Biochemistry 2L06 (2009)

Instructor:
Dr. Felicia Vulcu (vulcuf@mcmaster.ca)

Undergraduate Laboratory Staff:
Adam Pyke (pykead@yahoo.com) - Coordinator

Teaching Assistants:

<table>
<thead>
<tr>
<th>Stephanie Au Young</th>
<th>Ryan Kelly</th>
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<tr>
<td>Melissa Ayers</td>
<td>Michael Lung</td>
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<tr>
<td>Iva Bruhova</td>
<td>Ye Xu</td>
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<td>Vanessa D’Costa</td>
<td>Kate Pengelly</td>
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<td>Carmen Giltner</td>
<td>Peter Spanogiannopoulos</td>
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<td>Sean Jackson</td>
<td>Morgan Wyatt</td>
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<td>Seiji Sugiman-Marangos</td>
<td>Eric McNicholl</td>
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Lecture: every Monday 9:30am (MDCL-1309)
Labs: Every Wed/Thurs/Fri 2:30-6:30pm

ALL LECTURES AND LABS ARE MANDATORY!
Attendance to lectures and labs is mandatory! One missed lab (or lecture) without proper documentation from the Associate Dean’s office constitutes a warning and a mark of 0 on the lab notebook, the pre-lab exercise and the quiz (this mark will count towards your final mark); two missed labs (or lectures) will result in a mark of 0 for this term. Students are NOT to change lab periods unless specified by the Associate Dean’s office. Please bring your lab courseware to ALL labs and lectures.

**Introduction to 2nd term:**

The 2L06 lab course is designed to introduce students to the inner-workings of project development and execution. Though the majority of the project development has already been established students are responsible for the execution and understanding of the main goals of this project. The 2L06 project objective is to characterize a mutant form of DHFR. The first part of this project was completed in term one upon creation of both a wildtype \textit{folA} gene and a mutant \textit{folA} gene. This term focuses on teaching a number of technical skills in the laboratory in the context of working with the products of these two genes: DHFR protein. The first 4 labs are designed to teach students 4 basic and highly utilized experimental techniques: 1. Protein quantification, 2. Column chromatography, 3. SDS-PAGE and 4. Protein function determination (enzyme kinetics). Lectures for this portion of the course will emphasize the step-by-step technical protocols, the advantages and disadvantages of each technique along with a working knowledge of each technique. It is the responsibility of all students to read the lab each week PRIOR to coming to both the lecture and the lab. A short pre-lab exercise will be completed by students each week in lecture and handed back at the end of the lecture. At the beginning of the lab (each week) students will be expected to complete a short quiz designed to test their knowledge of the day’s lab. The weekly pre-lab exercise and quiz will continue for the entire semester.

This term also features a PBL component in which students will be assigned to groups of 6 and will be expected to research the role of DHFR and one technique related to its expression/purification/activity that will be performed later on in the term. Students are expected to put together a 15 minute presentation along with a 2-page handout which will be distributed to the course instructor (Dr. Felicia Vulcu), Adam and the Teaching Assistants PRIOR to the presentation. A 10-15 minute question period will follow each presentation.

The remaining 6 labs will allow students to further exercise their skills and expertise obtained during the first part of the term to express, purify and test the activity of their \textit{folA} and \textit{folA}\textit{I115M} gene products obtained during the first term. The wild type and mutant \textit{folA} genes will be transcribed and translated and the resulting over-expressed DHFR proteins will be purified using Ni-NTA and tested for activity. This is the main purpose of the 2L06 course: characterization of the I115M mutant DHFR protein.
Course Layout:

<table>
<thead>
<tr>
<th>Week of:</th>
<th>Description</th>
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<tbody>
<tr>
<td>Jan 5</td>
<td>Lab # 1</td>
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<tr>
<td>Jan 12</td>
<td>Lab # 2</td>
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<tr>
<td>Jan 19</td>
<td>Lab # 3</td>
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<tr>
<td>Jan 26</td>
<td>Lab # 4</td>
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<tr>
<td>Feb 2</td>
<td>PBL Week (group presentations!)</td>
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<tr>
<td>Feb 9</td>
<td>Lab # 5</td>
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<tr>
<td>Feb 16</td>
<td>READING WEEK!!!</td>
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<td>Feb 23</td>
<td>Lab # 6</td>
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<tr>
<td>Mar 2</td>
<td>Lab # 7</td>
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<tr>
<td>Mar 9</td>
<td>Lab # 8</td>
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<tr>
<td>Mar 16</td>
<td>Lab # 9</td>
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<tr>
<td>Mar 23</td>
<td>Lab # 10</td>
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<tr>
<td>Mar 30</td>
<td>Report # 2 tutorial</td>
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<tr>
<td>Mar 31</td>
<td>Report # 2 DUE!</td>
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Mark Distribution:
1. PBL component (7.5%), Term 2
2. Midterm (7.5%), Term 1
3. Report 1 (5%), Term 1
4. Report 2 (15%), Term 2
5. Lab Participation (15%), Terms 1+2
6. Quizzes and Prelabs (15% each), Terms 1+2
7. Notebooks (20%), Terms 1+2

Due dates:

Pre-Lab Exercises – in-class exercises due at the end of each lecture in class. If not turned in during class time you will receive a mark of 0 for that exercises (this mark will count towards your final mark).
Quizzes – in-lab quizzes at the beginning of every lab.
Lab Notebooks – due at the end of each lab period
Lab Notebook Discussions – due the week following the actual lab. All discussions need to have 2 primary literature references embedded in the text that pertain directly to specific points in the discussion. (Please ensure that you read the journal article you are referencing!). The references have to be different from lab to lab but can be used again for the final report write-up.
Final Report # 2 – Due March 31st by 2:30pm (NO LATER, 20% deduction if handed in later than 2:30pm, followed by 20% deduction each following day).

Please follow the same protocol for maintaining notebooks as highlighted in first term (posted on WebCT).
**Lecture/ tutorial outline:**
The Lecture component for this term will be in the format of a weekly tutorial. Lecture notes will NOT be posted on WebCT so take notes and pay attention!

<table>
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<tr>
<th>Date</th>
<th>Description</th>
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<tbody>
<tr>
<td>Jan 5</td>
<td>• Introduction to the 2nd term (includes handing out custom courseware, describing the PBL component, etc.)&lt;br&gt;• Brief background knowledge of Bradford/Lowry assays&lt;br&gt;• Overview of Lab # 1 (TA)&lt;br&gt;• Pre-lab exercise # 1</td>
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<td>Jan 12</td>
<td>• Brief overview of column chromatography (includes gel filtration and affinity chromatography, advantages and disadvantages, etc.)&lt;br&gt;• Overview of Lab # 2 (TA)&lt;br&gt;• Pre-lab exercise # 2</td>
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<tr>
<td>Jan 19</td>
<td>• Brief overview of SDS-PAGE&lt;br&gt;• Overview of Lab # 3 (TA)&lt;br&gt;• Pre-lab exercise # 3</td>
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<td>Jan 26</td>
<td>• Brief overview of alkaline phosphatase&lt;br&gt;• Overview of Lab # 4 (TA)&lt;br&gt;• Pre-lab exercise # 4</td>
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<td>Feb 2</td>
<td>• Guest lecturer</td>
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<td>Feb 9</td>
<td>• Tutorial on lab report writing (writing an introduction)&lt;br&gt;• Overview of lab # 5&lt;br&gt;• Pre-lab exercise # 5</td>
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<td>Feb 23</td>
<td>• Tutorial on lab report writing (figures/figure captions)&lt;br&gt;• Overview of lab # 6&lt;br&gt;• Pre-lab exercise # 6</td>
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<td>Mar 2</td>
<td>• Tutorial on lab report writing (materials and methods)&lt;br&gt;• Overview of lab # 7&lt;br&gt;• Pre-lab exercise # 7</td>
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<td>Mar 9</td>
<td>• Tutorial on lab report writing (results)&lt;br&gt;• Overview of lab # 8&lt;br&gt;• Pre-lab exercise # 8</td>
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<tr>
<td>Mar 16</td>
<td>• Tutorial on lab report writing (discussion)&lt;br&gt;• Overview of lab # 9&lt;br&gt;• Pre-lab exercise # 9</td>
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<td>Mar 23</td>
<td>• Tutorial on lab report writing (lab report guidelines)&lt;br&gt;• Overview of lab # 10&lt;br&gt;• Pre-lab exercise # 10</td>
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<tr>
<td>Mar 30</td>
<td>• No class</td>
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PBL guidelines (week of February 2nd)

Each group will be assigned a lab corresponding to the custom courseware. The students are to explain the background of this lab in detail, the purpose of this step in the overall context of the course, the advantages and disadvantages of this technique and elaborate on other techniques (which will be specified) that can be implemented in place of the technique in question. Also, all the students are to introduce the function of DHFR along with the purpose of this study including the mutant DHFR that was created. The presentation and handout guidelines are described below.

Labs to be assigned and points that need to be mentioned in the presentation ON TOP of explaining the purpose (BACKGROUND KNOWLEDGE) and procedure of the lab:

1. **Lab 5 – folA Expression (cell harvesting and buffer preparation)**
   - Centrifugation (principle of centrifugation for separation of mixtures)
   - Why do bacteria pellet at a specific speed?
   - How can ultracentrifugation be used to separate membranes from cytoplasm, ribosomes, mitochondria, ER, etc.?

2. **Lab 6 – DHFR Cell Lysis**
   - Alternative methods for cell lysis (French press, sonication, enzyme lysis)

3. **Lab 7 – Ni-NTA Purification of His(6)-DHFR**
   - Alternative tag purification (HA, Flag, GST)

4. **Lab 8 – DHFR Characterization (Protein visualization using SDS-PAGE)**
   - Alternative methods for protein visualization (Western blots, Blue Natives, IEF)

5. **Lab 9 – DHFR Characterization (protein concentration determination: Bradford assay)**
   - Alternative methods (BCA assay, A280)

6. **Lab 10 – Kinetics of DHFR**
   - Alternative methods for enzymatic activity (how would you measure the substrate or product of the reaction)?

Layout of presentation:

1. What is the purpose of the 2L06 project (full year)?
2. What is DHFR? Describe its function, enzyme mechanism, current state in the field, general unknowns, etc. In particular, why is DHFR such an important target for anticancer, antibacterial, etc. drugs?
3. What is the purpose of your study on DHFR? Focus on the bacterial DHFR and its importance.
4. What are the questions you are trying to answer in this lab course pertaining to DHFR?
5. How are you answering these questions?
6. What is your specific technique and how does it fit in this project?
7. How does the technique work, advantages and disadvantages?
8. What are other techniques that can be used? (must discuss at LEAST the information highlighted in the bullets above)
9. How does this study fit into the general knowledge of the DHFR field?
A two-page (double spaced, maximum) handout (Times New Roman font size 12, 1 inch margins all around) containing the points outlined above is to be handed out to the course instructor (Dr. Felicia Vulcu), Adam and the Teaching Assistants.

**Final Report Guidelines:**

This report encompasses the entire year and should be written in the style of a lab project. Figures that do not pertain directly to the development and understanding of the project should not be included. The project itself was highlighted by each group during the PBL component of the lab and in general terms should characterize DHFR. This includes the function of DHFR, the creation of a mutant form of \textit{folA}, the expression, purification and activity of mutant DHFR compared to wild type DHFR and a specific understanding of why this characterization is important for the overall comprehension of DHFR function. Figures that were generated in labs designed primarily to develop skills in certain techniques do not add to this project and should NOT be included. It is up to each student to decide which data generated throughout the year should be included in the report and the mark will reflect whether or not the student understood the purpose of the course. Marks will be deducted for either not including all the pertinent data or for including too much data.

The final report should be 20 pages of text (MAXIMUM LIMIT) double-spaced with 12-point font (Times New Roman) and 1-inch margins all around. Marks will be deducted for not following this format. Title page and references are the only things that do not count for the 20-page limit. The pages should be numbered. Note: YOU MAY NOT LABEL ANYTHING ON THIS REPORT WITH PEN/PENCIL, ETC. ALL FIGURES ARE TO BE LABELED USING APPROPRIATE SOFTWARE. A MARK OF 0 WILL BE RECEIVED IF THE FIGURES ARE LABELED USING PEN/PENCIL/MARKER, ETC.!

Late reports will receive a 20% deduction if handed in later than 2:30pm and an additional 20% deduction/day.

The manuscript should follow this order: A maximum of 2 figures/page is allowed! Include figure/table captions with figures!

1. Title (separate page)
2. Abstract
3. Introduction
4. Materials and Methods
5. Results
6. Discussion
7. Conclusion
8. Abbreviations
9. References
10. Figures/ Tables and Captions

- **Title:** should be short and straight to the point (no more than 2 printed lines). The title should describe the aim of the project. Please include it as part of a title page with other appropriate information.
- **Abstract:** should be clear and concise in its summary of your main finding(s). Should NOT exceed 300 words. MARKS will be deducted if greater than 300 words (1 mark deducted/character extra!)
Introduction: should clearly place your findings in the context of the field as a whole. This section should not be used as a long summary of the field. It should encompass two questions discussed in your text (pg. 42, the Biochemical Literature): “1. What is the current state of knowledge in the area? And 2. What are the significant unknowns?”. The introduction should start out general and become progressively specific to your project. The introduction should include a short background on DHFR, its function, work to date in the field and how your project fits into the already extensive field. At least 1 diagram explaining the overall purpose of this project should be included. This can follow the format of a flowchart as a tentative example. This diagram should include a descriptive figure caption (the diagram must be created by the student and NOT copied from other sources!) THIS SECTION REQUIRES REFERENCING OF PRIMARY JOURNAL ARTICLES!!!!

Materials and Methods: should be concise and easy to follow so that your experiments could be repeated by another student. The experiments must be clearly laid out and must spell out all pertinent information such as buffers used (including concentrations), equipment used, centrifuge rotors used, speeds of centrifuges, method of lysing cells, etc. However, care must be taken not to over describe this section and include information not relevant to the technique (i.e. too much information is not allowed in this section). Referencing techniques is allowed but all experiments performed by you must be laid out in a concise manner. Referencing techniques (I MEAN PRIMARY LITERATURE NOT THE LAB MANUAL) is imperative in this section.

Results: This section should describe the data presented in your figures. Care must be taken not to over-analyze or discuss the data in this section, but you must present the data clearly and state the main conclusion(s) from each figure/table presented. It is recommended that the results section is broken down into different subsections, each containing a 1-sentence heading highlighting the main point to be made in the subsection.

Discussion: This section is designed entirely for interpreting the data. You can include future experiments that need to be done, other controls that should be performed and even your opinion on what the data might mean to the field as a whole. You can even use a diagram to make your point clear. Care should be taken not to over-analyze your data. You should present your ideas in a clear, thought-out manner. Unlike the introduction section, the discussion starts out specific and tends to gravitate towards broad concepts.

Conclusion: This section should include a concise statement of your results, how they fit into the field as a whole and what you think future directions might be for this field (you can draw from your results). THINK GLOBAL!

Abbreviations: All abbreviations used in the text should be written out in long form the first time they are introduced, example PCR (polymerase chain reaction). This section should contain all abbreviations used along with their long form.

References: should be cited throughout the text by number, example (1). The references should follow the JBC (Journal of Biological Chemistry) format.

For example, journal (1) and book (2) references should be in the following styles:


Tables: Should contain a title and a short description of the table.

Figures/ Figure Captions: should have titles and figure legends describing the experiment in sufficient detail to allow readers to understand the figure in the absence of additional text. The figure legend should include scale bar information for images and details of data points (e.g. mean ± sem). All figures should be high quality and should be created with applications capable of producing high resolution files. The figures should be labeled appropriately.