

MCB150L Molecular Immunology Laboratory SPRING 2014

The objective of this course is to provide an introduction to experimental design and basic techniques commonly used in immunology research laboratories. The course is intended for undergraduate students majoring in immunology. The cellular immunology module provides experience in preparation of cell suspensions from lymphoid organs of mice, detection of activated T cells, production of monoclonal antibodies, and the following assays; enzyme-linked immunosorbent assay (ELISA), immunoprecipitation, SDS-PAGE, western blot, and flow cytometry. The molecular module provides experience in isolation of DNA, Southern blotting, restriction mapping, subcloning, and DNA sequencing. Emphasis will be placed on experimental design and the interpretation of data. A working knowledge of basic immunology is needed for the class; **either previous completion or concurrent enrollment in MCB 150 "Molecular Immunology" is an ABSOLUTE REQUIREMENT!**

The laboratory work will include making and working with cell suspensions from spleens and thymuses of mice that have been euthanized by the staff immediately before the laboratory period. It is not possible to immunize cell lines; cells from normal or immunized mice must be used for experiments that involve production of antibody responses in tissue culture. Therefore, this course is not suitable for students who object to the use of animals in teaching and/or research.

Quizzes

There will be short unannounced quizzes given at the beginning of some of the laboratory periods during each module of the course. The quizzes will be based on material from the lectures and experimental protocols in the manual or ask you to explain sample data obtained in the lab.

Journal Club

Basic science research articles will be posted online and students will be asked to answer questions and discuss the articles in class and/or in problems sets.

Laboratory reports

In the Cellular Immunology Module you will report your work in laboratory reports written in a format similar to that used in scientific journals. Details will be provided in separate Report Format handouts. In the Molecular Immunology Module you will do problem sets that involve the experimental protocols and the analysis of the data. Due dates are listed in the course schedule and summarized below. Late work will be penalized by 5 points per class. If you are unable to meet a deadline because of circumstances beyond your control such as job or grad school interviews or illness, please discuss this with the instructors before the due date of the assignment.

Although all of the experiments you do will be performed in groups of two or more, **all written assignments** are intended to be **individual efforts**. This is not to say that you shouldn't communicate with anyone else about your assignments. We strongly encourage you to discuss procedures, data, results, interpretations, analysis, etc. among yourselves and with the teaching staff. However, keep in mind that other than figure legends you must NOT have the same sentences in your report or problem set as another student. We want the work that you hand in to be **your own individual work**, based on the synthesis of **your** thoughts, questions and discussions concerning the experiment. **Any duplicate work will be penalized.**

Laboratory notebooks

You will be expected to keep a laboratory notebook for this course. The lab notebook can be either a hardbound notebook or a binder. The purpose of the notebook is to give you practice in recording laboratory procedures and data in an easily readable format that will enable you to locate all of the necessary information about an experiment when it is time to write a report or paper. You will need to read the relevant protocols cited in the Class Schedule before each laboratory session and have your laboratory notebook set up in flow sheet or outline form at the beginning of each laboratory period. The purpose of preparing your notebook ahead of time is to enable you to begin work on the day's experiment promptly so that you can finish the lab on time and to enable you to enter all critical information pertaining to the day's experiment (cell counts, concentrations, volumes, etc.) directly into your notebook during each laboratory period. Grading the lab notebooks will be determined by checking that **relevant data and brief outlines of experimental protocol** have been included. Putting photocopies of data printouts and graphs into the notebook is acceptable but some sort of handwritten values for cell counts and calculations is necessary. Notebooks will be graded during each half of the semester.

Grading

Cellular Immunology

	Module 1	POINTS
Lac Z assay for T cell activation	Data summary	25
Immunoprecipitation	Lab report	70
Production of monoclonal antibodies	Lab report	80
Notebooks		30
Journal club		25
Lab Performance		20
Unannounced quizzes		50
Midterm I		100

Molecular Immunology

	Module 2	
Analysis of Ig gene rearrangement by Southern hybridization	problem set	45
Restriction analysis of Ig gene rearrangement	problem set	45
DNA Sequencing	problem set	40
PCR analysis	problem set	45
Notebooks		50
Lab Performance		25
Unannounced quizzes		50
Midterm II		100
<hr/> TOTAL		800

MCB 150L SPRING 2014
CLASS SCHEDULE
Cellular Immunology Module

January 22/23	W/Th	<p>Introduction. Film: Laboratory Safety. Lecture 1: Cell counting and cell viability Laboratory: <u>Cell Count and Cell Viability:</u> (pp 16-20)</p>
January 27/28	M/T	<p>Lecture 2: Fusion to Produce B cell Hybridomas. Laboratory: <u>Cell Fusion for Production of Hybridomas:</u> Parts A and B, Steps 1-13. Perform fusion and plate hybridomas into HAT medium in 96-well plates. (pp 26-30).</p>
January 29/30	W/Th	<p>Lecture 3: Flow cytometry Laboratory: <u>Flow cytometry:</u> Stain cells and analyze (pp 59-60). Evaluations. Review for exam.</p>
February 3/4	M/T	<p>Lecture 4: Lac Z T cell activation assay. Protein Assay Laboratory: <u>LacZ T cell Activation Assay:</u> Part A. Set-up cultures for T cell activation. (pp 21-23) <u>Protein Assay</u> (p 25)</p>
February 5/6	W/Th	<p>Laboratory: <u>Lac Z T cell Activation Assay:</u> Part B. Add CPRG substrate. (p24) Lecture 5: ELISA Journal Club 1 Laboratory: <u>Enzyme-Linked Immunosorbent Assay (ELISA) for Antibody:</u> DAY 1 steps 1-2: Coat plates. (pp 33-35) <u>Cell Fusion for Production of Hybridomas:</u> Part B, step 14. Observe and feed cultures remove 100 μl and add 100 μl. (p 30)</p>
February 10/11	M/T	<p>Laboratory: <u>ELISA for Antibody:</u> DAY 2. Block plate before lecture. (p35) Lecture 6: Cloning Laboratory: <u>ELISA for Antibody:</u> DAY 2. (pp 35-36) <u>Cell Fusion for Hybridomas:</u> Part C. Transfer supernates to corresponding wells of transfer plate and feed cultures on hybridoma master plate. (p 31) <u>LacZ T cell Activation Assay:</u> Analyze data in computer room.</p>
February 12/13	W/Th	<p>Laboratory: <u>ELISA for Antibody:</u> DAY 3. (p37) Complete ELISA assay and check results with Instructors before selecting well from hybridoma master plate to use for cloning. <u>Cloning:</u> Parts A-C. Select antigen specific -hybridoma from hybridoma master plate. (pp 42-44) <u>Cell Fusion for Production of Hybridomas:</u> (pp 31-32) <u>Record growth of hybridoma cultures from master plate.</u></p>

February 17/18	M/T	NO CLASS: President's Day
February 19/20	W/Th	Data Summary on LacZ Assay for T Cell Activation due. Lecture 7: Immunoprecipitation. Laboratory: <u>Immunoprecipitation:</u> Part A: Label mouse hybridoma cells with biotin, prepare cell lysate. Part B. Pre-clear lysate. (pp 47-48)
February 24/25	M/T	Lecture 8: SDS PAGE and Western Blot <p style="text-align: right;">Journal Club 2</p> Laboratory: <u>Immunoprecipitation:</u> Part C: Precipitate mouse IgG from cell lysates with goat anti-mouse IgG-agarose and prepare samples for electrophoresis. (p 49)
Feb 26/27	W/Th	Laboratory: <u>SDS-PAGE:</u> Parts A -C. Prepare samples, run gels. (pp 50-52) <u>Western Blot:</u> Parts A and B. Perform electrophoretic transfer of proteins from slab gel to nylon membrane. (pp 53-55) <u>Cloning:</u> Part D. Observe and feed cloning plate. (p 45)
March 3/4	M/T	Laboratory: <u>Western Blot:</u> Part C. Add antibodies to nylon membranes before lecture (pp 55-56). Lecture 9: Science Writing Lecture. Laboratory: <u>Western Blot:</u> Parts C and D. Develop nylon membranes with labeled antibodies and plot MW standard curve. (pp 57-58) <u>ELISA for Antibody.</u> DAY 1. Steps 1-2; Coat plates with antigen (p 35) <u>Antigen Capture ELISA:</u> DAY 1. Coat plates with antibody. (pp 38-40)
March 5/6	W/Th	Block ELISA plates before lecture Laboratory: <u>Cloning:</u> Record growth and transfer culture supernatants to antigen-coated plate for assay (pp 45-46). Laboratory: <u>ELISA for Antibody.</u> DAY 2. Add samples (p 36) <u>Antigen Capture ELISA:</u> DAY 2. Steps 3-6. Add samples (p 41)
March 10/11	M/T	Laboratory Report on Immunoprecipitation due Laboratory: Add secondary antibody before lecture. Laboratory: Finish <u>ELISA for Antibody.</u> DAY 3. Steps 9-16 (p 37) <u>Antigen Capture ELISA:</u> DAY 3. Steps 7-10 (p 41). Discussion of monoclonal antibody lab report: Criteria for completion of cloning and evaluation of specificity.
March 12/13	W/Th	MIDTERM I (2 hours)
March 19/20		Laboratory Report on Production of Monoclonal Antibodies Due

**MCB 150L Spring 2014
CLASS SCHEDULE**

Molecular Immunology Module

March 17/18	M/T	<p>Lecture: Introduction of purification of genomic DNA</p> <p>Laboratory: Extraction and purification of B cell genomic DNA.</p>
March 19/20	W/Th	<p>Lecture: Overview of DNA module and introduction to V(D)J recombination.</p> <p>Laboratory: Set up restriction digestions; pore agarose gels; test the digestions.</p> <p>Detailed Schedule:</p> <ol style="list-style-type: none"> 1. Set up restriction digestions according to the manual; 2. Pour agarose gel for testing the digestion; 2. Lecture at ~ 2:30 pm.; 3. Run the gel to test the digestions.
March 24-28		SPRING BREAK
March 31/ April 1	M/T	<p>Lecture: Southern Blotting.</p> <p>Laboratory: Gel electrophoresis (Staff will start it on Tuesday); Southern transfer (done in class).</p>
April 2/3	W/Th	<p>Lecture: Genomic phage libraries and DNA cloning vectors.</p> <p>Laboratory: Pre-hybridize and hybridize filters from Southern transfer; Set up digestions of plasmid DNA for restriction mapping.</p> <p>Detailed Schedule:</p> <ol style="list-style-type: none"> 1. Pre-hybridize the Southern Blot filters; 2. Lecture ~ 1:30 pm; 3. Set up plasmid DNA digestions for restriction mapping; 4. Hybridize the filters overnight (Staff will wash the filters).
April 7/8	M/T	<p>Lecture: Restriction mapping (~ 1:30 pm).</p> <p>Laboratory: Develop Southern Blots; Plasmid DNA restriction mapping and analysis; Prepare insert and vector for subcloning: set up digestions; isolate insert and vector.</p> <p>Detailed Schedule:</p> <ol style="list-style-type: none"> 1. Pore agarose gels for restriction mapping; 2. Incubate membranes with anti DIG - AP conjugated antibody; 4. Run gels for DNA restriction mapping; 5. Conduct the rest of the detection steps for Southern Blots; 6. Photograph the agarose gels; 7. Set up digestions for isolating insert and vector.
April 9/10	W/Th	Lecture: Sub-cloning.

		<p>Laboratory: Separate digests in agarose gel; purify the DNA; set up ligation.</p> <p>Detailed Schedule:</p> <ol style="list-style-type: none"> 1. Load the samples and run agarose gel; 2. Lecture ~1:30 pm; 3. Purify DNA fragment and set up ligation; 4. Discuss Southern Blot data.
April 14/15	M/T	<p>Problem Set on Analysis of Ig Gene Rearrangement by Southern Blot due.</p> <p>Lecture: Plasmid miniprep.</p> <p>Laboratory: Transform bacteria. Set up first PCR reactions.</p>
April 17/17	W/Th	<p>Lecture: DNA sequencing: Maxam & Gilbert and Sanger's methods. <u>Antibody DNA sequence data and bioinformatics.</u></p> <p>Laboratory: Plasmid DNA Mini-prep and analysis by restriction digestions; Set up sequencing reactions. Set up second PCR reactions.</p>
April 21/22	M/T	<p>Problem Set on restriction mapping and sub-cloning due.</p> <p>Lecture: PCR as a general technique and in analysis of V(D)J recombination.</p> <p>Laboratory: Run agarose gel with all PCR products. Work on problem set with sequencing data.</p> <p>Detailed Schedule:</p> <ol style="list-style-type: none"> 1. Pour agarose gel; 2. Lecture ~1:30 pm; 3. Run agarose gel; 4. Work on problem set.
April 23/24	W/Th	<p>Problem Set on DNA sequencing due.</p> <p>Lecture: Summary of DNA module.</p> <p>Laboratory: Analyze PCR data and complete problem set in lab.</p>
April 28/29	M/T	<p>Problem Set is due for PCR analysis of D-J Rearrangements.</p> <p>Question Review; Course evaluation. Exam Review.</p>
April 30/ May 1	W/Th	<p>MIDTERM II (2 hours)</p>