



## Biochemistry Lab II Semester Guide



### Dr. Provost's Safari Guide to Hunting Malate Dehydrogenase

Or

How to bag the elusive mutant MDH

*"There must be no barriers for freedom of inquiry. There is no place for dogma in science. The scientist is free, and must be free to ask any question, to doubt any assertion, to seek for any evidence, to correct any errors."*

**Robert Oppenheimer**

***This document is to serve as a guide to help you find your way through this lab.*** It is NOT the answer to "what do I need to do now?" That answer is for you to provide, or at least search for. This guide is meant to point you in the right direction and give you the bigger picture of what your semester will be. There are several phases to the semester. Each is described below with critical points and questions for each phase that you should be concerned with. Remember, this is new ground for many of you. The goal is to push you into a place where you may feel uncomfortable where you find the answers yourself, and will not have your hand held. *"Fear is the little mind killer. Fear is the little-death that brings total obliteration. I will face my fear". (Frank Herbert, Dune)* And now without further adieu...

**Semester Project Overview:** First, you will work on your own through the first phase of the semester, where you will learn to work on the DNA and amino acid sequences and learn how to search and render the three dimensional structures. Then you will form groups of two or three, research MDH, propose a project based on scientific evidence, plan and execute the plan and then present the work

### **Phase I Bioinformatics, Structure and MDH Background:**

- Before starting any research project or work in industry, it is critical to first learn the tools and get enough background. So each student will first read and follow the instructions on the Modeling Workshop Handout on the class website. There are several tutorials linked with the handout. Read each step of the handout, watch each of the tutorials and then complete the homework assignment at the end of the handout. Once you are finished you should be able to search and find DNA, aa, and structural sequences for any protein including MDH.
- The next thing you need to do is to learn about the protein you will be working with. While part of the homework in the handout, it is key that you know the protein you are working with. The easiest way to do this is to answer a handful of questions.
  - o What is MDH?
  - o What reaction does it catalyze? What is the function of MDH in the cell?
  - o What is the molecular weight of the enzyme? Is MDH a monomer or does it have several subunits?
  - o Is the enzyme regulated?
  - o What are the domains / key amino acids for binding the substrate and the reaction?
  - o Is there more than one isozyme? What are the differences between enzymes from various sources (rice, yeast, liver, ect...).
  - o What is the aa and / or nucleotide sequence for the protein?



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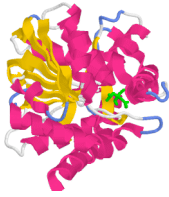
Now you should be asking yourself, how the heck do I find this information? Information of this sort isn't found in a textbook. And that is exactly the point. Very little of what you need after college is in a textbook. The information comes primarily from publications and databases. There is a ton of information out there and the strong will learn how to separate and scan through all of the important data. So your first task is to answer these questions by searching pub med and other literature and internet sources. **After finishing the homework in the Modeling Handout, take the time to answer each of these questions in your notebook.**

### **Phase II Designing the Experiment:**

- Now that you have already determined what MDH is, what it does (both reaction and function in metabolism), it is time to decide what you are going to do.
- Form into groups of 2 or 3.
- On the web, you will find a list of different wild-type MDH isozymes and several point mutations as well as a list of possible project ideas. Your job will be to look at the list, consider the key characteristics, critical amino acids and important domains of MDH (learned in phase I), review the papers you found and think/create an observation (from the literature) that you can create a hypothesis using the clones on the list. Remember, you need to move beyond "consumer science" (which soap is better...) and think about a true hypothesis. You are NOT limited to the list of suggested experiments on the web. If you have other MDH or MGH related ideas, talk to your instructor to further develop these ideas.

**QUESTIONS/POINTS of CONSIDERATION:** Start with an observation about the enzyme(s) (this will come from the literature), create a scientific question and then a hypothesis. Look up the scientific process on either the internet or from your cell biology lab notes! Now consider how will you test your hypothesis. You should be thinking of general ideas not minutia at this time.

- Once you've gotten a hypothesis and a general idea to test, plan the expression (see the link on the web), the purification (with detail) of your protein, and the experiments you plan to conduct to test your hypothesis. Your experiments should be prepared with enough detail so that you can easily do the experiment with limited additional work. Enzyme assays are linked on the web. Additional resources can come from your instructor, the web or other sources.
- For expression, each group will have to start from a single colony growing on a culture plate and culture the bacteria in liquid media with antibiotics, expand the culture, induce the expression, harvest and lyse the cells. Instructions for this will be specific and placed on the class web page.
- Review your plan with your instructor to refine and approve the project. Ensure that your instructor knows well in advance which clones you will need. You will be given an LB agar plate with a streak of bacteria that will express your protein.



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MSUM Biochemistry

- Prepare a 10-15 minute presentation to be given to the class. This is to be a simple, short and informal presentation on your hypothesis, the reasoning you got to this hypothesis, the clones you are using and an outline of the experiment with expected results!

### **Phase III Conducting the Experiment:**

- Start by expressing your protein, creating the lysate and use the Ni-Affinity column to purify the protein. SDS-PAGE (not western blot) analysis can be conducted at the users discretion.
- Test your hypothesis.
  - Don't forget to review the data as you go! Don't fall into the trap of analyzing the results later. You will never know what worked or didn't and needs to be repeated this way.
  - Plan how long your experiments will take BEFORE starting. This include planning some time to make solutions/buffers and for CLEANING UP! Use backward planning - plan what time you want to be done and then go backwards with the time for each step to see when you should start.
- Prepare a final Presentation of the results. Details for your presentation will be loaded onto the web.