MCB 150L FALL 2015
CLASS SCHEDULE
Cellular Immunology Module

August 26	W	**Introduction.** **Film: Laboratory Safety.** **Lecture 1**: Cell counting and cell viability **Laboratory**: Cell Count and Cell Viability:
August 31	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.
September 2	W	**Lecture 3:** Flow cytometry **Laboratory:** Flow cytometry: Stain cells and analyze.
September 7	M	LABOR DAY HOLIDAY
September 9	W	**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. **Laboratory**: Protein Assay
September 14	M	**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. Cell Fusion for Production of Hybridomas: Part B, step 14. Observe and feed cultures remove 100 µl and add 100 µl.
September 16	W	**Laboratory:** ELISA for Antibody: DAY 2. Cell Fusion for Hybridomas: Part C. Transfer supernatants to corresponding wells of transfer plate and feed cultures on hybridoma master plate. LacZ T cell Activation Assay: Analyze data in computer room.
September 21	M	**Laboratory:** ELISA for Antibody: DAY 3. Add secondary antibody before lecture. **Lecture 6:** Cloning 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 23	W	**Data Summary on LacZ Assay for T Cell Activation due.** **Lecture 7:** Immunoprecipitation. **Laboratory:** Immunoprecipitation: Part A: Label mouse hybridoma cells with biotin, prepare cell lysate. Part B. Pre-clear lysate.
September 28	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 30	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 5	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 7	W	**Block ELISA plates before lecture** **Lecture: Exam Review** **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
October 12	M	**Laboratory Report on Immunoprecipitation due** **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.
October 14	W	**MIDTERM I (2 hours)**
October 21	W	**Laboratory Report on Production of Monoclonal Antibodies Due**

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Molecular Immunology Module

October 19 **LAB 1**	M	**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 21 **LAB 2**	W	**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 26 **LAB 3**	M	**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 28 **LAB 4**	W	**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 2 **LAB 5**	M	**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 4 **LAB 6**	W	**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 9 **LAB 7**	M	**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 11	W	**VETERAN'S DAY HOLIDAY**

November 16 **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 18 **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 23 **LAB 10**	M	**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
November 25	W	*THANKSGIVING NO CLASS*
November 30	M	Summary/ Question Review of DNA Module Course Evaluations
December 2	W	**MIDTERM II (2 hours)**
December 7	M	**1. Problem Set on DNA sequencing and Subcloning due**