BIO 13 Protein Investigation

Bradford Assay

Abstract

The use of inquiry-based activities in a team-based learning environment to develop quantitative reasoning, critical thinking, computational, and writing skills

F. Tamari and I. Ho

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I. Purpose and student learning outcomes

The primary purpose of this exercise is to determine protein concentrations using spectrophotometry and construct a standard curve representing concentrations of known proteins. The secondary learning purpose of this exercise is to create a team-oriented inquiry-based learning activity that promotes the development of critical thinking and learning skills parallel to the goals of National Science Foundation's *Vision and Change: A Call to Actions.*

The student learning outcomes for this exercise are:

1. Gain knowledge of proteins, protein concentrations and spectrophotometry
2. Perform an inquiry-based learning exercise in a group setting
3. Develop critical thinking skills through analysis of data and construction of graphs and/or tables to summarize data
4. Learn basic computational skills using Excel
5. Develop scientific writing skills

II. Introduction

Central Dogma of Genetics and Basic Proteomics

The Central Dogma of Genetics summarizes the processes that DNA can be involved in, namely DNA replication or transcription. Transcription may then be followed by translation. DNA replication is necessary for processes that involve proliferation of cells by mitosis. Both transcription and translation are involved in gene expression.

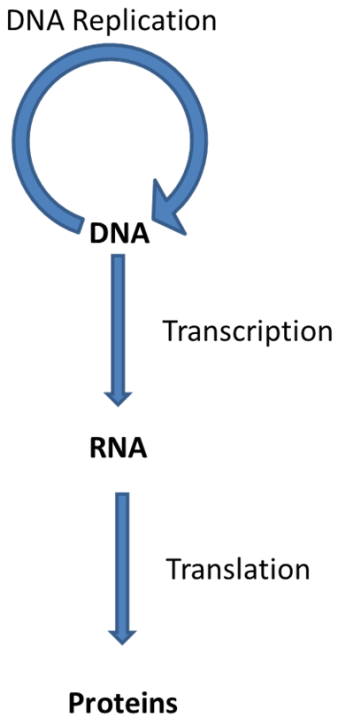

Figure 1: Central Dogma of Genetics. DNA can be involved in replication or transcription (which may be followed by translation).

Proteins produced as a result of gene expression can be quantified. Spectrophotometry is one way to determine protein concentrations. It can be done using multiple samples (up to 96 at a time) with a microplate reader. A common procedure used to quantify proteins is called the Bradford assay.

The Bradford Assay: An Example

Your protein quantification activity is a modification of a commonly used technique called the Bradford assay (Bradford, 1976). Known concentrations of a protein called Bovine Serum Albumin (BSA) will be placed in a 96-well dish at 0, 2, 4, 6, 8, 10 and 20 µg/µL concentrations. (BIO 65 students, in one well, a protein with an unknown concentration will be placed). (Figure 1).

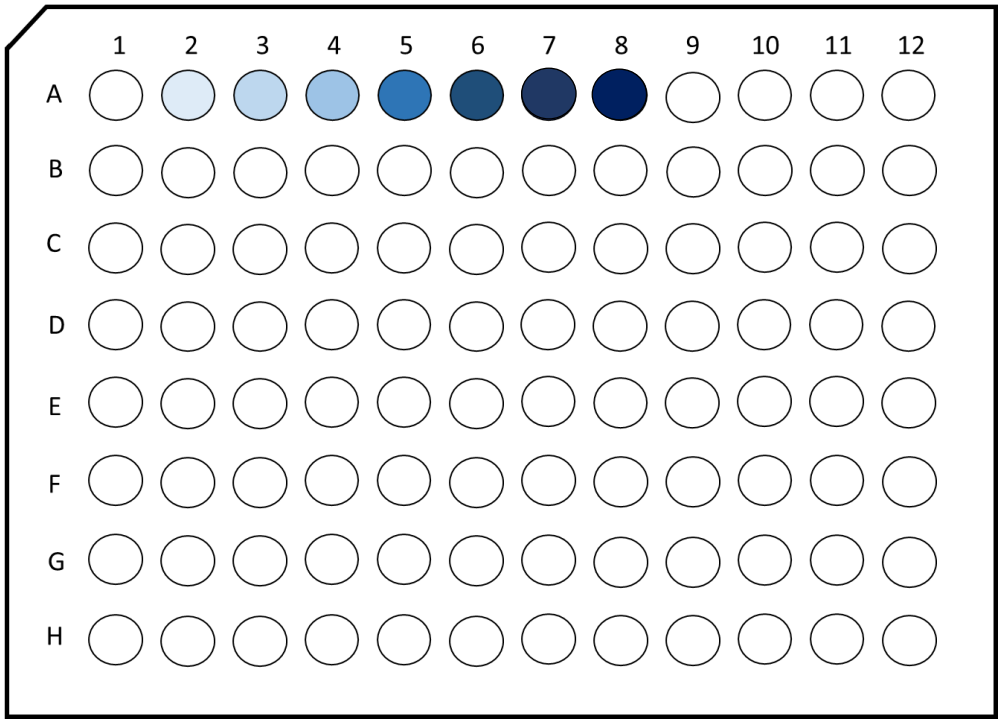

Figure 2: The use of a 96-well dish for samples A1-A8

The Bradford reagent reacts with proteins and produces a colorimetric reaction with colors of various intensities which correlate directly with protein concentrations (higher protein concentrations produce more intense colors). The produced colors can be quantified by using a microplate reader (spectrophotometer). Following the collection of this data (Table 1), a standard curve can be constructed with an equation of the line being used to determine the unknown concentration of a protein (Figure 2).

Table 1: Sample collected data

[Protein] µg/µL	**Absorbance**	**Well**
0	0	A1
1	0.05	A2
2	0.10	A3
4	0.25	A4
6	0.3	A5
8	0.38	A6
10	0.55	A7
20	0.77	A8

III. Pre-lab Activities

1. Define spectrophotometry.

2. Define absorbance.

3. Prelab Activity:
   In groups of 4 students, use the data from Table 1 (previous pages) to create a standard curve. You can use the graph paper below to do this activity. Once you have created the graph, discuss whether the graph can be used to determine protein concentrations for proteins with known OD readings.

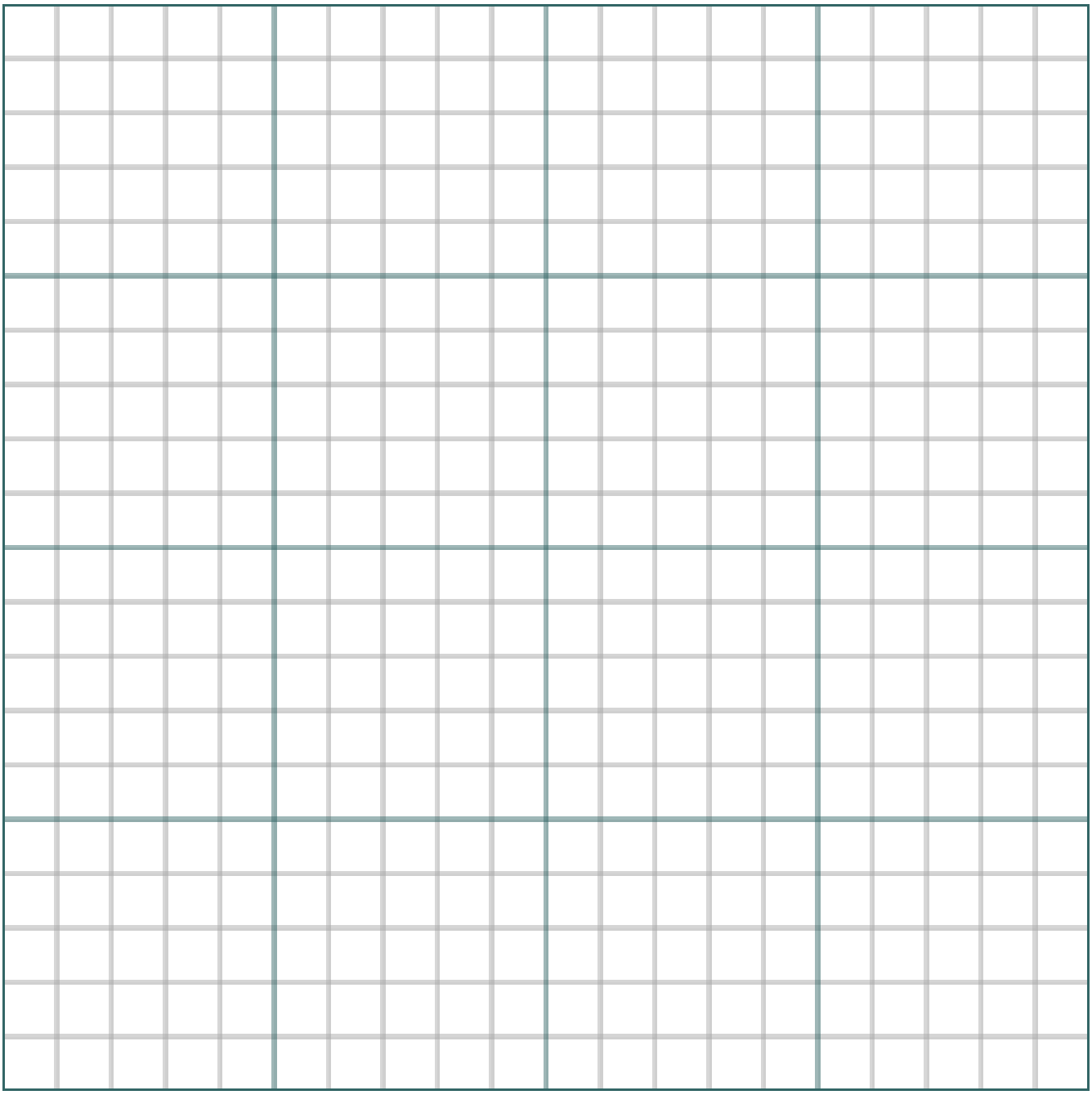

Figure 3: Standard curve plot of OD 595 nm against protein concentration

4. You are placing the following quantities of BSA (1 µg/µL) in wells A1-A8 for a total volume of 200 µL in each well. Calculate the volumes of each reagent that needs to be added and complete the table below.

Well	**[BSA] µg/µL**	**BSA (µL)**	**Water (µL)**	**Bradford Reagent (µL)**	**Total volume (µL)**	**Absorbance OD 595nm (A_{595})**
A1	0		100	100	200	0
A2	1		99	100	200	0.05
A3	2	2		100	200	0.10
A4	4	4		100	200	0.25
A5	6	6		100	200	0.3
A6	8			100	200	0.38
A7	10			100	200	0.55
A8	20			100	200	0.77

Table 2: Calculation of volumes to add for experiment

IV. Materials

Micropipettes: P20, P200, P1000

Microcentrifuge/1.5mL tube with Bovine serum albumin (BSA, 1 µg/µL)

96-well microplates

Distilled water

iMark Absorbance Microplate Reader

Bradford reagent

V. In Lab Activities

1. Obtain the following items from the instructor's bench:

    Micropipettes (P20 and P200)

    Microcentrifuge tube containing BSA (1µg/µL)

    Microcentrifuge tube containing dH_2O

    96 Well dish

    Bradford reagent

Place the calculated quantities from Table 2 in wells A1-A8. Make sure the correct quantities are placed in each well. First add BSA (A2-A8), then dH_2O, then the Bradford reagent. Check with your instructor if you have questions. Read absorbance (OD595nm) and record it for each sample in the following table. Your instructor will help with this.

Well	**[BSA] µg/µL**	**Absorbance OD 595nm**
A1	0	
A2	1	
A3	2	
A4	4	
A5	6	
A6	8	
A7	10	
A8	20	

Table 3: OD 595nm readings

VI. Results and Analysis

Generate a table similar to Table 3 using Excel. Use a scatterplot to plot OD595nm reading (Y axis) against [BSA] (X axis). Draw a line of best fit through your graph and display the equation of the best-fit line on the graph as well.

As an example, we have plotted the data in Table 1 in the graph below. Your graph will look similar to the graph below.

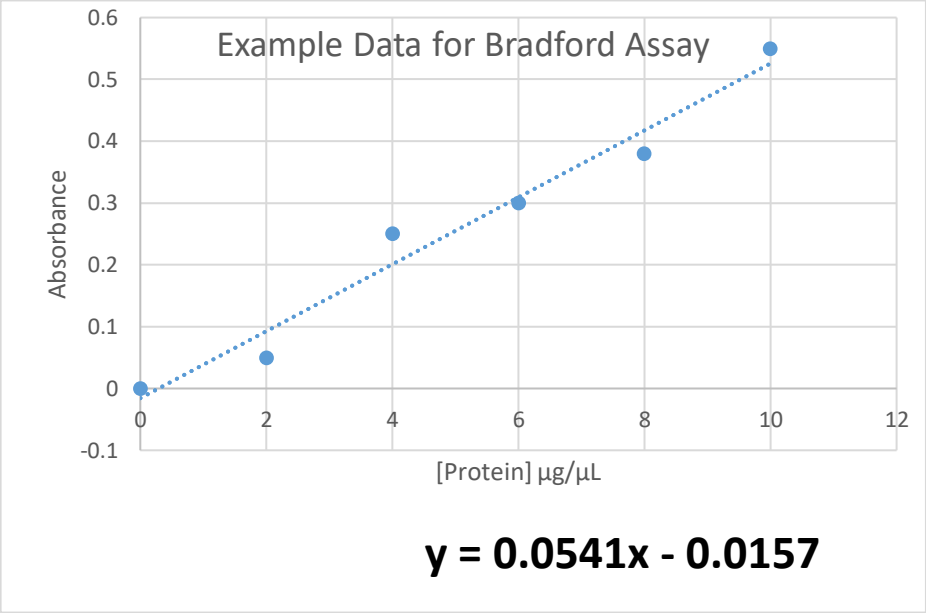

Figure 4: Example plot of OD595nm vs [protein]

VII. Post-lab Activities

Use quantitative reasoning skills to determine the unknown concentration for a protein by either using the equation of the line, or through using the graph. For example, using figure 4, you can solve for x (protein concentration) by substituting in the absorbance reading (y) to determine the unknown concentration of the protein in question. Here's how:

$x = (y+0.0157)/0.0541$ (µg/µL)

$x = (0.43+0.0157)/0.0541$ (µg/µL)

$x = 8.24$ (µg/µL)

Assignment 1:

In the space below, using your own graph and equation of the line, try to do this for an unknown [protein] with OD595nm=0.635

Assignment 2:

Write up one-page report and in it include 1. description and 2. Interpretation of your results. Submit this 1 page report with your Excel-generated graph for grading.

VIII. Appendix

Place the following quantities of BSA in each well following the table below:

Well	[BSA] µg/µL	BSA (µL)	Water (µL)	Bradford Reagent (µL)	Total Volume (µL)	Absorbance OD 595nm
A1	0	0	100	100	200	0
A2	1	1	99	100	200	0.05
A3	2	2	98	100	200	0.10
A4	4	4	96	100	200	0.25
A5	6	6	94	100	200	0.3
A6	8	8	92	100	200	0.38
A7	10	10	90	100	200	0.55
A8	20	20	80	100	200	0.77

Table 4: Volumes added for experiment