APPLIED CELL BIOLOGY - SYLLABUS
(V23-0037)

Instructor: Ignatius P. Tan, Ph.D.  Writing Tutor:
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Phone: 212-998-8295  Guest Lecturer:
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Course Description:
Various methodology used to study cell structure and function will be examined. In the laboratory the students will study the fundamentals of cell biology and the experimental approaches used to examine the cell. Experimental topics will cover cellular, subcellular and macromolecule localization, biochemical analysis of the cell, and cell culture techniques. Discussion will also cover protein and antibody microarray techniques used in proteomics.

Course Hours:
Lab:  Tues 12:30 - 3:15
      Thurs 12:30 - 3:15

Objectives of the Course:
1. Provide students with laboratory experience using various techniques to study the cell.
2. The various techniques will be introduced to the students via a project. The project will be designed so that the results require that the student critically think about the information before continuing with the next set of techniques.
3. Students will also be introduced to the literature, so that at the conclusion of the project, the student should be able to discuss the results of their project in reference to published papers.
4. Students will work with writing fellows to develop the skills and experience in writing scientific papers.

Course Policy and Grading:
- Lecture and Laboratory attendance required.
- Students are responsible for laboratory assignments, which may require extra time in the lab.
- Grade will be based on 2 lecture-lab exams (60%), and lab notebook/report and presentations/discussions (40%).
- Exams will be based on lecture notes and lab exercises.
- Presentations are short 10 - 15 min discussions on student project.
- Students will work with the writing teaching fellow on their lab papers. Students are required to devote time outside class to meet with the teaching fellow.
- Lab notebooks are to be kept current.
- Notebooks are subject to unannounced review.

Each lab report will be graded and the following grading system will be given:

- Missing or incomplete sections.
- All sections are OK.
+ Exceptional work - Extra credit given.

Late submissions to the Writing Fellows will result in a grade reduction.

Writing Fellows:
- Writing Tutors will work with the students to improve their draft. All students are expected to meet with the Tutors to discuss their writing.
- Writing Tutors are NOT teaching assistants for the class. All grading will be done by the instructor.
- The Handbook on Writing Tutors describes their role in the class:

  What the Writing Tutors Do
  Because Tutors are peer mentors, not graders, they serve as facilitators rather than judges. As peers, they are in a unique position to advise, encourage, and challenge students on the often-sensitive issues found in their own writing. Tutors seek to demystify the conventions of academic discourse and to help their peers advance their aims as writers.

  Some Things that Tutors Don't Do
  Tutors are not teaching assistants: they address writing issues, not course content. They focus their comments on questions including: Does this draft respond effectively to the assignment? Is the argument, hypothesis, or research question clearly and compellingly set up? Are the readings of data or texts both rigorous and persuasive? Is the paper's discussion or exposition developed cogently and in a way that shows facility with and knowledge of the discipline's writing conventions? While Tutors will doubtless engage in spirited discussions about course content during their conferences, they will do so only as interested peers. They keep in mind the writing goals [the Instructor] sets for the assignment as well as what they have learned in their workshops about tutoring. [The Instructor is] alone are responsible for assessing any paper's content.

  Tutors are also not proofreaders. While the Tutors will identify patterns of grammatical or mechanical error and explain how to avoid them, their main goal is to help their peers become better writers over the long term, not to fix all of the issues in the paper at hand.

Textbook and Suggested References:
- There is no textbook for this course.
- During the course handouts will be distributed.

References:
Molecular Biology: A Problem Solver, By Alan s. Gerstein
Molecular Cloning: A Laboratory Manual, By Sambrook, Fritsch, Maniatis.
Molecular Cell Biology, By Loddisch, Baltimore, Berk, Zipursky, Matsudaira, Darnell.
- WEB Sites:
  Entrez, BLASTt, Restriction Mapping: http://www.arabidopsis.org/seqtools.html

PAPERS TO BE READ AND DISCUSSED IN CLASS:


Lab Notebooks:
Lab notebooks should be individual work, legible and organized. It should be written so that other people can follow your work exactly to achieve the same result(s). For each week's lab the following format should be followed:

Title and Date: Provide a descriptive title of the week’s lab and date the work.

Purpose: Briefly describe the purpose of the lab. (Min.: 2-3 sentence). You may also explain the significance of the procedure or the type of application that can be used with the procedure.

Materials: Specific information on materials used other than regular reagents.
-Example of information to be recorded: Specific activity, Company/Lot #, Concentration, Nucleotide sequence, type of DNA plasmid, DNA probes sequence, enzymes, label, etc.

Procedure: Handouts will be provided. These handouts should be put into or the procedure written into your notebook. In some cases there will be some modification or changes in the procedure. This should be recorded to show the changes. Information on volume, concentration and time should be added when needed.

Results: Your results should be labeled and have enough information so that an outside reader can understand your results. The results should also be referenced appropriately (Fig. 1, ... or Table 1, ...) so that you can discuss the results in the discussion section.

Discussion: Summarize your result(s). Explain the outcome of the procedure. Was there anything you could have done to better your result(s).
LAB SAFETY and RULES:

1. No eating, drinking, or smoking in the lab.

2. Wear protective gear when appropriate. Gloves when working with radioactive materials, ethidium bromide, acrylamide, phenols. UV goggles when using the UV transilluminator or working with isotopes. LAB COATS recommended.

3. CLEAN UP after yourselves. Wash your hands before and after working in the lab.

4. Spillage of hazardous materials should be reported immediately. Waste materials must be properly disposed.

5. Be careful about using common or stock solutions. If you feel you may have contaminated the solution, notify the TA. You are responsible for maintaining purity/sterility of your own working solutions.

6. Label your samples/tubes appropriately. Many of the samples will have to be used later in the course.

OUTLINE OF LAB EXPERIMENTS

SUMMARY: Each group will work with an organelle specific vector. Cultured cells will be transfected with the vector and aspects of cell biology will be studied by the group and presented to the class in the form of class presentations. Topics that will be discussed as a result of using these vectors are: membrane structure and function, expression of genetic information, function and transduction of cellular membrane systems, and cellular interactions.

I. Vectors: Nuclear, ER, Golgi, Mitochondria, Plasma Membrane, Actin.
   - Regulatory sites on vector.
   - Expression vector properties.
   - Translation control
   - Organelles properties and function

II. Cell Culture and Transfection:
   - Culture techniques.
   - Transfection techniques.
   - Microscopes: DIC, Phase, Fluorescent, Confocal.
III. Cell Fractionation:
- Methods to fractionate cell
- Purification of cellular components.

IV. Protein Analysis:
- Western blots.
- Protein extraction and purification procedures.

V. PCR Technology:
- Design of primers.
- RT-PCR, Real-time PCR, Quantitative PCR.

VI. Topics covered from assigned research articles:
- Yeast one, two-hybrid system
- EST
- Phage-based expression systems
- Complementation analysis
- Gene suppressioin: knockouts, transgenics, RNAi techniques

VII. Genomics and Proteomics
- Methods to access DNA and protein sequences
- Bioinformatic approaches to gene expression.
- Microarray, Mass spectrophotometers.
- Protein analysis: Structure, multiple sequence alignment, molecular phylogeny and evolution.
### LECTURE/LAB SYLLABUS - Spring 2012

<table>
<thead>
<tr>
<th>DATE</th>
<th>TOPIC</th>
<th>STUDENT PROJECTS</th>
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<tbody>
<tr>
<td></td>
<td>No class</td>
<td>No individual projects: Vectors are to be grown by all students.</td>
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<tr>
<td>#1</td>
<td>Introduction to course.</td>
<td>Vectors that code for the following will be used to transfet TM4 cells:</td>
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<tr>
<td></td>
<td>Vectors for the propagation, manipulation and delivery of specific DNA sequences into a host cell.</td>
<td>- β-Galactosidase.</td>
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<td>#3</td>
<td>Transform bacteria with specific vector.</td>
<td>- Human cytoplasmic β-Actin.</td>
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<td>#4</td>
<td>Isolate transformant and setup mini-culture.</td>
<td>- Golgi Fussion Protein</td>
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<td>Mini-plasmid prep.</td>
<td>- Nuclear Protein</td>
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<td>#6</td>
<td>Agarose gel of mini-plasmid prep.</td>
<td>- Plasma membrane Protein</td>
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<td><strong>Final Data Collection for lab report # 1</strong></td>
<td>- Mitochondria protein</td>
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<td>- Tubulin</td>
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<td>- Endocytosis via c-Myc</td>
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<td></td>
<td></td>
<td>See attached information sheets on specific vectors.</td>
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<td>II:</td>
<td>Cell Culture and Transfection.</td>
<td>II: Cell Culture and Transfection</td>
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<td></td>
<td>#7 Introduction to microscopes. Culturing cells. <strong>Draft of 1st Lab Report Due</strong> - Does not include Abstract. (Final version of Report # 1 due 3/7)</td>
<td>Projects:</td>
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<td></td>
<td>#8 Setup culture cells for Immunofluorescence (2/28) and Cell fractionation (3/5).</td>
<td>A. Alter cell culture condition(s) and observe effect on cell morphology. - Observe effect on cell's cytoskeletal system, primarily polymerization of actin.</td>
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<td>#9 Transfect cultured cells with vectors. <strong>Student Presentations of vectors</strong></td>
<td>B. Examine cell-cell communication. - Culture cells transfected with β-gal vector and non-transfected cells.</td>
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<td>#10 Immunofluorescence - Guest Lecturer</td>
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<td>III:</td>
<td>Cell Fractionation.</td>
<td>III: Cell Fractionation.</td>
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<td>#11 Cell fractionation of cells - Part I. Transfection.</td>
<td>Projects: Each group of students will isolate specific organelle.</td>
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<td>#12 Cell fractionation of cells - Part II. RNA and Protein Extraction. <strong>Revised 1st Lab Report Due</strong> - Includes Abstract</td>
<td>A. Isolate specific organelles using density gradients. - ER, Golgi, Nucleus,</td>
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<td>B. Isolated organelle will be prepared for microscopic examination.</td>
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<td>#13 Review</td>
<td>C. Samples will also be collected for PAGE.</td>
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<td>#14 <strong>Exam # 1</strong></td>
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<td>IV.</td>
<td>Protein Analysis.</td>
<td>IV.</td>
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<td>#15 Prepare samples for SDS-PAGE for Western Analysis.</td>
<td>A. Determine whether the protein is located in the cytoplasm or organelle bound.</td>
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<td>#16 Design probes for RT-PCR</td>
<td>B. Determine properties of protein,</td>
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<td>#17 Discussion of Papers</td>
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<td>#18 Setup polyacrylamide gels. Transfer protein to membrane.</td>
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<td>#19 Detect proteins via antibody.</td>
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<td><strong>Final Data Collection for Lab Report # 2.</strong></td>
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<tr>
<td>V:</td>
<td>Genomics and proteomics.</td>
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<td>#20 Computer exercise on BLAST</td>
<td>Projects: Each group will examine their protein of interest.</td>
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<td>#21 Genome wide analysis of RNA and protein</td>
<td>Methods to assess DNA and protein sequences.</td>
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<td>#22 Proteomics</td>
<td>Bioinformatic approaches to gene expression.</td>
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<td>Gene expression: Microarray data analysis</td>
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<td>Protein Analysis: Structure, Multiple Sequence Alignment, Molecular Phylogeny and Evolution.</td>
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<td>VI:</td>
<td>PCR Amplification of Message.</td>
<td>VI:  PCR Amplification of Message.</td>
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#23  | Transfect cells for RNA Extraction | Projects: Examine the transcripts level and correlate to protein expression levels. |
#24  | Extract and Purify RNA. | |
#25  | PCR Amplification of amplicons. | |
#26  | Agarose gel of amplicons. | |
#27  | **Student Presentations** | |
#28  | Exam #2 | |
|      | 2nd Lab Report Due | |

**Reading Assignments:**


Assigned Reading on Web Site:

PCR Primer Design and Reaction Optimization: [http://www.mch.uct.ac.za/peroptim.htm](http://www.mch.uct.ac.za/peroptim.htm)