BI 171 Lab # 8

Part 1. Pre-Lab Assignment: *Tetrahymena* Biology

Using PubMed, find two articles related to (1) the function of Rad51, either in *Tetrahymena* or another organism; and (2) the environmental impact you are proposing to expose your *Tetrahymena* cultures to. One of these articles can be a review article, but you will need at least one primary literature article. Furthermore, you must acquire articles that are different from your other group member(s). Upload your two articles to the link posted on Moodle by the indicated due date. You will not be given credit for abstracts; the entire article must be uploaded for you to receive credit!

Part 2. Discovery Lab: How good are you at micropipetting – FOR REAL?

The objective of this experiment is to accurately measure microvolumes using a micropipet. The experimentally determined extinction coefficient of ρ-nitrophenol will help determine accuracy.

Introduction

Micropipets are the "workhorses" of small [microlitre (10-6 L), µL] biochemical measurements frequently used in molecular biology procedures. A reaction volume of 20 µL containing 4 or more reagents is very common. Consequently, proficiency in using micropipettes to achieve the accuracy needed in measuring reagents is essential.

The primary goal of this exercise is to verify and/or improve your proficiency in micropipetting technique using the experimentally derived extinction coefficient for **ρ-nitrophenol (PNP)**. The extinction coefficient can be calculated using **Beer's Law** ($A = c \cdot \Sigma \cdot l$, where A = absorbance, c = concentration (Molarity), Σ = extinction coefficient, lcm = pathlength). Beer's Law relates the dependence of the absorbance of a solution to its concentration via its extinction coefficient. It is also referred to as the absorptivity coefficient or the molar extinction coefficient. The accepted **extinction coefficient** for PNP is 1.7 x 104 M-1cm-1.

In this exercise, you will prepare four dilutions of a 4.12 x 10-3 M PNP stock solution. The absorbance of each dilution will be determined using a spectrophotometer set at 400 nm (the wavelength at which this compound best absorbs light). The absorbance values obtained will be then be used to determine the extinction coefficient which will, in turn, be used to determine the accuracy of your micropipetting. You will be using Linear Regression Analysis in order to analyze your data; the link to a **YouTube video** that describes how to perform Linear Regression using Excel has been posted to this week's Moodle page. You should refer to this video during lab to help with your analysis.

Procedure

1. Set the spectrophotometer to 400 nm.

2. Add 3mL of 0.10 M sodium carbonate to each of four 13 x 100mm glass test tubes.

3. Add the indicated volume of ρ-nitrophenol (see Table 1) to each of your 4 test tubes (1 test tube per dilution) and mix briefly by pipetting up and down.

4. Blank the spectrophotometer using 1mL of .1M sodium carbonate and record the absorbance of 1mL of each PNP dilution at 400 nm both in Table 1 and in an Excel spreadsheet.

Table 1

Tube Number	0.1M Carbonate	PNP Volume	Final PNP Molarity	Absorbance (400nm)
1				
2				
3				
4				

Results and Analysis

1. Calculate the Final PNP Molarity (M) of the PNP dilutions in each of the tubes; these values are the **Expected concentrations** of PNP for each of your dilutions. Record the values in Table 1 and in your Excel spreadsheet.

2. Plot Absorbance (Y ordinate) versus Final PNP Molarity (M) (X abscissa) for each dilution and fit a straight line to your data set (most substances obey Beer's Law and produce a straight line). Use (0,0) as the origin for your graph.

3. The experimentally determined molar extinction coefficient (Σ) for PNP can be determined from the slope of the best-fit line generated from your data (Why? *Hint: Solve Beer's law equation below for* Σ.). Include the appropriate units on the Σ values obtained.

4. In Excel, use the experimentally determined molar extinction coefficient (Σ) for PNP to calculate the experimentally Observed PNP concentrations for each of your four dilutions.

5. Determine the % error of each data point from the best-fit line using the following formula:

   ((Expected PNP concentration – Observed PNP concentration)/Expected PNP concentration)(100%))

   You must repeat the above analysis for any dilution that has an error greater than 5% !

Upload the following to Moodle by the end of lab today:

1. The graph demonstrating that you were successful at pipetting the indicated volumes within 5% error.
2. A brief discussion regarding the accuracy of your micropipetting technique.

Part 3. Inquiry Lab: Effect of environmental factors on the expression of *Rad51* in *Tetrahymena*

Experimental Design:

As we discussed last week, the remainder of the semester will be devoted to testing the effect environmental changes have on the expression of the *Rad51* gene. The *Rad51* gene encodes the Rad51 protein which is involved in repairing single- and double-stranded DNA damage in the organism *Tetrahymena thermophila* (and in us!). During today's lab, your group will be designing an experiment to test the effect of environmental changes on *Rad51* expression in lab cultures of *Tetrahymena thermophila*. There are several environmental factors that are currently affecting the livelihood of these microscopic Protists including temperature, pollution, and pH. You will want to make sure that the environmental changes you plan to study are reasonable (in other words, they won't immediately kill the organisms!) and relevant to environmental changes

the *Tetrahymena* are currently facing. During next week's lab you will be presenting your proposal to the entire lab class and we will be deciding together which lab group's experimental design will be implemented for the remainder of the lab module.

Part 4. Post-Lab Assignment: *Tetrahymena* research proposal

With your lab partners, write a one-page outline of your proposed study that includes the following content:

1) Title: Should give the reader a clear idea of the specific question being addressed.
2) Authors: Names of all the participant investigators.
3) Question: Restate the research question by including the specific variable you intend to investigate.
4) Hypothesis: Stated in a manner that is **quantifiable** and **observable**.
5) Methods: How you propose to test your hypothesis.
   a) Be certain your methods section includes definitions of your variables (dependent and independent), a description of your control and treatment conditions, how you intend to randomize the treatments, and how many replicates you intend to manage.
   b) Briefly describe your set-up and how you will be collecting the data.
6) Prediction: Be sure to include a description of your variables and how you predict the independent variable will affect the dependent variable.
7) Materials: Please provide us with a list of materials we need to have available for next week's lab.

After typing your outline, designate one person from your group to immediately (before leaving lab) submit your outline in the form of a WORD file on MOODLE, in the designated assignment titled **Post-lab Assignment 7**. Your research proposal will be evaluated on the following criteria: completeness, organization, clearly articulated hypothesis and prediction, a materials list, and basic writing skills (spelling, grammar, sentence structure, etc.).