Cell & Molecular Biology Laboratory:

Enzymology (Tyrosinase) Report, Expts. 5 & 6

Due Tues., Mar. 19, 2018 (5-6 *typed* text pages, MAXimum!!); **50 points total!**

Note: *Please write your reports in PAIRS (2-3 students, maximum), but ONLY* ***use your OWN members' words*** *and* ***only tabulate and graph data from your group****. You may refer to other groups' data for comparison.* ***Both authors must hand-sign the report, and include a statement that you BOTH contributed EQUALLY*** *(or an estimated percentage contribution each partner made) to performing the experiments, presenting the data, & conducting the analysis and writing of the report.* ***Please type with 12 point font, with 1.5 spacing between lines****.*

- ***Thoughtful analysis, careful explanations, and well-organized, well-presented, and well-explained data are the keys to your grade!!! Use BOLD headings for each section.***
- *The attached grading rubric is a general guideline for grading biology lab reports. Not all standards may apply here.*

1. **Introduction** (0.5-1 page; **6** points): In your own words and from your own point of view, give a thoughtful overview of experiments 5 & 6. Recognizing the *SIGNIFICANCE of your scientific investigations* is always crucial to scientific professions. Therefore, you should include some ***background information on tyrosinase, DOPA and/or melanin***. Also ***discuss enzymes and why they are important*** to biology, the environment, medicine, and/or our scientific understanding of life in general.
   a) Be sure to cite any outside sources according to the following format (see also the *Cell* journal example with the link below)**: Author - Last, First name(s). (Year published). "Title of Article or chapter." *Title of Journal or Book*. Publisher and Location, if a book.** *Parenthetical In-Text Citations (eg: APA style or CBE/CSE) are preferred.* ***[see Syllabus for more on citations]***
   b) **What is the Question/Hypothesis we are testing in each experiment** (from YOUR point of view? – Write the Hypothesis and your Predictions for the Experiment in**: *"If____, then ____" format).*** What is the overall **purpose** of the experimental investigations? **Why** are we doing these experiments (not only from an educational perspective, but why is it important to understand tyrosinase and how enzymes work in general?). **How** are we performing the investigations? What laboratory techniques, experimental methods (in general), and general strategies are we using? What predictions can we make based on our hypothesis, & how will we make *unambiguous interpretations* of the results *(hint: consider the* ***control samples*** *we used, & if there are others we could/should have used)*?

2. **Methods** (0.5-1 page; **6** points): These do not to be as detailed as the examples given in the professional versions below. Mainly, just reference the lab manual, but ***include any special alterations we or you made to the experimental procedures***. Be sure to **include** any important details of conditions, reagents, and control samples used in your Self-Designed Investigation for **Experiment 5.3,** and the **precise conditions we decided to use for in-gel enzyme detection** for Experiment. 6. ***What was the purpose of the different buffers and reagents we used in the experiment (****why do they contain the chemical ingredients that they have****)?***

3. **Results & Discussion** (3-4 pages of text; **26** points): Include **ALL DATA** in the form of Tables, Charts/Graphs, & Image/Photoscan Figures. Be sure to **label each data figure/table with an appropriate Title** *(ie: pH data activity all on one line graph & your self-designed experimental data all on one graph. Also include the Rf graph from the protein molecular weight size standards!!).* You should also **include a complete legend & caption, briefly describing the samples in each figure and the conditions that were used. LABEL all lanes and protein bands on your gel.** *[Data doesn't count towards the limit on the # of text pages.]*

Be sure to ***discuss the implications of the data, what we learn from it, and what it means*** in the **big picture** of how biological systems/cells work. Consider the ***thought Questions*** included in the Lab Manual for each experiment – these address concepts that you should incorporate into your analysis of your experimentation and results. What conclusions can we make about the pH preferences of tyrosinase & it's mobility and activity in Native PAGE (see data images under Additional Materials on the course website)? *Did the data **prove your/our hypotheses**? If not, how should we alter our hypotheses and retest them?*

- **The Results/Data and Discussion section should have THREE main PARTS:**
  - **a)** [Expt. 5.1-5.2] Enzyme extraction and colorimetric (spectrophotometric) assay -- I want LOTS of explanation on how we do these and WHY we use the buffers, reagents and conditions we use (eg: I even want you to mention why we keep extracts, especially protein extracts, on ICE!). ***I always want you to be THOROUGH but BRIEF in your explanations (ie: explain the main things well, but get to the point!).***
  - **b)** [Expt. 5.3] Student Investigation -- as for all the report, you need NOT list much of the procedural details from the Manual that were used (just reference the lab manual for basic procedures). However, you MUST give a DETAILED description of your self-designed experimental conditions -- temperatures, buffers, reagent concentrations, CONTROLS, times for readings, etc. There might have been mistakes or difficulties with your experimental methods, but ***if there were additional CONTROLS you should have done*** in hindsight, or unexpected variables you could not control, these must at least be DISCUSSED in the report.
  - **c)** [Expt. 6] Native PAGE and Activity Staining: Basic explanation of PAGE and how it works, all data and graphical analysis from the gel (with complete legends), why we used the sample, electrophoresis, and activity buffers that we did. ***What did we do to speed-up the in-gel reaction?*** You should give precise concentrations and names of reagents.
  - **d)** NOTE: Data tables, charts, and images may take up AS MUCH SPACE as you need, and do not count towards your written text page length.

4. **Conclusions** (0.5 page; **6** points): Summarize the major "take-home" deductions that we can make from the data, and that are most significant to our understanding of enzymes and biology. **WHAT were the major hypotheses that you tested? Were these hypotheses proven or disproven? Explain.**

*Were you able to form an **unambiguous answer** to your tested questions/ hypotheses? If not, **what additional experiments, samples, or controls should we have used**?*

What other investigations could you conduct with our newly-learned lab methods, or what other aspects of tyrosinase, DOPA, or other enzyme activities do you think would be topics of interesting investigations? In other words, try to think outside what we did in our little laboratory experiment. ***Write a hypothesis statement for one of these experiments*** *(Use **"If… (this is a true answer to our SCIENTIFIC question), then…(this will happen as a result of our experiments)."** format).*

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5. **REFLECTION** (0.5-1 page; **6** points):
EACH Lab Partner must include a PERSONAL REFLECTION *about your own learning experience in the laboratory, following the guidelines below. This may be part of the Conclusions.*

a) ***What did you do well?***

- **What went best about the lab, and that you were strongest at completing?**
  - **Eg: Forming hypothesis, designing the experimental methods, collecting and analyzing data, organizing and presenting Data in Graphical format, and/or writing conclusions and discussions about results and how they relate to Cellular and Molecular Biology??**

b) ***What problems or challenges did you encounter in completing the assignment? How did you resolve them?***
   - ❖ **What was toughest to do or complete during the process of designing, experimenting, and writing the report on your results (see above sub-sections).**
   - ❖ **What difficulties delayed successful experimentation, data collection, analysis/interpretation of data, or writing of the report? Were certain lab techniques difficult to learn and/or master? (Social/communication difficulties with lab partners are legitimate complications as well!)**
   - ❖ **How did you find a solution to these difficulties?**

c) ***What aspects of the project are you especially proud of? Explain.***
   - ❖ **Where in the process of experimentation and writing the report do you feel you really excelled, and would like to show-off to your friends/family or to the world?? ☺**
     - **Explain why you are particularly proud of these parts of the project.**

d) ***What processes led to the successful completion of your assignment.***
   - ❖ **What did you have to do mentally/intellectually, socially/communication-wise, and physically to do a good job with the experimentation, analysis, data presentation, and writing of the report?**

e) ***How would you improve the assignment if you had additional time and resources?***
   - ❖ **What other questions or hypotheses would you like to investigate about Cellular Metabolism, Protein function, or Enzyme function?**
   - ❖ **What other experiments or procedures would you incorporate into your Investigation?**
   - ❖ **What other equipment or resources would help you produce better (eg: more consistent) results or analysis of your data?**

❖ ***It is STRONGLY recommended that you have your instructor give you a brief review of your semi-final Report (draft) at least a few days before it is due!!!***

Here are some real-life, good examples of professional scientific articles. Three are even related to our lab topic! Don't get overwhelmed by the technical content, just look at the format, organization & Citations, and read the abstracts / summaries.

- **http://www.cell.com/abstract/S0092-8674(09)01043-5**
- **http://www.jbc.org/cgi/reprint/279/9/7971**
  - *Open the PDF file to see a very standard format for biology papers.*
- **http://www.jbc.org/cgi/reprint/191/2/799.pdf#search=%22Dopa%20tyrosinase%22**

Laboratory Report (50 Points total) *Possible* Grading Rubric:
Tyrosinase Activity: Spectrophotometry and Native PAGE Labs

Professor Name: **Dr. Nathan Staples**

Student Name(s): _____

CATEGORY	Excellent (6 or 7 points)	Good (4-5 points)	Fair (2-3 points)	Poor (0 - 1 points)
1. Introduction: Background & Sources	Introduction demonstrates good background research on enzymes and tyrosinase, with scientific citations.	All required elements are present.	One required element is missing, but additional elements that add to the report (e.g., thoughtful comments, graphics) have been added.	Several required elements are missing.
2. Introduction: Goals & Hypothesis	The purpose of the lab or the question to be answered during the lab is clearly identified and stated. The hypothesis and strategy for the experiments are clearly stated and thoroughly explained.	The purpose of the lab or the question to be answered during the lab is identified, but is stated in a somewhat unclear manner.	The purpose of the lab or the question to be answered during the lab is partially identified, and is stated in a somewhat unclear manner.	The purpose of the lab or the question to be answered during the lab is erroneous or irrelevant.
3. Materials and Methods	Procedures are listed in clear steps. Each step is a complete sentence. Strategy is mentioned, and details focus on specific alterations of the provided procedure by one's own lab group (esp. experimental Conditions and concentrations of reagents used).	Procedures are listed in a logical order, but steps are not in complete sentences, or important details are missing.	Procedures are listed but are not in a logical order or are difficult to follow, and are missing important details.	Procedures do not accurately list the steps of the experiment or specific details (concentrations of reagents for eac part, etc.).
4. Colorimetric/ Spectro-photometric Data (7 points)	Professional looking and accurate representation of the normalized data in both tables and graphs. Graphs and tables are accurately labeled and titled, and all graph lines or bars are distinguishable by pattern or color. Experimental design is a well-constructed test of the stated hypothesis. Investigators planned and used proper control samples for comparisons.	Accurate representation of the data in tables and/or graphs. Graphs and tables are labeled and titled.	Accurate representation of the data in written form, but no graphs or tables are presented.	Data are not shown OR are inaccurate.
5. Electrophoresis Gel Data (7 points)	A clearly labeled (each lane) and captioned photo of your PAGE gel is included. A standardized Molecular Weight graph (Rf vs. kDa MW) is included, and shows interpolated kDa estimations for Tyrosinase activity band(s).	Gel Data and graphed standards are included, with captions, but are not complete.	Gel Data OR graphed standards are included, with captions, but one is missing and is not complete.	Most gel data and graphs are missing or are not properly labeled or analyzed.
6. Analysis and Discussion *(More points may be assigned to this section!)*	The relationship between the variables is discussed and trends/patterns logically analyzed. Predictions are made about what might happen if part of the lab (variables or methods) were changed or how the experimental design could be changed and improved.	The relationship between the variables is discussed and trends/patterns logically analyzed.	The relationship between the variables is discussed but no patterns, trends or predictions are made based on the data.	The relationship between the variables is not discussed.
7. Conclusion	Conclusion includes whether the findings supported the hypothesis, possible sources of error, and what was learned from the experiment.	Conclusion includes whether the findings supported the hypothesis and what was learned from the experiment.	Conclusion includes what was learned from the experiment.	No conclusion was included in the report OR shows little effort and reflection.
8. Personal Reflections	Brief but thorough and insightful thoughts are given about the process of experimentation, working with peers, and how to devise new or improve future investigations.	Thoughts are given, but 1 or 2 are neither complete nor well-conceived.	Thoughts are given, but most are neither complete nor well-conceived.	Very little thought and/or effort is put into the reflection.