabs spin columns
- ☐ BCA assay reagents (BSA or other protein standard, working reagent dye concentrate)

- ☐ Restriction digest reagents (appropriate restriction enzymes, digestion buffer)
- ☐ Gel electrophoresis reagents (agarose powder, TAE stock solution, loading buffer, GelRed or other gel stain, appropriate molecular weight ladders)
- ☐ Primers for amplification, colony PCR, and Sanger sequencing
- ☐ PCR reagents (dNTPs, DNA polymerase, buffers, and PCR clean-up kit like the QIAquick)
- ☐ DNA ligase and buffer; ATP mix
- ☐ Chemically competent *E. coli* cells
- ☐ Whatman #1 filters; air-permeable filters for PCR plates
- ☐ 96-100% and 70% ethanol
- ☐ Molecular grade and DDI water

Each lab module is written for use with an Opentrons for Education protocol, which can be downloaded from the Opentrons Protocol Library. Instructions for use of these protocols are included in the corresponding lab module.

New to Opentrons?

Opentrons is on a mission to put laboratory automation in the hands of today's students and the educators that shape their futures. You can join our education initiative at Opentrons for Education.

This course uses the Opentrons OT-2 Liquid Handler with two classroom outcomes in mind: first, to allow students to gain valuable skills in automation, and second, to increase reproducibility and save time in classroom experiments. The OT-2 is a bench-top liquid handling robot that fits on half of a standard lab bench. The robot has space for two pipettes, either single or 8-channel, to pipette volumes from 1-1000 uL. In addition, the OT-2 can control Opentrons modules, including thermocycler, heater-shaker, and temperature modules, to handle your classroom workflows smoothly.

The "Course materials" section summarizes modules and recommended labware specified in the protocols which accompany this course. Out of the box, the OT-2 can support any automation-compatible, ANSI/SLAS compliant well plates, in addition to a range of supported labware in our Labware Library. Labware already in your lab can also be used by creating a custom labware definition for the robot in the Opentrons Labware Creator.

In this course, students will gain experience with the use of protocols, or code that directs the robot to perform commands in your experimental workflow. The Opentrons Protocol Library includes protocols designed for use in this course and for other common experiments. Students will also use the no-code, visual Protocol Designer tool to develop their own protocols with ease.