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Prep Sheet

Measurements and Statistical Analysis

Authored by Kennedy Bae, Ph.D. and Kinnari Watson, Ph.D.

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 authors, Kennedy Bae, at kennedy@opentrons.com, or Kinnari Watson, at kinnari@opentrons.com.

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Educator Guide

Measurements and Statistical Analysis

Authored by Kennedy Bae, Ph.D. and Kinnari Watson, Ph.D.

Purpose

This lab aims to develop student understanding of using serial dilutions to generate a standard curve, and the use of standard curves for interpolating sample values. This includes an appreciation of:

- Basic pipetting skills
- Serial dilutions
- Measuring replicates
- Range of the standard curve
- Use of statistics to assess significance

Students perform manual pipetting in parallel with automated liquid handling, allowing students to see the strengths and opportunities of both techniques.

Student Audience

This lab was designed for use in entry to mid-level undergraduate biology courses. It is flexible to accommodate any number of students that are enrolled in the class.

Background Knowledge

Students should have a conceptual understanding of pipetting, spectrophotometry, standard curves, and statistical analysis.

Core Competencies

Laboratory Skills

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

Data Analysis

Developing standard curves, interpolation, and statistical analysis

Critical Thinking

Interpretation of experimental data, troubleshooting, and drawing reasonable conclusions

Supplies

Opentrons Protocol

- ☐ Download protocol from https://protocols.opentrons.com/protocol/customizable_serial_dilution_ot2
  - Select the following parameters on the webpage according to your setup and supplies:
    - Pipette Type
    - Mount Side
    - Tip Type
    - Trough Type
    - Plate Type
  - Keep the default settings for:
    - Dilution factor (3)
    - Number of dilutions (10)
    - Total mixing volume (150)
    - Blank in well plate (yes)
    - Tip use strategy (use one tip)
    - Volume of air gap (10)

Opentrons Equipment

- ☐ Opentrons OT-2 Automated Liquid Handling Robot
- ☐ Opentrons p300 8-channel pipette

Non-Opentrons Equipment

- ☐ Plate-based spectrophotometer
- ☐ Software for data analysis (ex. Excel or dedicated statistics software)
- ☐ P1000 variable volume manual pipettes (one per student)
- ☐ P100 variable volume manual pipette (only one required, for demonstrating Part A)

Labware

- ☐ Opentrons 300 µL Tip Rack
- ☐ 12-well trough
- ☐ 96-well Flat Bottom plate
- ☐ Test tubes (6 per student)
- ☐ Dropper (1 per student)

Reagents

- ☐ 1000 mL diH20 per student

- [ ] FD&C Blue No. 1 (McCormick® was used in the development of this lab)

Experimental Duration

Required Class Sessions

1

Lab Run Time

Estimated total time: 2.5-3 hrs

Introduction and Calibration Curve Generation: 35 minutes

Sample Preparation and Pipetting: 1 hour

Data Collection: 30 minutes

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 starting 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 spectrophotometry–The educator will be running the spectrophotometer for this lab
- Understanding of standard curves, including knowledge of the equation that defines the curve, and the ability to interpolate values
- A strong grasp of basic statistical analysis, including calculating and interpreting means, standard deviations, and R^2

Procedure Guide

1. Lab Introduction ~ 15 minutes

Ideally, students should read the lab guide before coming to class, however, plan to spend 10 minutes discussing pipetting techniques, review of standard curves, and interpolating sample values.

2. Calibration Curve Generation ~ 20 minutes

Next, demonstrate standard curve generation (Part A) for the class (this lab assumes that the educator will be the sole operator of the OT-2 robot and spectrophotometer):

1. Prepare the blue dye solution with 50 µL of blue food dye and 10 mL of diH2O.
2. Add 200 µL of the blue dye solution to the wells A1-H1 of the 96-well flat bottom plate.
3. Add 20 mL of water to A1 well of the trough reservoir.

(Note: The column after the last dilution is the default location of the blanks. The last well of the trough/reservoir is the default liquid trash.)

4. Arrange labware as follows:
    a. Slot 1 = Opentrons 300 µL tip rack
    b. Slot 2 = 12-channel reservoir
    c. Slot 3 = 96-well flat bottom plate
5. Proceed to the OT-2 run app to run the protocol.
6. Quantify the absorbance of the 96-well plate at 450 nm.

3. Discussion ~ 5 minutes

After running the serial dilutions and generating the standard curve, you may wish to come back together as a class and discuss the strengths and opportunities of automation.

4. Part B: Manual Sample Preparation ~ 1 hour

During this portion of the lab, students will be asked to independently complete Part B of the lab:

1. Use one test tube to collect a 200 µL aliquot of the concentrated dye.
2. Use a second test tube to collect 4 mL of the diluent (diH2O).
3. Pipette at least 800 µL of diH20 into each of the 4 remaining tubes. These will become your samples.
4. Use the dropper to dispense varying amounts of the concentrated dye into each sample tube.
5. Pipette 200 µL of your first sample into a well of the 96-well plate. Repeat this 3 more times so that you have 4 replicates of that sample in 4 wells of the plate. Write down which wells you placed this sample into (you will likely be sharing your 96-well plate with other students - you want to know which samples are yours!).
6. Repeat the above step for your remaining 3 samples. You should 4 wells of each sample, for a total of 16 wells, filled in the 96-well plate.
7. Quantify the absorbance of the 96-well plate at 450 nm.

While students complete Part B, plan on circulating around the lab to answer questions and observe student's pipetting technique, reminding students to record which wells they're using, and providing individual help as needed.

5. **Data Collection ~ 30 minutes**

Allow 30 minutes for data collection.

6. **Breakdown ~ 10 minutes**

Leave time at the end of the lab to briefly introduce the lab report and provide students with sufficient time to clean their stations and ask remaining questions.

Lab Report

Instructions

Assign students to prepare a comprehensive lab report that includes calibration curve data, interpolated sample values, and statistical analysis. Provide guidelines for report structure and data presentation. Some ideas for exploration are below.

- Establish the standard curve
    - Aggregate the individual values for your standards.
    - Find the mean and standard deviation in each.

    - Use the mean values to define the standard curve.
    - Determine how well the calculated curve fits the data points by calculating the R2 value. Recall that a line that fits the data points perfectly has an R2 value of 1. It's important to create a new standard curve with a calculated R2 value for each experiment.
- Interpolation of Sample Values
    - Use your calculated standard curve to interpolate sample values manually.
    - What is the standard deviation between replicates of each of your samples?
    - What are some possible reasons for this variance?
    - What are the benefits of measuring replicates?
- What trends can you observe amongst different sets of samples? Are any of the observed differences statistically significant?
    - Are there any outliers? How does the use of statistics allow for the assessment of outliers vs cherry-picking data?

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Student Guide

Measurements and Statistical Analysis

Purpose

This lab will develop your understanding of using serial dilutions to generate a standard curve, and the use of standard curves for interpolating sample values. This includes an appreciation of:

- Basic pipetting skills
- Serial dilutions
- Measuring replicates
- Range of the standard curve
- Use of statistics to assess significance

In this lab students will use data obtained by manual pipetting as well as the OT-2 automated liquid handler, allowing students to see the strengths and opportunities of both techniques.

Required Equipment

- Opentrons OT-2 Automated Liquid Handling Robot
- Opentrons p300 8-channel pipette
- Opentrons 300 µL Tip Rack
- Manual pipette (P1000)
- 12-well trough
- 96-well Flat Bottom plate
- 1000 mL deionized water (diH2O)
- FD&C Blue No. 1 (McCormick® was used in the development of this lab)
- 6 Test Tubes
- Dropper

Experimental Procedure

Part A: Observe Standard Curve Generation

The OT-2 will perform automated pipetting of the serial dilutions. When your educator measures these standards with the spectrophotometer they will be able to generate a standard curve.

Your educator will follow these instructions to prepare and run the OT-2 robot. Steps 1-4 will be completed manually (by your

educator or student volunteers) before running the automated OT-2 robot steps:

1. Prepare the blue dye solution with 50 µL of blue food dye and 10 mL of diH2O.
2. Add 200 µL of the blue dye solution to the wells A1-H1 of the 96-well flat bottom plate.
3. Add 20 mL of water to A1 well of the trough reservoir.

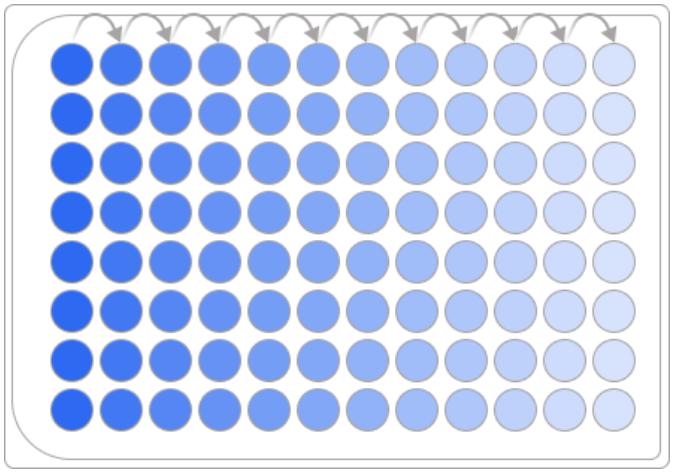
   (Note: The column after the last dilution is the default location of the blanks. The last well of the trough/reservoir is the default liquid trash.)

4. Arrange labware as follows:
   - Slot 1 = Opentrons 300 µL tip rack
   - Slot 2 = 12-channel reservoir
   - Slot 3 = 96-well flat bottom plate
5. Proceed to the OT-2 run app to run the automated protocol.

   - Download protocol from https://protocols.opentrons.com/protocol/customizable_serial_dilution_ot2
     - Select the following parameters on the webpage according to your setup and supplies:
       - Pipette Type

        - Mount Side
        - Tip Type
        - Trough Type
        - Plate Type
    - Keep the default settings for:
        - Dilution factor (3)
        - Number of dilutions (10)
        - Total mixing volume (150)
        - Blank in well plate (yes)
        - Tip use strategy (use one tip)
        - Volume of air gap (10)
6. Quantify the absorbance of the 96-well plate at 450 nm.

Note: Serial dilutions are performed by sequentially transferring aliquots of a standard into increasing amounts of diluent (see diagram). The OT-2 will aspirate an aliquot of the concentrated standards and dispense it into the diluent. It will then mix the concentrated dye and the diluent by repeatedly pipetting the liquid up and down. Then the robot will aspirate an aliquot of the first mixed standard and dispense it into the next column of diluent.



Part B: Conduct Manual Sample Preparation

1. Use one test tube to collect a 200 µL aliquot of the concentrated dye.
2. Use a second test tube to collect 4 mL of the diluent (diH2O).
3. Pipette at least 800 µL of diH20 into each of the 4 remaining tubes. These will become your samples.
4. Creating different samples with differing amounts of concentrate versus diluent: Use the dropper to dispense varying amounts of the concentrated dye into each sample tube.
5. Pipette 200 µL of your first sample into a well of the 96-well plate. Repeat this 3 more times so that you have 4

replicates of that sample in 4 wells of the plate. Write down which wells you placed this sample into (you will likely be sharing your 96-well plate with other students - you want to know which samples are yours!).

6. Repeat the steps 5 and 6 for your remaining 3 samples, so you end up with 4 replicates of each sample in 4 wells. You should 4 wells of each sample, for a total of 16 wells, filled in the 96-well plate.
7. Quantify the absorbance of the 96-well plate at 450 nm.

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Student Quiz

Measurements and Statistical Analysis

Student Quiz

1. What is the benefit of using serial dilutions to generate a standard curve?
2. Why is the generation of calibration curves important in biological measurements?
3. Name one potential challenge in generating calibration curves and suggest a solution.
4. Describe the role of the equipment used in this lab.
5. What are common sources of variability from manual pipetting?
6. Why is it useful to measure replicates of each sample?
7. What is the purpose of interpolating sample values in this experiment?
8. How can statistical analysis help interpret differences among various samples?
9. How might the findings from this lab be relevant for biological research?