MCB150L Molecular Immunology Laboratory SPRING 2016

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.

Faculty	GSIs
Robert Beatty 642-0671 prbeatty@berkeley.edu Office Hour: Friday 1-2 pm 176 LSA	Gwen Tindula gntindula@berkeley.edu Office hour: Monday 11-12 4051 VLSB
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Instructional Support Staff Jennifer Zeitler 642-0742 jenniferzeitler@berkeley.edu

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 (2 quizzes for 25 points each)		50
Midterm I		100

Molecular Immunology
Module 2

PCR analysis	problem set #1	45
Analysis of Ig gene rearrangement by Southern hybridization	problem set #2	45
Restriction analysis of Ig gene rearrangement	problem set #3	45
DNA Sequencing	problem set #4	40
Notebooks		50
Lab Performance		25
Unannounced quizzes		25
Journal Club		25
Midterm II		100
TOTAL		800

MCB 150L SPRING 2016
CLASS SCHEDULE
Cellular Immunology Module

January 20/21	W/Th	**Introduction.** **Film: Laboratory Safety.** **Lecture 1**: Cell counting and cell viability **Laboratory**: Cell Count and Cell Viability: (pp 16-20)
January 25/26	M/T	**Lecture 2:** Fusion to Produce B cell Hybridomas. **Laboratory**: Cell Fusion for Production of Hybridomas: Parts A and B, Steps 1-13. Perform fusion and plate hybridomas into HAT medium in 96-well plates. (pp 23-27).
January 27/28	W/Th	**Lecture 3:** Flow cytometry **Laboratory:** Flow cytometry: Stain cells and analyze (pp 28-29). Evaluations. Review for exam.
February 1/2	M/T	**Lecture 4**: Lac Z T cell activation assay. Protein Assay **Laboratory**: LacZ T cell Activation Assay*:* Part A. Set-up cultures for T cell activation. (pp 30-33) Protein Assay (p 34)
February 3/4	W/Th	**Laboratory:** Lac Z T cell Activation Assay: Part B. Add CPRG substrate. **Lecture 5:** ELISA **Journal Club 1** **Laboratory:** Enzyme-Linked Immunosorbent Assay (ELISA) for Antibody: DAY 1 steps 1-2: Coat plates. (pp 35-39) Cell Fusion for Production of Hybridomas: Part B, step 14. Observe and feed cultures remove 100 µl and add 100 µl. (p 27)
February 8/9	M/T	**Laboratory:** ELISA for Antibody: DAY 2. Cell Fusion for Hybridomas: Part C. Transfer supernates to corresponding wells of transfer plate and feed cultures on hybridoma master plate. LacZ T cell Activation Assay: Analyze data in computer room.
February 10/11	W/Th	**Laboratory:** ELISA for Antibody: DAY 3. (p37) **Lecture 6:** Cloning during incubation. Complete ELISA assay and check results with Instructors before selecting well from hybridoma master plate to use for cloning. Cloning*:* Parts A-C. Select antigen specific -hybridoma from hybridoma master plate. (pp 40-43) Record growth of hybridoma cultures from master plate.
February 15/16	M/T	**NO CLASS: President's Day**

February 17/18	W/Th	**Data Summary on LacZ Assay for T Cell Activation due.** **Laboratory:** Immunoprecipitation: (pp 45-47) Part A: Label cells with biotin. **Lecture 7:** Immunoprecipitation. (lecture during biotin labeling) **Laboratory:** Part A. prepare cell lysate. Part B. Pre-clear lysate.
February 22/23	M/T	**Lecture 8**: SDS PAGE and Western Blot **Laboratory:** Immunoprecipitation: Part C: Precipitate mouse IgG from cell lysates with goat anti-mouse IgG-agarose and prepare samples for electrophoresis.
Feb 24/25	W/Th	**Laboratory:** SDS-PAGE: Parts A -C. Prepare samples, run gels. (pp 48-50) **Journal Club 2 while gel is running** Western Blot: Parts A and B. Perform electrophoretic transfer of proteins from slab gel to Zeta-probe nylon membrane. (pp 51-54) Cloning: Part D. Observe and feed cloning plate.
Feb 29/ March 1	M/T	**Laboratory:** Western Blot: Part C. Add antibodies to nylon membranes before lecture (pp 51-57). **Lecture 9:** Science Writing Lecture. **Laboratory:** Western Blot: Parts C and D. Develop nylon membranes with labeled antibodies and plot MW standard curve. ELISA for Antibody. DAY 1. Coat plates with antigen (pp 35-39) Antigen Capture ELISA: DAY 1. Coat plates with antibody. (pp 58-60)
March 2/3	W/Th	**Laboratory Report on Immunoprecipitation due** **Laboratory:** Cloning: Record growth and transfer culture supernatants to antigen-coated plate for assay **Laboratory:** ELISA for Antibody. DAY 2. Add samples. Antigen Capture ELISA: DAY 2. Steps 3-6. Add samples.
March 7/8	M/T	**Laboratory:** Finish ELISA for Antibody. DAY 3. Steps 9-16. Antigen Capture ELISA: DAY 3. Steps 7-10. **Discussion of monoclonal antibody lab report:** Criteria for completion of cloning and evaluation of specificity.
March 9/10	W/Th	**MIDTERM I (2 hours)**
March 18	Friday	**Laboratory Report on Production of Monoclonal Antibodies Due**

MCB 150L Spring 2016
CLASS SCHEDULE

Molecular Immunology Module

March 14/15 **LAB 1**	M/T	**1.** Lecture: Overview of PCR and use in analysis of V(D)J recombination **2.** Wet Lab: Set up 1st PCR reactions **3.** Lecture: Genomic DNA purification **4.** Wet Lab: Extraction and purification of B cell genomic DNA **5.** Wet Lab: Set up 2nd PCR reactions
March 16/17 **LAB 2**	W/Th	**1.** Wet Lab: Set up restriction enzyme digestions of B cell DNAs **2.** Wet Lab: Pour agarose gels for testing the digestion and PCRs **3.** Lecture: Overview of molecular half and review of V(D)J recombination **4.** Wet Lab: Run the gel to test the digestions ***5.*** Wet Lab: Run the gel to visualize the PCR reactions
March 21-25		**SPRING BREAK**
March 28/29 **LAB 3**	M/T	**1.** Lecture: Southern blotting and hybridization **2.** Wet Lab: Southern transfer of gels run on previous day by staff **3.** Lecture: Bioinformatics and Ig gene organization **4.** Dry Lab (4051): Bioinformatic analysis of IgH locus **Answer bioinformatic questions in manual**
March 30/31 **LAB 4**	W/Th	**1.** Wet Lab: Pre-hybridize Southern blot filters **2.** Lecture: Restriction enzyme mapping **3.** Wet Lab: Hybridize filters overnight (Staff will perform washes) **4.** Dry lab (4051): Design J_H restriction mapping strategies with lab partner **5. Turn in mapping strategies at end of class** **6. Order sequencing oligo for LAB 9**
April 4/5 **LAB 5**	M/T	**1. Problem Set is due for PCR analysis of D-J Rearrangements.** 2. Wet Lab: Incubate membranes with anti DIG - AP conjugated antibody 3. Tables meet to decide RE strategy to present for a single J_H 4. Wet Lab: Conduct remaining detection steps for Southern blots 5. Four tables each present their RE mapping strategy to use in LAB 6
April 6/7 **LAB 6**	W/Th	**1.** Staff will post Southern blot data **2.** Lecture: Discussion of Southern blot data and intro to subcloning **3.** Wet Lab: Set up subcloning digests for vector and insert **4.** Wet Lab: Set up restriction mapping digests decided in LAB 5
April 11/12 **LAB 7**	M/T	**1. Problem Set is due for Ig Gene Rearrangement by Southern Blot** 2. Wet lab: Load two gels for subcloning and RE mapping 3. Lecture: Ligation in subcloning and assessing efficiency of ligation (pt 1) 4. Wet Lab: Purify DNA fragments. Set up ligations. Take gel photos.

April 13/14 **LAB 8**	W/Th	1. Lecture: Transformation in subcloning and blue-white selection (part 2) 2. Wet Lab: Transform bacteria with ligation mixtures 3. **Journal Club Paper Discussion**
April 18/19 **LAB 9**	M/T	**1. Problem Set on Restriction Mapping due** 2. Lecture: Discussion of subcloning results and intro to plasmid preps 3. Wet lab: Plasmid DNA mini-prep and check for subcloning success 4. Wet lab: Set up sequencing reaction
April 20/21 **LAB 10**	W/Th	**1.** Lecture: DNA sequencing: Maxam & Gilbert, Sanger, and next generation. Bioinformatic analysis of BCR and antibody rearrangements. 2. Dry lab (4051): Work on problem set analyzing sequencing data
April 25/26	M/T	**Problem Set on DNA Sequencing and Subcloning due.** **Question Review; Course evaluation. Exam Review.**
April 27/28	W/Th	**MIDTERM II (2 hours)**