Prep Sheet

Lab Module 8: DNA Amplification with PCR

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 PCR Preparation 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
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
Lab Module 8: DNA Amplification with PCR

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

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Contents

Included in this document are the following sections:

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

This lesson plan includes suggestions to allow for instructor flexibility in student learning outcomes. 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.

Purpose

Students will amplify their DNA via PCR for cloning, building on concepts learned in the previous class (primer design for classical cloning and PCR experiment design). Here, students will assemble master mix manually, and the OT-2 will pipette master mix into a 96-well plate. Thermocycler reactions will be carried out in a bench-top thermocycler. Afterwards, PCR products should be run on an agarose gel, followed by PCR clean-up methods. In this lab class, students gain experience with:

- Design and analysis of PCR experiments
- Experimental planning ahead of complex experiments
- Laboratory automation of pipetting tasks

Core Competencies

Laboratory Skills:

- Design and completion of PCR experiments
- Basics of Golden Gate cloning methods

Automation Skills:

- Automation of pipetting tasks
- Protocol customization

Background Knowledge

Students should begin this lab with an understanding of the basic cloning workflow used in this course, as well as an understanding of the components used in a PCR reaction. The included pre-lab reading introduces specific information about the Golden Gate cloning method and “one-pot” digestion and ligation ahead of the following class. *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
- ☐ OT-2 P-20 Single-Channel GEN2 pipette (in left mount)
- ☐ Temperature Module (in deck slot 3)

Opentrons Protocol

- ☐ PCR Preparation protocol

Non-Opentrons Equipment

- ☐ Benchtop thermocycler
- ☐ Tabletop microcentrifuge

Labware

- ☐ Opentrons OT-2 96 Filter Tip Rack 20 µL in deck slot 1
- ☐ NEST 96 Well Plate 100 uL PCR Full Skirt in deck slot 2
- ☐ Opentrons 24 Well Aluminum Block with NEST 2 mL Snapcap tubes (on the Temperature Module in deck slot 3)

Reagents and Other Materials

- ☐ 10 μM primer working stocks (forward and reverse; diluted from dry oligos in Lab Module 7)
- ☐ 1.5 and 2 mL DNAse- and RNAse free microcentrifuge tubes for PCR clean-up
- ☐ Molecular grade water, such as HyClone Water (sufficient volume for PCR reaction and DNA elution in clean-up)
- ☐ Green HF (high fidelity) Buffer Pack (5X), such as Phusion® HF Buffer Pack, for use with Phusion High Fidelity DNA Polymerase
- ☐ Phusion High Fidelity DNA Polymerase, such as Phusion™ High-Fidelity DNA Polymerases
- ☐ dNTPs mix (10 mM)
- ☐ DNA template to be amplified
- ☐ PCR clean-up kit, such as the QIAquick PCR Purification Kit
- ☐ 96-100% ethanol
- ☐ 3M sodium acetate, pH 5.0

Experimental Duration

Required Class Sessions

1

Lab Run Time

This lesson plan was prepared for a traditional laboratory class time of 80-90 minutes, and includes three major steps: first, the OT-2 combines manually assembled master mix with DNA samples. Next, students amplify their DNA samples via PCR, followed by gel electrophoresis and PCR clean-up. To save time in class, gels should be poured ahead of class. PCR products can be stored at -20 °C until sufficient time is available for gel electrophoresis and PCR clean-up.

Basic Troubleshooting and Tips

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

- Sticky end cloning is used in Golden Gate assembly methods; however, this BiteSize Bio article can be helpful in understanding both sticky and blunt end cloning.

- PCR cleanup of amplified DNA may be appropriate in this lab module ahead of MoClo assembly in the following class. This lesson plan is written to include reagents and methods from the QIAquick PCR Purification Kit . Additional columns from Syd Labs- Spin Columns for Plasmid Miniprep may be helpful. Refer to the manufacturer instructions and relevant handbook for your kit of choice.

- If students are using *standardized parts* (such as MoClo plasmid vectors), the previous miniprep isolation is sufficient, and no further PCR cleanup should be required before the "one-pot" digestion and ligation in the following class.

Procedure Guide

Before Class

1. To save time in class, 0.7% agarose gels should be prepared and poured ahead of time. See Lab Module 6 for detailed agarose gel preparation instructions.
2. Pre-chill a NEST 96 Well Plate (100 uL, PCR Full Skirt) at 4 °C.

3. Add 96-100% ethanol to Buffer PE from the Qiagen kit before use. Follow directions on the bottle label for exact volume.
4. Add 1:250 volume of pH Indicator I to Buffer PB from the Qiagen kit before use (for example, add 120 µL of pH Indicator I to 30 mL Buffer PB). Next, add pH Indicator I to the entire buffer PB.

Lab Introduction

1. Instructors may wish to review the PCR reaction with students. Students should understand *why* the two PCR reactions they completed experimental design for in Lab Module 7 (with the PCR and Primer Design worksheet) are different.

Master Mix Assembly

1. Students should assemble a master mix for their PCR reaction based on their Primer and PCR Design Worksheet (section **A**) from the previous class. The master mix should include:
    - **Green HF Buffer (5X)**; 4 µL
    - **dNTPs** (10 mM); 0.4 µL
    - **Forward and reverse primers** (10 µM working dilution stocks); 1 µL *each*

- **Phusion DNA polymerase**; 0.25 µL
- **Hyclone molecular grade water**; 13.35 µL (to a final master mix volume of 20 µL)

An example table from the worksheet is shown below:

# Reactions and µL needed	**1**	**4**		**Thermocycler Conditions**	**°C**	**min:sec**	
HyClone Water	13.35			1. Initial Denaturation	98	0:30	
Green HF Buffer (5x)	4			2. Denaturation	98	0:10	5x
dNTPs (10 mM)	0.4			3. Annealing	**60**	0:20	
F Primer (10 µM)	1			4. Extension	72	**0:40**	
R Primer (10 µM)	1			5. Denaturation	98	0:10	30x
DNA Template				6. Anneal and Extend	72	**0:40**	
Phusion DNA Polymerase	0.25			7. Final Elongation	72	5:00	
Total	20.00			8. Storage	12	∞	

2. Students should prepare as many tubes as required (our example uses 3 master mix tubes), each containing 2 mL of master mix.
3. Store master mix on ice.

PCR Prep- Master Mix and DNA Sample Pipetting

1. Download and open the PCR Preparation protocol file in a code editing program to customize for your experiment. As written, the protocol will distribute three different master mixes into various wells of a 96-well plate.
2. Using the Excel template created in Lab Module 4, edit your tables as follows:
    a. Edit your "Step 1" table to include your initial volumes of master mix. Here, the protocol can be customized for the number of different master mixes required.

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
	2mL_tubes	A1	2	Master Mix #1	This is master mix #1	#33A4FF
	2mL_tubes	A2	2	Master Mix #2	This is master mix #2	#63FE82
	2mL_tubes	A3	2	Master Mix #3	This is master mix #3	#F5FE63

The example table shown here includes manually assembled master mix in 2mL_tubes (NEST 2 mL snapcap tubes on the temperature module).

b. Next, edit your “Step 2” table to include the transfer steps (from the 2mL_tubes source to your tc_plate, or 96-well plate, destination) and transfer volume.

Step 2: Put columns H-L into "csv_transfer_data_raw". Volume is in uL. This is the table for transferring liquid.	Source_Labwar	Source_Well	Destination_Labware	Destination_Wel	Transfer_Volum	Pick_Up_Tip
	2mL_tubes	A1	tc_plate	A1	12	TRUE
	2mL_tubes	A1	tc_plate	A2	12	FALSE
	2mL_tubes	A1	tc_plate	A3	12	FALSE
	2mL_tubes	A1	tc_plate	A4	12	FALSE
	2mL_tubes	A1	tc_plate	A5	12	FALSE
	2mL_tubes	A1	tc_plate	A6	12	FALSE
	2mL_tubes	A1	tc_plate	A7	12	FALSE
	2mL_tubes	A1	tc_plate	A8	12	FALSE
	2mL_tubes	A1	tc_plate	A9	12	FALSE
	2mL_tubes	A1	tc_plate	A10	12	FALSE
	2mL_tubes	A1	tc_plate	A11	12	FALSE
	2mL_tubes	A1	tc_plate	A12	12	FALSE
	2mL_tubes	A1	tc_plate	B1	12	FALSE
	2mL_tubes	A1	tc_plate	B2	12	FALSE
	2mL_tubes	A1	tc_plate	B3	12	FALSE
	2mL_tubes	A1	tc_plate	B4	12	FALSE
	2mL_tubes	A1	tc_plate	B5	12	FALSE
	2mL_tubes	A1	tc_plate	B6	12	FALSE
	2mL_tubes	A1	tc_plate	B7	12	FALSE
	2mL_tubes	A1	tc_plate	B8	12	FALSE
	2mL_tubes	A1	tc_plate	B9	12	FALSE
	2mL_tubes	A1	tc_plate	B10	12	FALSE
	2mL_tubes	A1	tc_plate	B11	12	FALSE
	2mL_tubes	A1	tc_plate	B12	12	FALSE
	2mL_tubes	A1	tc_plate	C1	12	FALSE
	2mL_tubes	A1	tc_plate	C2	12	FALSE
	2mL_tubes	A1	tc_plate	C3	12	FALSE
	2mL_tubes	A1	tc_plate	C4	12	FALSE
	2mL_tubes	A1	tc_plate	C5	12	FALSE

The example table shown here details transfer of master mix (from 2mL_tubes) to the tc_plate (a NEST 96 well plate in deck slot 2).

3. As in previous lab modules, copy and paste your data tables into the “Modify” sections at the beginning of the protocol.
4. Save your protocol file and import into the Opentrons App.
5. Set up your labware and liquids:
    a. Opentrons OT-2 96 Filter Tip Rack with 20 µL tips in deck slot 1
    b. Pre-chilled NEST 96 Well Plate 100 µL PCR Full Skirt in deck slot 2
    c. Opentrons 24 Well Aluminum Block with NEST 2 mL Snapcap tubes in deck slot 3, on the Temperature Module. The snapcap tubes contain the various master mixes required for the class.
6. Run your modified PCR Preparation protocol.
7. The OT-2 will set the Temperature Module to 4 °C.
8. The OT-2 will dispense 12 µL of chilled master mix to each specified well of the 96-well plate.
9. When the OT-2 is finished with the protocol run, students can remove the 96-well plate and add their DNA samples. Remind students to take notes on which wells contain which samples.

PCR Reaction

1. Students should load the NEST 96-well plate into a benchtop thermocycler.

2. Set the thermocycler reaction steps according to section **A** of the Primer and PCR Design worksheet and run the reaction accordingly.
3. End the program, open the thermocycler lid, and remove the plate.
4. Students should ensure plate is properly labeled and/or take notes including a “plate map.”

Gel Electrophoresis

1. Students (or instructors) can place the solidified agarose gel into the gel box.
2. Students should add an appropriate amount of loading buffer to their PCR samples.
3. Students should fill the gel box with 1X TAE until the gel is covered.
4. Students (or instructor) can load the molecular weight ladder into the first lane of the gel.
5. Students will load samples (5 µL each) into the other lanes of the gel. Remind students to take notes on which lanes samples are loaded into.
6. Gel should be run for 60 minutes at 120 volts (time and voltage adjusted as needed).
7. If only the desired band is seen on the gel, clean up the remaining PCR products with a spin column.

PCR Clean-Up

Note: all centrifugation steps are carried out in a standard benchtop microcentrifuge at 17,900 *xg* (13,000 rpm) at room temperature.

1. Add 5 volumes of Buffer PB to 1 volume of your PCR sample (for example, add 500 µL of Buffer PB to 100 µL of the PCR sample).
2. Check that the mixture's color is yellow. If orange or violet, add 10 µL of 3M sodium acetate (pH 5.0) and mix. Wait for the mixture to turn yellow.
3. Place a spin column in a 2 mL collection tube.
4. *Binding DNA:* apply the PCR sample + Buffer PB mixture to the spin column and centrifuge for 30-60 seconds.
5. Discard flow-through. Place the spin column back into the same collection tube.
6. *Washing DNA:* add 0.75 mL Buffer PE to the spin column and centrifuge for 30-60 seconds.
7. Discard flow-through. Place the spin column back into the same collection tube. Centrifuge for an additional 1 minute.
8. Place the spin column into a clean 1.5 mL microcentrifuge tube.
9. Add 50 µL Buffer EB or molecular grade water to the center of the spin column membrane. Let stand for 1 minute (can let stand for up to 4 minutes to increase DNA yield).

10. *Eluting DNA:* centrifuge for 1 minute.
11. Store purified PCR products at -20 °C.

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 in class today.
- What are the differences between this PCR reaction and the other PCR reaction you prepared for (section B of your Primer and PCR Design worksheet, for genotyping with PCR)?
- Describe in your own words the molecular events that will take place in the *one-pot digestion and ligation* you will complete in the next class.

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Student Guide

Lab Module 8: DNA Amplification with PCR

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Pre-Lab Reading

In today's class, you'll perform a PCR (**p**olymerase **c**hain **r**eaction) to prepare for the next steps in the cloning process. Let's revisit the cloning workflow:

After the PCR reaction you'll complete today, the next step is assembly of your vector (plasmid backbone) with other components (like a gene of interest) in a process called *ligation.* In this course, we've also referred to this step as a *"one-pot" digestion and ligation*. This means that in a single tube, in a single thermocycler program, we can complete two steps: a digestion, or cutting of your DNA pieces with restriction enzymes, and a ligation: sealing together your cut pieces of DNA into a single assembled plasmid.

The cloning method used in this course is *modular cloning,* a specific Golden Gate cloning method. Let's break down what this means, since there are many different cloning methods. In traditional, also known as classical cloning, restriction enzymes are used to cut DNA fragments or plasmids. Often, two different restriction enzymes are required to cut both, one for the plasmid and one for the DNA insert. When two different

restriction enzymes are used, the cut ends of the two pieces of DNA are not compatible (or complementary) to one another, as the enzymes cut at different recognition sites. In addition, restriction enzymes can generate two different kinds of *ends* when they cut a strand of DNA- *sticky* or *blunt* ends.

End Type	**Experimental Considerations**
blunt	• enzyme cuts at the same location on the top and bottom strands • parts are always compatible, no matter what enzyme was used to cut the DNA • easier to insert, but with less control
sticky	• enzyme cuts at different locations on the top and bottom strands; leaves an “overhang” (one strand is longer than the other, with unpaired bases “hanging” off) • only DNA pieces with the complementary sequence can be ligated together

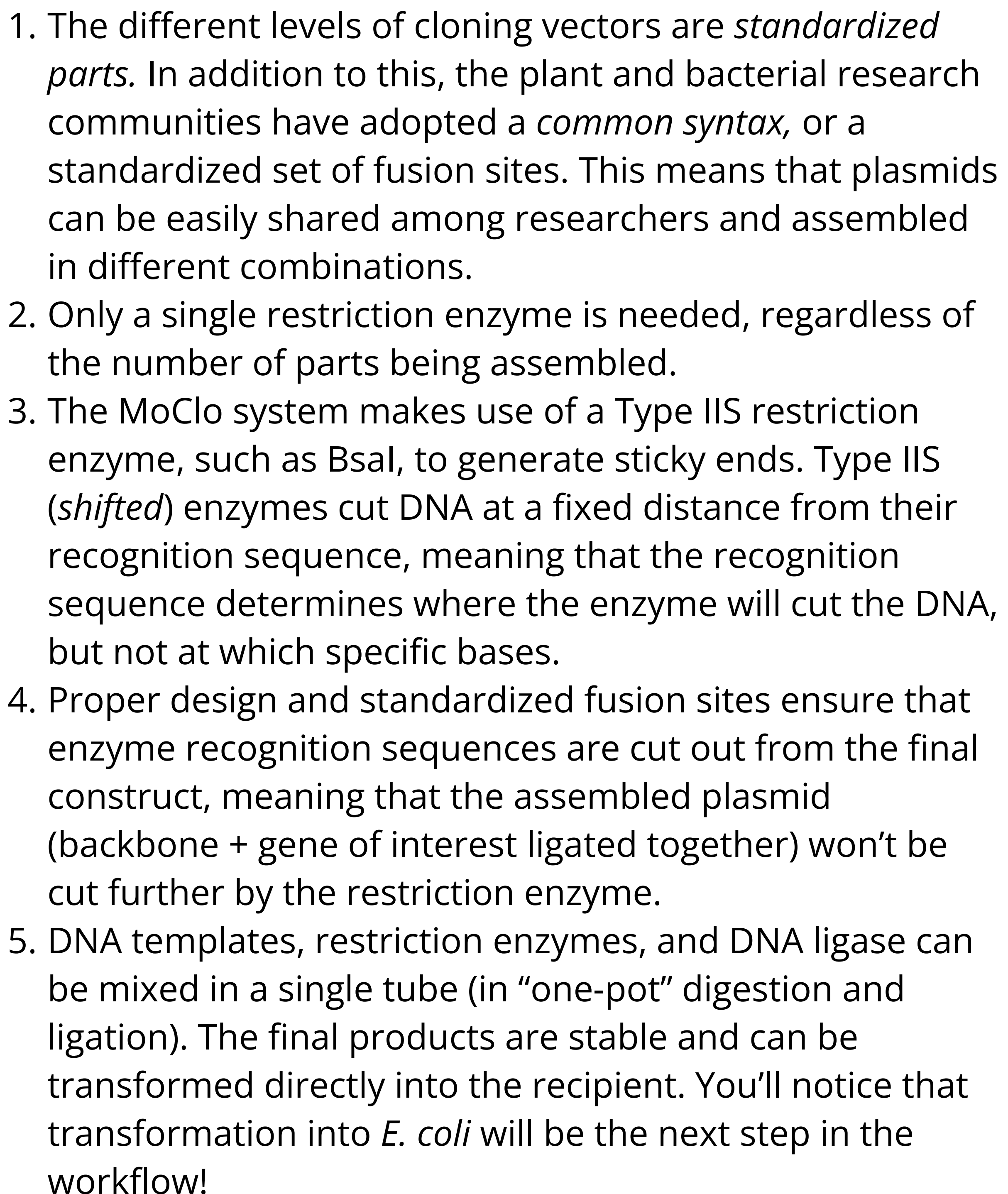
	• easy to adhere together, and with more control

For more information on blunt and sticky ends in restriction enzyme cloning, you can read the following article: Restriction Enzyme-based cloning and ligation.

Now, let's compare this to *modular cloning,* often referred to as "MoClo." MoClo is a Golden Gate assembly method that specifically uses a single Type IIS restriction enzyme (such as BsaI, which is used in this class). Golden Gate cloning is a type of *hierarchical assembly*, in which you can complete multiple steps of cloning to build larger molecular constructs. There are three sets of cloning vectors used in the MoClo system that can be added together in successive steps:

Level 0: a plasmid containing one or more basic DNA parts (like a promoter or coding sequence)
Level 1: a vector in which Level 0 parts can be assembled to create a *transcriptional unit* (combines that promoter and coding sequence together with a terminator, for example).
Level 2: up to six Level 1 modules can be assembled into a Level 2 vector

There are a number of unique aspects to the MoClo system. Many of them are why scientists like to use this system in their cloning experiments. We'll cover just a few here:



1. The different levels of cloning vectors are *standardized parts.* In addition to this, the plant and bacterial research communities have adopted a *common syntax,* or a standardized set of fusion sites. This means that plasmids can be easily shared among researchers and assembled in different combinations.
2. Only a single restriction enzyme is needed, regardless of the number of parts being assembled.
3. The MoClo system makes use of a Type IIS restriction enzyme, such as BsaI, to generate sticky ends. Type IIS (*shifted*) enzymes cut DNA at a fixed distance from their recognition sequence, meaning that the recognition sequence determines where the enzyme will cut the DNA, but not at which specific bases.
4. Proper design and standardized fusion sites ensure that enzyme recognition sequences are cut out from the final construct, meaning that the assembled plasmid (backbone + gene of interest ligated together) won’t be cut further by the restriction enzyme.
5. DNA templates, restriction enzymes, and DNA ligase can be mixed in a single tube (in “one-pot” digestion and ligation). The final products are stable and can be transformed directly into the recipient. You’ll notice that transformation into *E. coli* will be the next step in the workflow!

The PCR reaction you'll complete today is an important step in cloning workflows for a few reasons. If you aren't working with standardized MoClo parts, PCR ahead of a Golden Gate cloning process can add the correct restriction and adapter sequences to your amplified DNA. In addition, you'll also make more copies of the proper DNA sequence needed for your experiment!

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

Purpose

Today, you will amplify your DNA via PCR for cloning by using primers and PCR design from the previous class. You'll assemble master mix manually, and the OT-2 will pipette the master mix into a 96-well plate. Thermocycler reactions will be carried out in a bench-top thermocycler. Afterwards, PCR products should be run on an agarose gel, followed by PCR clean-up methods.

Learning Outcomes

- Design and analysis of PCR experiments
- Experimental planning ahead of complex experiments

- Laboratory automation of pipetting tasks and protocol customization

Supplies

Opentrons Equipment

- ☐ OT-2 automated liquid handling robot
- ☐ OT-2 P-20 Single-Channel GEN2 pipette (in left mount)
- ☐ Temperature Module (in deck slot 3)

Opentrons Protocol

- ☐ PCR Preparation protocol

Non-Opentrons Equipment

- ☐ Benchtop thermocycler
- ☐ Tabletop microcentrifuge

Labware

- ☐ Opentrons OT-2 96 Filter Tip Rack 20 µL in deck slot 1
- ☐ NEST 96 Well Plate 100 uL PCR Full Skirt in deck slot 2

- ☐ Opentrons 24 Well Aluminum Block with NEST 2 mL Snapcap tubes (on the Temperature Module in deck slot 3)

Reagents and Other Materials

- ☐ 10 µM primer working stocks (forward and reverse; diluted from dry oligos in Lab Module 7)
- ☐ 1.5 and 2 mL DNAse- and RNAse free microcentrifuge tubes for PCR clean-up
- ☐ Molecular grade water, such as HyClone Water (sufficient volume for PCR reaction and DNA elution in clean-up)
- ☐ Green HF (high fidelity) Buffer Pack (5X), such as Phusion® HF Buffer Pack, for use with Phusion High Fidelity DNA Polymerase
- ☐ Phusion High Fidelity DNA Polymerase, such as Phusion™ High-Fidelity DNA Polymerases
- ☐ dNTPs mix (10 mM)
- ☐ DNA template to be amplified
- ☐ PCR clean-up kit, such as the QIAquick PCR Purification Kit
- ☐ 96-100% ethanol
- ☐ 3M sodium acetate, pH 5.0

Procedure Guide

Before Class

1. Complete the pre-lab reading.
2. You (or your instructor) should prepare and pour a 0.7% agarose gel prior to class. See Lab Module 6 for detailed gel preparation instructions.

Master Mix Assembly

1. First, you will assemble a master mix for your PCR reaction based on your Primer and PCR Design worksheet (section **A**) from the previous class. Fill in the blank table from the worksheet, shown below, as you did in the previous class.

<table>
<tr><th># Reactions and µL needed</th><th>1</th><th>4</th><th></th><th>Thermocycler Conditions</th><th>°C</th><th>min:sec</th><th></th></tr>
<tr><td>HyClone Water</td><td></td><td></td><td></td><td>1. Initial Denaturation</td><td></td><td></td><td></td></tr>
<tr><td>Green HF Buffer (5x)</td><td></td><td></td><td></td><td>2. Denaturation</td><td></td><td></td><td rowspan="3">5x</td></tr>
<tr><td>dNTPs (10 mM)</td><td></td><td></td><td></td><td>3. Annealing</td><td></td><td></td></tr>
<tr><td>F Primer (10 µM)</td><td></td><td></td><td></td><td>4. Extension</td><td></td><td></td></tr>
</table>

R Primer (10 μM)				5. Denaturation			30x
DNA Template	1.35			6. Anneal and Extend			
Phusion DNA Polymerase				7. Final Elongation			
Total	20.00			8. Storage	12	∞	

2. Manually assemble your master mix with the following components. You will need to prepare as many different master mixes as are required. Each tube should contain 2 mL of master mix.
    a. **Green HF Buffer (5X)**; 4 μL
    b. **dNTPs** (10 mM); 0.4 μL
    c. **Forward and reverse primers** (10 μM working dilution stocks); 1 μL *each*
    d. **Phusion DNA polymerase**; 0.25 μL
    e. **Hyclone molecular grade water**; 13.35 μL (to a final master mix volume of 20 μL)
3. Store master mix on ice.

PCR Prep- Master Mix and DNA Sample Pipetting

1. As a class, download and open the PCR Preparation protocol file in a code editing program to customize for your experiment. As written, the protocol will distribute

three different master mixes into various wells of a 96-well plate.

2. Using the Excel template created in Lab Module 4, edit your tables as follows:
    a. Edit your "Step 1" table to include your initial volumes of master mix. Here, the protocol can be customized for the number of different master mixes required.

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
	2mL_tubes	A1	2	Master Mix #1	This is master mix #1	#33A4FF
	2mL_tubes	A2	2	Master Mix #2	This is master mix #2	#63FE82
	2mL_tubes	A3	2	Master Mix #3	This is master mix #3	#F5FE63

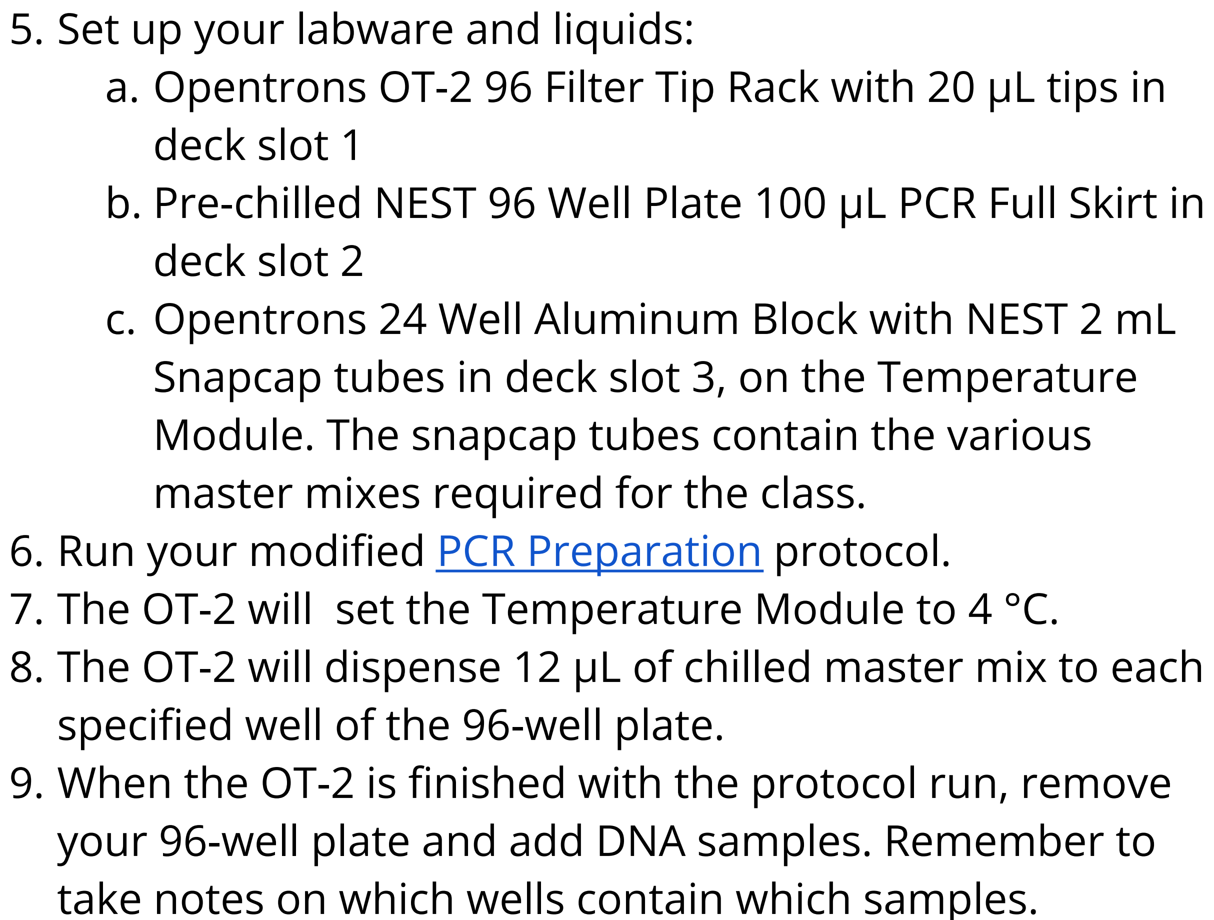
The example table shown here includes manually assembled master mix in 2mL_tubes (NEST 2 mL snapcap tubes on the temperature module).

    b. Next, edit your "Step 2" table to include the transfer steps (from the 2mL_tubes source to your tc_plate, or 96-well plate, destination) and transfer volume.

Step 2: Put columns H-L into "csv_transfer_data_raw". Volume is in uL. This is the table for transferring liquid.	Source_Labwar	Source_Well	Destination_Labware	Destination_Wel	Transfer_Volum	Pick_Up_Tip
	2mL_tubes	A1	tc_plate	A1	12	TRUE
	2mL_tubes	A1	tc_plate	A2	12	FALSE
	2mL_tubes	A1	tc_plate	A3	12	FALSE
	2mL_tubes	A1	tc_plate	A4	12	FALSE
	2mL_tubes	A1	tc_plate	A5	12	FALSE
	2mL_tubes	A1	tc_plate	A6	12	FALSE
	2mL_tubes	A1	tc_plate	A7	12	FALSE
	2mL_tubes	A1	tc_plate	A8	12	FALSE
	2mL_tubes	A1	tc_plate	A9	12	FALSE
	2mL_tubes	A1	tc_plate	A10	12	FALSE
	2mL_tubes	A1	tc_plate	A11	12	FALSE
	2mL_tubes	A1	tc_plate	A12	12	FALSE
	2mL_tubes	A1	tc_plate	B1	12	FALSE
	2mL_tubes	A1	tc_plate	B2	12	FALSE
	2mL_tubes	A1	tc_plate	B3	12	FALSE
	2mL_tubes	A1	tc_plate	B4	12	FALSE
	2mL_tubes	A1	tc_plate	B5	12	FALSE
	2mL_tubes	A1	tc_plate	B6	12	FALSE
	2mL_tubes	A1	tc_plate	B7	12	FALSE
	2mL_tubes	A1	tc_plate	B8	12	FALSE
	2mL_tubes	A1	tc_plate	B9	12	FALSE
	2mL_tubes	A1	tc_plate	B10	12	FALSE
	2mL_tubes	A1	tc_plate	B11	12	FALSE
	2mL_tubes	A1	tc_plate	B12	12	FALSE
	2mL_tubes	A1	tc_plate	C1	12	FALSE
	2mL_tubes	A1	tc_plate	C2	12	FALSE
	2mL_tubes	A1	tc_plate	C3	12	FALSE
	2mL_tubes	A1	tc_plate	C4	12	FALSE
	2mL_tubes	A1	tc_plate	C5	12	FALSE

The example table shown here details transfer of master mix (from 2mL_tubes) to the tc_plate (a NEST 96 well plate in deck slot 2).

3. As in previous lab modules, copy and paste your data tables into the “Modify” sections at the beginning of the protocol.
4. Save your protocol file and import into the Opentrons App.



5. Set up your labware and liquids:
    a. Opentrons OT-2 96 Filter Tip Rack with 20 µL tips in deck slot 1
    b. Pre-chilled NEST 96 Well Plate 100 µL PCR Full Skirt in deck slot 2
    c. Opentrons 24 Well Aluminum Block with NEST 2 mL Snapcap tubes in deck slot 3, on the Temperature Module. The snapcap tubes contain the various master mixes required for the class.
6. Run your modified PCR Preparation protocol.
7. The OT-2 will set the Temperature Module to 4 °C.
8. The OT-2 will dispense 12 µL of chilled master mix to each specified well of the 96-well plate.
9. When the OT-2 is finished with the protocol run, remove your 96-well plate and add DNA samples. Remember to take notes on which wells contain which samples.

PCR Reaction

1. Load the NEST 96-well plate into a benchtop thermocycler.
2. Set the thermocycler reaction steps according to your above table (the same as section **A** of your Primer and PCR Design worksheet) and run the reaction.
3. End the program, open the thermocycler lid, and remove the plate.

1. Make sure that your plate is properly labeled. You can include a “plate map” in your notes.
2. Store PCR products at -20 °C for the following class.

Discussion Questions

Discuss the lab activities with your labmates.

- Describe in your own words what took place in the PCR reaction you ran in class today.
- What are the differences between this PCR reaction and the other PCR reaction you prepared for (section **B** of your Primer and PCR Design worksheet, for genotyping with PCR)?
- Describe in your own words the molecular events that will take place in the *one-pot digestion and ligation* you will complete in the next class.