Opentrons for Education

Document Version 1 (04/12/24)

Prep Sheet

PCR Colony Screening

Authored by Clay Wright, Ph.D.

Virginia Tech, Department of Biological Systems Engineering

Opentrons for Education

Document Version 1 (04/12/24)

Educator Guide

PCR Colony Screening

Authored by Clay Wright, Ph.D.

Virginia Tech, Department of Biological Systems Engineering

Contents

Included in this document are the following sections:

Purpose

The goal today is for students to identify colonies that contain correctly assembled plasmid DNA using PCR that is implemented in the OT-2 liquid handling robot and the Opentrons Protocol Designer.

To do this, students will need to:

- Identify primers that will amplify a fragment spanning the junction between the vector backbone and insert
- Understand the manual *E. coli* PCR colony screening protocol
- Use the Protocol Designer to develop an OT-2 *E. coli* PCR colony screening protocol

Student Audience

This lab was designed for mid to upper-level undergraduate students in the college of Biological Systems Engineering at Virginia Tech (BSE 3984).

The lab can accommodate most class sizes, as the OT-2 protocol will be run by the instructor, rather than individual students.

Background Knowledge

Students should come to the lab with a conceptual understanding of manual pipetting, colony screening, DNA amplification, and running/interpreting gel electrophoresis results.

Core Competencies

Laboratory Skills

Pipetting, sample preparation, and the use of laboratory automation equipment

Critical Thinking

Interpreting gel electrophoresis results, troubleshooting, designing automation for efficiency, considering approaches to automation

Supplies

Opentrons Equipment

- ☐ P20 Single-Channel GEN2
- ☐ P300 Single-Channel GEN2
- ☐ Opentrons 96 Tip Rack 20 µL
- ☐ Opentrons 96 Tip Rack 300 µL
- ☐ Heater-Shaker GEN1
- ☐ NEST 96 Well Plate 100 µL PCR Full Skirt
- ☐ Opentrons 24 Tube Rack with Eppendorf 1.5 mL Safe-Lock Snapcap

Reagents

- ☐ DI water
- ☐ One*Taq*® 2X PCR Master Mix with Standard Buffer
- ☐ Forward Primer 1
- ☐ Reverse Primer 1
- ☐ Forward Primer 2
- ☐ Reverse Primer 2

Experimental Duration

Required Class Sessions

1

Lab Run Time

Estimated total time: 2.5-3 hours

- Overview and Review of Manual Protocol (Part One): 10 minutes
- Drafting OT-2 Protocol on Opentrons Protocol Designer (Part Two): 35 minutes
- Presenting Protocols, Deciding on Consensus Protocol: 15 minutes
- Running Protocol, Colony Dip, PCR as a Class (Part Three): 1.5 hours

Basic Troubleshooting

1. Do a trial run before class; this way any unexpected occurrences can be resolved before students arrive.

2. Issues with tips striking plates are almost always due to using alternate labware or robot calibration. If you experience this and have confirmed the correct labware, try re-calibrating the robot.
3. If you need to reach out to Opentrons Support, please inform them that you are part of our Opentrons for Education program and the date of your next lab class.

Required Pre-Lab Activities

Prior to this lab, students should have the following technical abilities and theoretical knowledge:

- Load a pipette tip onto a manual pipette
- Volume adjustments for manual pipettes
- Theoretical knowledge of DNA amplification and gel electrophoresis

Note: Experience with coding (Python or otherwise) is not required for this lab.

Procedure Guide

1. **Overview and Review of Manual Protocol (Part One) ~ 10 minutes**

Students should read over the lab before coming to class, but plan on taking a few moments to outline the lab session, explain the purpose, and answer student questions.

The manual *E. coli* screening PCR colony protocol students will be incorporating automation into is as follows:

Prepare the PCR master mix:

1. Calculate the volume of each mastermix to make with the *E. coli* colony PCR calculator.
2. Using the “uL for master mix” column add the calculated amount of each reagent to each master mix in a 1.5 mL tube.
3. Aliquot 20 µL of master mix to each well of a strip or plate.

Colony dip and run the PCR (Note there is no way to automate these steps in our current OT-2):

4. Take a very small amount of a colony from a plate and dip it into the first PCR tube.
5. Repeat for each of the remaining PCR tubes, using a fresh tip for each colony.

6. Cap the PCR tubes, move them to the thermal cycler and run the appropriate protocol.

2. Drafting OT-2 Protocol on Opentrons Protocol Designer (Part Two) ~ 35 minutes

Each student will use the Opentrons Protocol Designer to create their protocol, using the following instructions:

1. You will be instructed to describe the desired setup for the robot, what tools and parts you will need and where they go. This is the “File” tab on the left side.
2. You will then be asked to describe the liquids involved in the protocol. These only need to be the starting liquids, not anything that you will be making.
3. Finally you will need to specify where all of the liquids will be moved to in order to perform the desired protocol, “Design”ing your protocol.

During this portion of the lab, plan to individually check-in with students and see how they’re getting along. Remind them to think efficiently and avoid potential cross-contamination.

3. **Presenting Protocols, Deciding on Consensus Protocol ~ 15 minutes**

Students should briefly present their protocols, along with their rationale. As a class, students will identify a consensus protocol to be run by the instructor.

Plan to create some space during this time for healthy debate and critical thinking.

4. **Running Protocol, Colony Dip, PCR as a Class (Part Three) ~ 1.5 hours**

Once the class has decided on a consensus protocol, you will run the protocol, colony dip, and PCR.

You may decide to make this section more engaging by bringing up student volunteers to do various parts of this lab. And while the PCR runs, you may choose to discuss the drawbacks and opportunities of automation as a class, answer questions, or allow students to work on the lab report in small groups.

Lab Report

Instructions

Assign students to prepare a comprehensive lab report that helps them reflect on their results and on the OT-2 automation. Some ideas for exploration are included below:

1. Code shuffling activity: All the pages of the Opentrons code blew off your desk when you opened the door! Figure out what's happening on each page of code and then put them back in the correct order.
    a. Blue
    b. Green
    c. Red

2. Refer back to the OT-2 protocol you prepared. Can you identify any opportunities to make your automation more efficient?

3. Identify a few critical points in your protocol where things could go wrong during automation (ex: cross-contamination or running out of a reagent). How might you address these?

4. Review and attach an image of the gel electrophoresis results to this lab. How do you interpret these results?

Opentrons for Education

Document Version 1 (04/12/24)

Student Guide

PCR Colony Screening

Authored by Clay Wright, Ph.D.

Virginia Tech, Department of Biological Systems Engineering

Purpose

Your goal today is to identify colonies that contain correctly assembled plasmid DNA using PCR that is implemented in the OT-2 liquid handling robot and the Opentrons Protocol Designer.

To do this you will need to:

- Identify primers that will amplify a fragment spanning the junction between the vector backbone and insert
- Understand the manual *E. coli* PCR colony screening protocol
- Use the Protocol Designer to develop an OT-2 *E. coli* PCR colony screening protocol

Required Equipment

Pipettes and Tips:

- P20 Single-Channel GEN2
- P300 Single-Channel GEN2
- Opentrons 96 Tip Rack 20 µL
- Opentrons 96 Tip Rack 300 µL

Modules and Labware:

- Heater-Shaker GEN1
- NEST 96 Well Plate 100 µL PCR Full Skirt

- Opentrons 24 Tube Rack with Eppendorf 1.5 mL Safe-Lock Snapcap

Reagents:

- DI water
- One*Taq*® PCR master mix
- Forward Primer 1
- Reverse Primer 1
- Forward Primer 2
- Reverse Primer 2

Experimental Procedure

Background

To do PCR on DNA within E. coli and most other bacteria, as well as mammalian cells, we can just put a few cells of the organism into a PCR and the initial denaturation step will lyse the cells releasing the DNA. The most common problem that occurs with this protocol is that you put too many cells into the reaction and there is too much nonspecific DNA and other molecules that may interfere with the polymerase. Other organisms like fungi and plants contain so much interfering material, namely their cell walls, that more rigorous lysis is needed. We typically do either several freeze-thaw cycles, sodium hydroxide lysis, or grinding with liquid nitrogen to release the DNA from these more difficult organisms.

Regardless, the main point here is that you do not need many cells to have a lot of DNA in a reaction. In fact, for E. coli, you do not want the cell solution you plan to lyse and use for PCR to be cloudy, or "turbid" in microbiology jargon. You should barely be able to see the cells dispersing into the solution.

Typically, microbiologists will make up the PCR reaction mixture as a master mix (with all of the reactions needed in a single tube) and then aliquot this master mix to individual tubes. You don't need much volume, 20 µL is generally sufficient to see a band, especially if using a narrow-well comb when pouring your gel. You then dip a tip with a tiny amount of a colony into each PCR tube and give it a gentle swizzle. (A word of advice: Leave the tip in the solution until done picking colonies so you don't lose your place in the plate or strip of tubes.)

Today we will be using two different sets of primers to detect correct assembly of our plasmids for 15 different colonies as well as a negative control, our plasmid that we used for the backbone. This will be a total of 32 different PCRs. While it is not awful to make up colony PCRs you can begin to see how at this scale the pipetting gets tedious and making a mistake would be easy. The master mix method definitely helps, but for less repetitive things, the Opentrons helps a lot. Creating this protocol in the OT-2 will hopefully be a very achievable but demonstrative protocol to introduce you to lab automation.

Part One: Review Manual *E. coli* PCR Colony Screening Protocol

Your goal in this lab is to create an automated Opentrons OT-2 protocol that will replicate the following manual *E. coli* colony PCR protocol:

Prepare the PCR master mix:

1. Calculate the volume of each mastermix to make with the *E. coli* colony PCR calculator.
2. Using the “uL for master mix” column add the calculated amount of each reagent to each master mix in a 1.5 mL tube.
3. Aliquot 20 µL of master mix to each well of a strip or plate.

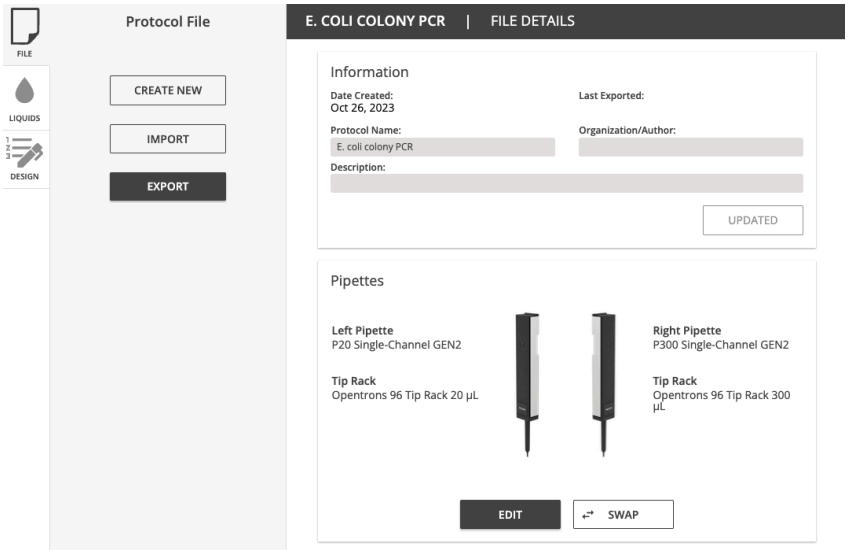
Colony dip and run the PCR (Note there is no way to automate these steps in our current OT-2):

4. Take a very small amount of a colony from a plate and dip it into the first PCR tube.
5. Repeat for each of the remaining PCR tubes, using a fresh tip for each colony.
6. Cap the PCR tubes, move them to the thermal cycler and run the appropriate protocol.

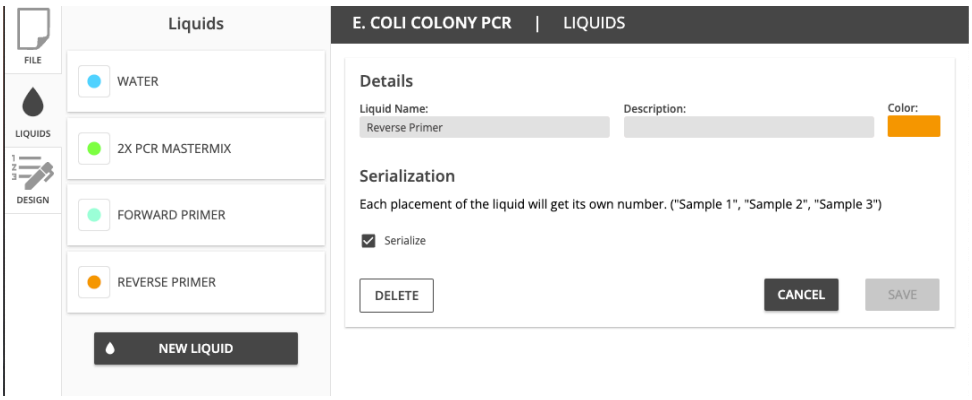
Part Two: Making Your OT-2 Protocol

To make your protocol, open the [Opentrons Protocol Designer].

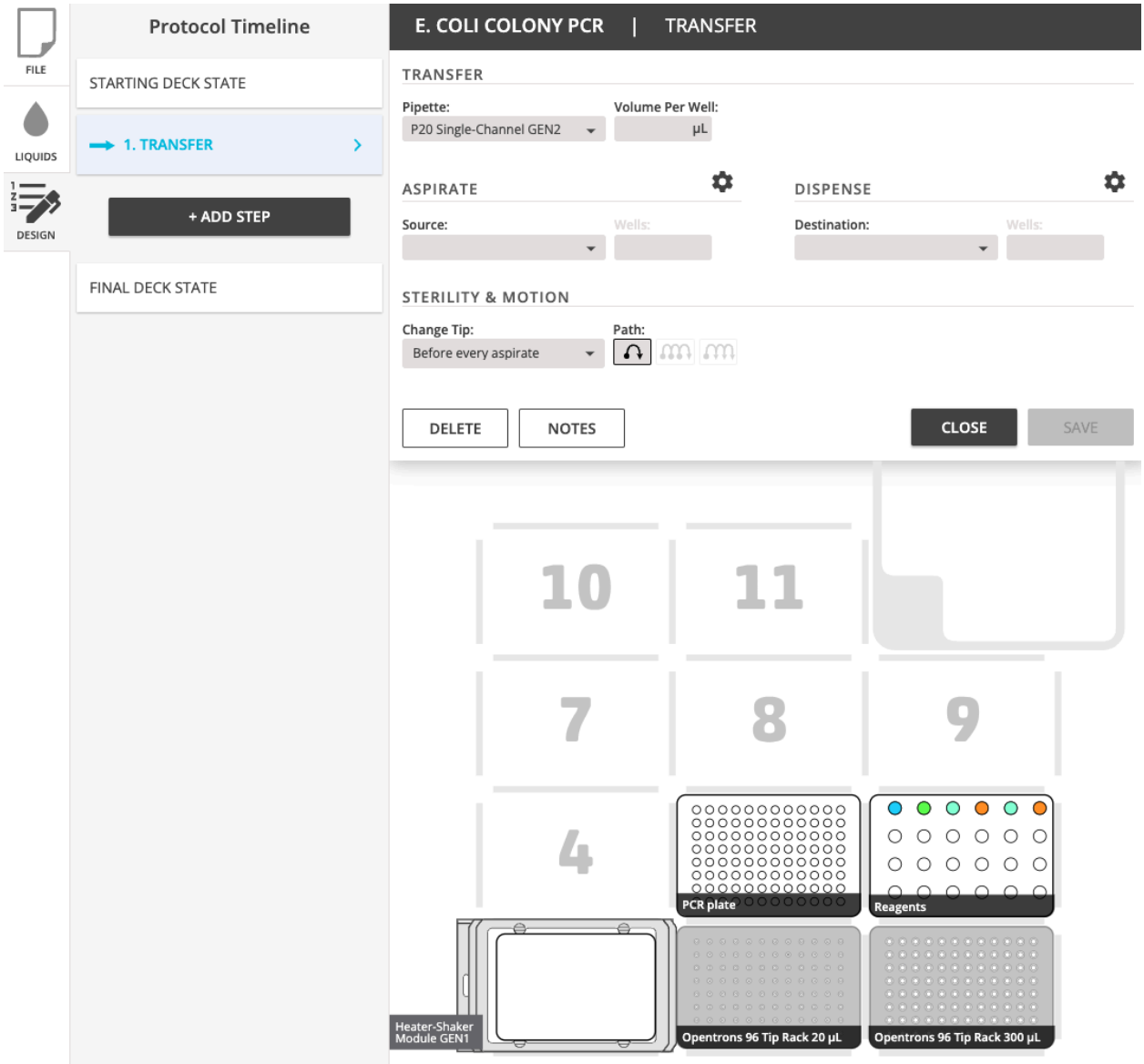
1. You will be instructed to describe the desired setup for the robot, what tools and parts you will need and where they go. This is the “File” tab on the left side.



2. You will then be asked to describe the liquids involved in the protocol. These only need to be the starting liquids, not anything that you will be making.



(Note that you can use the ‘Serialize’ Checkbox in the protocol designer to make these different primers. Also note that when specifying volumes this is something we can change later, just make sure the volume you put makes sense and is in excess of what you think you will need.)



3. Finally you will need to specify where all of the liquids will be moved to in order to perform the desired protocol, “Design”ing your protocol.

   When developing your OT-2 protocol think about how you can make it most efficient. Check out the advanced settings under the “gear icon”. You can also use different paths to save tips. Just be careful and make sure you don’t contaminate reagents or ruin your experiment with cross contamination. Along these lines, also try to think about things that could go wrong, like running out of a reagent, and make sure to document these, as it might be helpful in debugging your protocol and making sure the end user knows about critical points in your protocol.

   The software might take some time to get used to but you can click around and get used to it pretty quickly and in the end it’s pretty intuitive. As you will come to see though it requires a lot of clicking to specify a protocol that maybe could be more useful if it were written in Python and could be easily modified to add samples or new reagents.

   Once you have finished your protocol go back to “File” and export it. This will allow you to import it later, or upload it to the OT-2.

4. As a class, each student will briefly present their protocol and together we will decide on the final class protocol to run on the OT-2.

Part Three: Running The Protocol

Once the class has decided on a consensus protocol, your instructor will run the protocol, colony dip, and PCR.

You may want to take notes during this portion of the lab to prepare for your lab report.

Getting Started

Before teaching the lesson plan, complete the following steps prior to class.

- ☐ Unbox the OT-2
- ☐ Set up the Opentrons app
- ☐ Attach pipettes
- ☐ Calibrate the deck
- ☐ Calibrate tip length & pipette offset
- ☐ Import any related protocols to the app
- ☐ Test run the protocol on the OT-2

Need Additional Support?

For technical support, please check our Opentrons Help Center for relevant articles. If you need further support, please contact support@opentrons.com.

If you have questions related to the lesson plan, please reach out to the author, Clay Wright, Ph.D., at wrightrc@vt.edu.