MCB150L Molecular Immunology Laboratory FALL 2017

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, 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. **Previous completion or concurrent enrollment in MCB 150 "Molecular Immunology" is a REQUIREMENT!**

Faculty	GSIs
Robert Beatty 642-0671 prbeatty@berkeley.edu Office Hour: Fridays 2-3 pm 176 LSA	Valerie Vargas-Zapata vvargas+class@berkeley.edu Office hour: Tuesdays 5 - 6 pm 4051 VLSB
Bill Sha bsha@berkeley.edu Office hour: TBA	Michael Manoharan michael_manoharan@berkeley.edu Office hour: Tuesdays 5 - 6 pm 4051 VLSB

Instructional Support Staff Arash Ng 642-0742 arashng@berkeley.edu

Course Policies

Safe, Supportive, and Inclusive Environment

Whenever a faculty member, staff member, post-doc, or GSI is responsible for the supervision of a student, a personal relationship between them of a romantic or sexual nature, even if consensual, is against university policy. Any such relationship jeopardizes the integrity of the educational process. Although faculty and staff can act as excellent resources for students, you should be aware that they are required to report any violations of this campus policy. If you wish to have a confidential discussion on matters related to this policy, you may contact the Confidential Care Advocates on campus for support related to counseling or sensitive issues. Appointments can be made by calling (510) 642-1988.

The classroom, lab, and work place should be safe and inclusive environments for everyone. The Office for the Prevention of Harassment and Discrimination (OPHD) is responsible for ensuring the University provides an environment for faculty, staff and students that is free from discrimination and harassment on the basis of categories including race, color, national origin, age, sex, gender, gender identity, and sexual orientation. Questions or concerns? Call (510) 643-7985, email ask_ophd@berkeley.edu, or go to http://survivorsupport.berkeley.edu/.

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.

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** and if you are caught will result in a failing grade for the class. UC Berkeley's cheating policy (http://bulletin.berkeley.edu/academic-policies/#studentconductappealstext) will be followed. If you are **uncertain what constitutes cheating or plagiarism**, go to http://campuslife.berkeley.edu/conduct/integrity.

Grading

Cellular Immunology

Module 1		**POINTS**
LDH Assay	Data summary	25
Immunoprecipitation	**Lab report**	70
Production of monoclonal antibodies	**Lab report**	80
Notebooks		25
Journal club		30
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 FALL 2017
CLASS SCHEDULE
Cellular Immunology Module

August 23	W	**Introduction.** **Film: Laboratory Safety.** **Lecture 1**: Cell counting and cell viability **Laboratory**: Cell Count and Cell Viability:
August 28	M	**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.
August 30	W	**Laboratory:** Flow cytometry: Stain cells. **Lecture 3:** Flow cytometry during antibody incubation. **Laboratory:** Flow cytometry: Analyze stained cells in LSA.
September 4	M	LABOR DAY HOLIDAY
September 6	W	**Laboratory**: Cell Death Lactose dehydrogenase (LDH) Assay. **Lecture 4**: T cell activation assays and Inflammasome cell death assay. **Laboratory**: LDH Assay. Protein Assay
September 11	M	**Lecture 5:** ELISA **Laboratory:** Enzyme-Linked Immunosorbent Assay (ELISA) for Antibody: DAY 1 steps 1-2: Coat plates. Cell Fusion for Production of Hybridomas: Part B, step 14. Observe and feed cultures remove 100 µl and add 100 µl. LDH Assay: Analyze data in computer room.
September 13	W	**Laboratory:** ELISA for Antibody: DAY 2. Block plates. **Journal Club 1** **Laboratory.** ELISA for Antibody: DAY 2 and Cell Fusion for Hybridomas: Transfer supernatants to corresponding wells of transfer plate and feed cultures on hybridoma master plate.
September 18	M	**Laboratory:** ELISA for Antibody: DAY 3. Add secondary antibody before lecture. **Lecture 6:** Cloning **Laboratory:** ELISA for Antibody: DAY 3. 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. Cell Fusion for Production of Hybridomas: Record growth of hybridoma cultures from master plate.

September 20	W	**Data Summary on LDH Assay due.** **Lecture 7:** Immunoprecipitation. **Laboratory:** Immunoprecipitation: Part A: Label mouse hybridoma cells with biotin, prepare cell lysate. Part B. Experiment with half the class doing pre-clear of cell lysates and half the class doing IP without pre-clear.
September 25	M	**Lecture 8**: SDS PAGE and Western Blot **Journal Club 2** **Laboratory:** Immunoprecipitation: Part C: Precipitate mouse IgG from cell lysates with goat anti-mouse IgG-agarose and prepare samples for electrophoresis.
September 27	W	**Laboratory:** SDS-PAGE: Parts A -C. Prepare samples, run gels. Western Blot: Parts A and B. Perform electrophoretic transfer of proteins from slab gel to nylon membrane. Cloning: Part D. Observe and feed cloning plate.
October 2	M	**Laboratory:** Western Blot: Part C. Add antibodies to nylon membranes before lecture. **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. Steps 1-2; Coat plates with antigen Antigen Capture ELISA: DAY 1. Coat plates with antibody.
October 4	W	**Laboratory:** ELISA for Antibody. DAY 2. Add block. Cloning: Record growth and transfer culture supernatants to antigen-coated plate for assay. ELISA for Antibody. DAY 2. Add samples Antigen Capture ELISA: DAY 2. Add samples
October 9	M	**Laboratory Report on Immunoprecipitation due** **Laboratory:** Finish ELISA for Antibody. DAY 3. Steps 9-16 Antigen Capture ELISA: DAY 3. Steps 7-10.
October 11	W	**Discussion of monoclonal antibody lab report:** Criteria for completion of cloning and evaluation of specificity **Exam review**
October 16	M	**MIDTERM I (2 hours)**
October 21	F	**Laboratory Report on Production of Monoclonal Antibodies Due**

MCB 150L Fall 2017
CLASS SCHEDULE

Molecular Immunology Module

October 18 **LAB 1**	W	**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
October 23 **LAB 2**	M	**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
October 25 **LAB 3**	W	**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. **5. Answer questions and order sequencing oligo for LAB 9.** **6.**
October 30 **LAB 4**	M	**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**
November 1 **LAB 5**	W	**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
November 6 **LAB 6**	M	**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
November 8 **LAB 7**	W	**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 (part 1) 4. Wet Lab: Purify DNA fragments. Set up ligations. Take gel photos.

November 13 **LAB 8**	M	1. Lecture: Transformation in subcloning and blue-white selection (part 2) 2. Wet Lab: Transform bacteria with ligation mixtures 3. **Journal Club Paper Discussion**
November 15 **LAB 9**	W	**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
November 20 **LAB 10**	M	**1.** Lecture: DNA sequencing: Maxam & Gilbert, Sanger, and next generation. Bioinformatic analysis of BCR and antibody rearrangements. Dry lab (4051): Work on problem set analyzing sequencing data
November 22	W	*THANKSGIVING NO CLASS*
November 27	M	Summary/ Question Review of DNA Module Course Evaluations
November 29	W	**MIDTERM II (2 hours)**
December 6	W	**Problem Set on DNA sequencing and Subcloning due**