EC552/BE552 - Computational Synthetic Biology For Engineers

Homework 3
Spring 2020

April 18, 2020

Experimental Automation

Due: 4/30/20

Overview

Goal: In order to produce consistent, reproducible, and high-throughput results in synthetic biology-related fields and strengthen your skillset in lab automation, you will be required to create two protocol ”scripts” that utilize 1) an open-source Laboratory Information Management System (LIMS), Aquarium, developed by the Klavins Lab at the University Washington and 2) a Liquid-handling robot, OT2, from OpenTrons.

The homework is divided into two corresponding activities, each of the activities will reinforce your understanding of lab automation and how to approach the problem step-by-step toward the final solution.

Activities:

1. **(50 Points)** Find a synthetic biology-related protocol from the literature and create a Krill (Ruby on Rail) script for the protocol in Aquarium. Your report must include the following information about the protocol:

   - Protocol Motivation (REPORT)
   - Protocol Steps (SEPARATE, REPORT)
   - Protocol Visual Flow Chart (REPORT)
   - Protocol Materials, Labware, and Equipment (REPORT)
   - Protocol Cost and Time Estimate for Manual Execution (REPORT)
   - Protocol Script in Krill (Ruby on Rail) for Aquarium (SEPARATE)
   - Images that Capture the Content of the Aquarium DEF (Definition) Page (SEPARATE)

2. **(50 Points)** Understand the application of OpenTrons Liquid-handling robot, OT2, and construct a Python script that replicates the previously identified synthetic biology-related protocol in an automatic fashion. Your report must include the following attributes about your design:

   - Protocol Materials and Labware (REPORT)
   - Protocol Hardware Modules and Equipment (REPORT)
   - Protocol Cost and Time Estimate for Automated Execution (REPORT)
   - Determine the Cost and Time point(s) where the Automated Protocol Out Performs the Manual One (REPORT)
   - Protocol Script in Python for OT2 Liquid-handling Robot (SEPARATE)

Note 1: Grading will based solely on the the deliverables so please ensure that all the required deliverables are included correctly to ensure proper grading.

Note 2: The final design report submitted must in the format of a Lab on a Chip Communication (Microsoft Word Template, LaTeX Template, Overleaf LaTeX Templates)

Activity #1

In this activity, you will do 1) a literature search for a synthetic biology-related protocol, 2) create a visual representation of the protocol, 3) perform cost and time estimate of the protocol for the manual execution based on literature reviews and online sources, and 4) construct a script that can replicate the protocol procedures in Aquarium.

In order to set up your local version of Aquarium to test and compile the Krill script for this assignment, please read the entire homework assignment before starting your script. The script must compile to receive any credit, and points will be awarded based on the quality of the deliverable.

An example of a good protocol can be found here: Sample Protocol. Please attach a detailed description of the protocol involved.

Here is a list of protocols that CANNOT be done:

- Cell Transformation
- PCR, Colony PCR, and PCR Purification
- DNA and RNA Enrichment, Extraction, and Purification
- Bioanalyzer DNA and RNA Quality Analysis
- DNA and RNA Sequencing Analysis
- Gel Electrophoresis, Extraction, and Purification
- Primer Design
- Gibson Assembly
- Modular Cloning
- Modular Gibson Assembly
- Restriction Digestion and Ligation
- Mutagenesis and Mutagenesis by Inverse PCR
- Media, Plates, Reagents, Samples, and Culture Preparations and Storage
- Cell Growth with Fluorescence and Biomass Assay (Plate Reader)
- Cell Growth with Fluorescence Microscope Assay

- Cell Growth with Flow Cytometry Analysis

The deliverable for **Activity #1** is 1) a **report** and 2) a **Krill script** for Aquarium.

The report must **clearly** describe the following criteria and the Krill script must **replicate** the manual execution of the protocol:

For report:

- The synthetic biology-related protocol being investigated
- The importance of the protocol
- The protocol procedures and a visual representation of the protocol
- The reagents and corresponding volumes, vendors, and Catalog # of the protocol (in a table)
- The labware, equipment and corresponding volumes of the protocol (in a table)
- The manual protocol cost and time estimation based on literature reviews and online sources

For Krill script:

- A protocol script that replicates the manual execution of the experiment in Aquarium
- Images that capture the content of the Aquarium DEF (Definition) Page

Activity #2

In this activity you will understand the application of OpenTrons Liquid-handling robot, OT2, and construct a Python script that replicates the previously identified synthetic biology-related protocol in an automatic fashion with OT2.

In order to set up your local simulation for the OT2 protocol to test and compile the Python script for this assignment, please read the entire homework assignment and the list of links provided below before starting your script. The script must compile (pass the simulation on your computer) to receive any points and points will be awarded based on the quality of the deliverable.

The following list of websites are a great place to begin your studying for OT2 and the API:

- OT-2 Python Protocol API
- Labware Library
- Simulating OT-2 protocols on your computer

Here is a list of available OT2 pipettes and hardware modules:

- P10 Single
- P10 Multi
- P300 Single
- P300 Multi
- P1000 Single
- Temperature Module
- Magnetic Module

Note 3: OpenTrons Thermocycler Module is unavailable, instead, we will use standalone bench-top Thermocycler.
Note 4: Provide detail commands of the utilization conditions in the python script if the protocol requires standalone laboratory equipment.

The deliverable for **Activity #2** is 1) a **report** and 2) a **Python script** for OT2.

The report must **clearly** describe the following criteria and the Python script must **replicate** the manual execution of the protocol in an **automatic** fashion:

For report:

- The pipettes, labware, hardware modules, equipment and corresponding volumes of the protocol (in a table)
- The OT2 automated protocol cost and time estimate based on literature reviews and online sources
- Determine when cost and time of the automated protocol out performs the manual one (there may be multiple cross over points).

For Python script:

- A protocol script that replicates the manual execution of the experiment in an automatic fashion utilizing OT2 Liquid-handling robot, available OT2-related pipettes and hardware modules, and additional laboratory equipment

Aquarium Tutorial

Setup Your Local Version of Aquarium:

1. Clone the CIDAR Lab's branch of the Aquarium-CIDAR GitHub Repositories locally, and be sure to clone **an earlier version of damplab-latest** branch
   (a) In the top left corner there should be a dropdown menu. This should say **"Branch: damplab-latest"**
   (b) This will create a folder **damplab-aquarium-damplab-latest**
2. Install Docker for Mac or Docker Toolbox for Windows on your local machine
   (a) Mac
   (b) Windows
   NOTE 5: Docker isn't really compatible with the Windows' Operating System, if you have Windows, you might want to install **Linux**
3. Download the **Dump** file, containing all of the current data, form **Piazza** into **mysql_init** folder, and rename it to **dump.sql**. To get to **mysql_init** folder:
   (a) first go to **damplab-aquarium-damplab-latest** folder
   (b) then go to **docker** folder
   (c) identify **mysql_init** folder
4. Open Terminal in this local Aquarium folder, **damplab-aquarium-damplab-latest** folder
   (a) Run git checkout b8e2f53c0b3ea7cf26e10a2f7f4d4ef7977f0789
   (b) Run git branch to check you are using **an earlier version of damplab-latest branch**
   (c) Run docker-compose up –build , **be aware there are two dashed before build**
   (d) Wait until this command, [1] Use Ctrl-C to stop, shows up
5. Opening a new tab in the Terminal , and run additional data migration commands:
   (a) Run docker-compose exec app /bin/sh
   (b) Run rake db:migrate
   (c) Run rake collections:migrate
6. Go to your local Aquarium
   (a) Your username and password are:

- username: **ec/be 522**
- password: **spring2019**

Start Coding:

1. Go to the **DEVELOPER** tab on the upper right corner of Aquarium
2. Click on the **NEW** button below the **DEVELOPER** tab
3. Create protocol definitions in the **DEF** tab and provide the information for the protocol input(s) and output(s)
4. Input protocol title in the **Operation Name (id:)**, and Cloning in the **Category**
5. Go to the workspace on the right of the **DEF** tab and click on **PROTOCOL** tab
6. Start coding!!! Click on the protocols listed on the left side of the web page under the category **Cloning** if you want to view some example protocols

Test and Compile Your Code in Aquarium:

1. To test and compile your code, go to **TEST** Tab
2. Input **1** in the **Batch Size:**
3. Click on **GENERATE OPERATIONS** button
4. Click on the red **TEST** button
5. Informative results of your script will appear under **Backtrace** Section of the page, and scroll to the bottom of this section to view the **Completed** command

Additional Resources:

- AQUARIUM Overview
- AQUARIUM Protocol Workflow
- Trident: The Aquarium API
- Aquarium-CIDAR GitHub Repositories

OpenTrons Tutorial

Where to Begin for OpenTrons:

- Go to the OT-2 Python Protocol API
  - More information on Pipettes
  - More information on Labware
  - More information on Labware Library
  - More information on Hardware Modules
  - More information on Example Protocols
  - More information on API Version 2 Reference

Simulating OT-2 Protocols on Your Computer:

- Go to Simulating OT-2 Protocols on Your Computer to simulate protocols without being connected to a robot. You can simulate some example protocols before you **test and compile** your protocol

Additional Resources:

- Opentrons GitHub Repositories