**BIOSci 1950: MOLECULAR GENETICS LABORATORY - FALL TERM 2014**

**GENERAL INFORMATION**

Lecture: Tuesday, 12:00 – 12:50 PM  
Lab: Thursday & Friday Sections, 12:00 – 4:50 PM  
Langley A148  
Langley A148 & A146

**INSTRUCTORS:**  
Dr. Vern Twombly  
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Teaching Assistant: Jian Fei Hua  
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jih27@pitt.edu

**PREREQUISITE & COREQUISITE**

The pre-requisite for 1950 is BioSci 1000 or 1810 (passed with a C grade or higher) in order to register, while the co-requisite for 1950 in BioSci 1940. The following courses passed with a C or better also provide important background: General Chemistry I & II, Organic Chemistry I & II, and Calculus I.

**COURSE OBJECTIVES**

The objective of this course is to introduce you to the practice and theory of molecular biology. To do this, the lab will explore the splicing mechanism(s) of a Group II self-splicing intron *(pictured right)*. The lab is unique in that your entire semester will focus on accomplishing a single complex experiment. This provides motivation for students to focus their efforts, ownership of the project, and control of their own success.

Group II introns RNA fold such that they can splice themselves from a pre-mRNA without the aid of any accessory proteins, *in vitro*. Evidence indicates that nucleotides at the intron/exon junctions are recognized by the splicing machinery. However, the exact role of junction nucleotides remains unknown. Your goal is to mutate these nucleotides and functionally test your modified intron. This is a semester-long experiment for which we will provide materials and "safety nets" so that you can continue despite occasional experimental failure. In this way you’ll use molecular biology techniques and learn the fundamental chemical and physical principles that makes it work.
This course teaches core “wet-lab” techniques, advanced protocols, and challenges you with analytical approaches used in research labs. The course has been designed for Molecular Biology & Biochemistry Majors oriented toward a career as a research scientist. It’s an upper-level laboratory course designed to challenge you, and it will draw upon your experiences from other laboratory science courses. This course involves substantial effort. Importantly, this course will teach you to be more self-reliant in any research lab. The physical and chemical principles behind the lab experiments will be emphasized, so that when troubleshooting is required, you will be able to rationally find a solution. In addition, you will be expected to fill gaps in protocols, such as calculating the amounts of solutions needed to achieve a given molarity. By the end of the semester, you should be capable of independently performing a variety of basic, yet fundamental tasks in a biomedical research lab.

**Organization:** The lecture portion of this course will discuss topics concerning the practical approaches and physical chemistry of molecular biology of the experiments to be done. The lab will explore the splicing mechanism(s) of a Group II intron. The lab is unique in that your entire semester will focus on accomplishing a single complex experiment. Students will be organized in groups of two.

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### Techniques & Principles Covered

- Lab Safety
- Accurate & Precise Pipetting
- Solution Preparation
- Aseptic Technique
- Centrifugation (Sorvall & Microcentrifuge)
- Bacterial Growth & Quantitation
- Plasmid Manipulation & Extraction
- Spectrophotometric Quantitation of DNA
- Agarose Gel Electrophoresis
- Restriction Endonuclease Digests & Mapping
- Computer-Aided Database Access
- Virtual Digests
- Competent Bacterial Cells & their Preparation
- Bacterial Transformation with Plasmids
- Cloning Vector Preparation
- Generation of DNA Libraries
- Site-Directed Mutagenesis
- Oligonucleotide Design
- Understanding Plasmid Maps
- Phage Production & Titering
- Bacterial Transduction by Phage
- In vitro Transcription
- RNA Purification
- In vitro RNA Splicing
- Reverse Transcription
- Polymerase Chain Reaction
- Statistical Analysis of Results

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### Course Requirements

**Format and Time Expectations:**
The course will consist of a one-hour lecture on Tuesday at 12 noon and one five-hour lab period on either Thursday or Friday depending upon your section. The lecture will cover the experiments to be done and the theory behind them. You should expect to spend as much time outside of class preparing for the lab, (doing homework, reading background material, preparing presentations, and studying for exams) as you do in lab. There will be homework assignments, quizzes, presentations (one poster and one PowerPoint), plus a lab notebook requirement.
Text:
There is no textbook for the course. We will draw material for this course from primary research papers (peer reviewed publications), reviews, and third-party research protocols. Background information can be found in my library in the office. Two books that might unusually useful will be available. The first is posted under Course Documents. The 2nd Edition of the second book is available in the office and 1st Edition is posted under Course Documents.


Computers:
You will need to use computers extensively to label gel images, look up web-based material, produce PowerPoint presentations, and perform your lab work. You will perform bioinformatics searches and analyses. You will also examine DNA sequence maps of the plasmid vectors with DNA plasmid editing programs (Strider or APE). Computer Login = Students, Password = there is no password, just hit the return key.

Turning in your work
Some assignments will be turned as electronic files through email and / or CourseWeb. Assignment Files that you turn in MUST follow the first 2 rules or you will receive no credit, no exceptions.

1) Include your last name or first & last initials, followed by the assignment name.
   a) Jones_DNA Library1.docx and B.Smith_Splice Efficiency2.docx are good examples.
   b) Gel_file.docx is a very, very bad example.
2) Place your name inside, on the first page of the document.
3) File names should not be too long.
4) Never include unusual characters, such as the following: "/, !, @, $, %, ^, &, *, (, ), +, ?, <, >, ~".

Work Load:
You will have homeworks, two quizzes, a poster, and a 20-minute oral presentation. You also will be graded on the quality of your lab notebook. As stated above, you should expect to spend a substantial amount of time outside of class preparing for the lab session, doing homework, reading background material, preparing presentations, and studying for in-class exercises like exams.

Notebook
The notebook is a significant portion of the grade, because it is such an essential tool in any experimental lab. If one records the essential information in a notebook, then the notebook record allows experiments to be reproducible. Lab notebooks must be real-time documents of your actions and observations, much like an airplane black box. It must be written in permanent ink. Notes must be original. Notes should not to be transcribed or amended later, either the evening after the lab session or late in the term. Your lab notebook must have your primary data as you capture it during your experiment. The form – bound or loose-leaf – is not important. The lab notebook must be well organized.
Follow this outline form of the essential features of your lab notebook:

1) **Reserve the first two or three pages for your Table of Contents of the notebook.** In the Table of Contents, enter the dates, concise titles, and page numbers of your data pages every day.

2) **Organize each experimental entry with the headings:**
   - a. Goal,
   - b. Procedure,
   - c. Results,
   - d. Conclusions (or some reasonable equivalent).

3) Every page should include a date and a page number.

4) Arrange conceptually and/or historically ordered.

5) Write notes as you work in real-time or within the lab period of your experiment while your thoughts and memories remain clear.

6) **Include primary data, attaching photos (etc) with staples or tape.**

7) **Label gel pictures clearly or any attached data.**

8) **Produce good quality gel photographs and other data.** Clear data is respected data.

9) Include your actions, your observations, your rationale, your thoughts, and your interpretations in the record. Do not include printed protocols that we gave you, although you may choose wisely to outline these protocols in your notebook.

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**Grading Policy**

1) **Be prepared** by reading relevant protocols or background materials, as suggested,

2) **Show effort and focus** on your lab techniques and experimental interpretation,

3) **Show safe laboratory practices,**

4) **Work well with others,** most importantly with your lab partner, show good lab etiquette,

5) **Communicate often**—ask questions - respond to questions during lecture and lab.

The total points possible in this class are distributed as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab Attendance &amp; Performance</td>
<td>30%</td>
</tr>
<tr>
<td>Lab Homework</td>
<td>15%</td>
</tr>
<tr>
<td>Presentation</td>
<td>30%</td>
</tr>
<tr>
<td>Lab Notebook</td>
<td>25%</td>
</tr>
</tbody>
</table>

**Attendance is mandatory for all lab and lecture sessions.** All absences must be excused directly by the instructors prior to the class meeting. Email is the best method. All absences must be justified, and we may require documentation. When students are unable to attend, we will try to make the lab material and experiments available at alternative times, but this may not always be feasible.

**Policy on the Export or Import of Lab Materials**

We do not permit any student to take any teaching lab samples, reagents or other materials to their research lab or back from their research lab to the Langley Teaching Lab. This policy is directed to biological reagents and samples, and it does not include electronic or paper documents. When students take experiments back to another lab space, or carry supplies from their lab to the teaching labs, we cannot control the experiment or personal safety, which it is our responsibility to do.

**Disabilities Resource Services**

If you have a disability for which you are or may be requesting an accommodation, you are encouraged to contact both your instructor and Disability Resources and Services, 216 William Pitt Union, [412-648-7890/412-383-7355(TTY)] as early as possible in the term. DRS will verify your disability and determine reasonable accommodations for this course.
ACADEMIC INTEGRITY POLICY
Cheating/plagiarism will not be tolerated. Students suspected of violating the University of Pittsburgh Policy on Academic Integrity, noted below (**), will be required to participate in the outlined procedural process as initiated by the instructor. A minimum sanction of a zero score for a quiz, exam, or paper will be imposed.
** The integrity of the academic process requires fair and impartial evaluation on the part of faculty and honest academic conduct on the part of students. To this end, students are expected to conduct themselves at a high level of responsibility in the fulfillment of the course of their study. It is the corresponding responsibility of faculty to make clear to students those standards by which students will be evaluated, and the resources permissible for use by students during the course of their study and evaluation. The educational process is perceived as a joint faculty-student enterprise which will perforce involve professional judgment by faculty and may involve—without penalty—reasoned exception by students to the data or views offered by faculty. Senate Committee on Tenure and Academic Freedom, February 1974

EMAIL COMMUNICATION POLICY
Each student is issued a University email address (username@pitt.edu) upon admittance. This email address may be used by the University for official communication with students. Students are expected to read email sent to this account on a regular basis. Failure to read and react to University communications in a timely manner does not absolve the student from knowing and complying with the content of the communications. The University provides an email forwarding service that allows students to read their email via other service providers (e.g., Hotmail, AOL, Yahoo). Students that choose to forward their email from their pitt.edu address to another address do so at their own risk. If email is lost as a result of forwarding, it does not absolve the student from responding to official communications sent to their University email address. To forward email sent to your University account, go to http://accounts.pitt.edu/logintoyouraccount.click on Edit Forwarding Addresses, and follow the instructions on the page. Be sure to log out of your account when you have finished. (For the full E-mail Communication Policy, go to www.bc.pitt.edu/policies/policy09/09-01.html.)

OFFICE HOURS
Office hours for either the Instructor or the Teaching Assistant can be Monday-Friday between 9:30am and 5pm, but because of our uneven schedules Office hours must be arranged by appointment. All relevant contact information (Office location, phone number, and email addresses) can be found in the first page of syllabus.
## 1950 Schedule For 2014

<table>
<thead>
<tr>
<th>Week</th>
<th>General Goal(s)</th>
<th>Concepts &amp; Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lab Safety</td>
<td>Lab Safety - Proper Equipment Usage - Plasmids as tools, vectors &amp; sources of genetic traits. Basis &amp; practice of plasmid isolation. Diagnostic DNA digests &amp; interpreting gels.</td>
</tr>
<tr>
<td>2</td>
<td>Cloning Vector Preparation I</td>
<td>Emphasis on Reagent Preparation, DNA Restriction Enzymes &amp; Ligation: Generating reagents for long-term experiments. Cloning Vector/Plasmid DNA restriction digestion, Phosphatase Treatment, Gel purification of DNA &amp; DNA Ligation.</td>
</tr>
<tr>
<td>4</td>
<td>Vector Preparation III: Analysis</td>
<td>Emphasis on Data Interpretation and Analysis: Plasmids from transformants &amp; restriction analysis. Score by color, insert size. Representation &amp; coverage of the genome by library.</td>
</tr>
<tr>
<td>6</td>
<td>Bacteriophage Techniques I: Phage production &amp; tittering NO LECTURE on TUESDAY</td>
<td>Emphasis on Phage techniques: Serial dilution &amp; spot titration, plaque isolation, interfering particles, replication &amp; phage RF DNA. Infect CJ236 (pBI1) with phage M13K07.</td>
</tr>
<tr>
<td>8</td>
<td>Site Directed Mutagenesis II: Production of mutant strands</td>
<td>Emphasis on Physical Chemistry of First Strand DNA Synthesis: Annealing reaction, extension, ligation, transformation, kinasing of oligos.</td>
</tr>
<tr>
<td>9</td>
<td>Site Directed Mutagenesis III: Recovery and verification of mutant plasmids</td>
<td>Emphasis on Course Content Review: Plasmids of SDM transformants and Restriction Endonuclease (REND0) analysis. Plasmids cut as in vitro transcription templates.</td>
</tr>
<tr>
<td>10</td>
<td>Splicing accuracy I: In vitro transcription, RT, &amp; PCR</td>
<td>Emphasis on Phage RNA polymerase systems for in vitro RNA production. Self-splicing, reverse transcription, &amp; primary PCR.</td>
</tr>
<tr>
<td>11</td>
<td>Splicing accuracy II: Production of cDNA library</td>
<td>Project Completion I: Purify 1st PCR of cDNA, conduct 2nd PCR, purify &amp; cut secondary PCR, ligate &amp; transform cDNA library.</td>
</tr>
<tr>
<td>12</td>
<td>Splicing accuracy III: Analysis of cDNA library</td>
<td>Project Completion II: Colony analysis. Prepare plasmids from selected colonies, REND0 analysis, DNA sequencing reactions</td>
</tr>
<tr>
<td>13</td>
<td>Extra Time for additional Experiments &amp; Analysis</td>
<td></td>
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<tr>
<td>14</td>
<td>---- THANKSGIVING BREAK ----</td>
<td>NO LECTURE OR LAB</td>
</tr>
<tr>
<td>15</td>
<td>Final Presentations</td>
<td>PowerPoint presentations – 15-20 minute each</td>
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</tbody>
</table>

**NOTE:** This schedule may be modified during the semester. You will be notified of any such changes.
LAB SAFETY -- Our 3 Main & Simple Goals are for you to....

1. Learn substantial concepts and techniques,
2. Keeping you safe from any injury,
3. Do all this while not breaking OUR stuff.

General Rules of the Laboratory

- Know the Locations of Room Exits, Eye Wash Stations, Shower, and First Aid Kits: know their locations and how to use them – Lab Layout drawing is the last page of this document.
- **Hazardous Materials** (acids, bases, toxins, etc.): know where they are and after use, put them away - back where they came from.
- **Hazardous Waste Disposal**: use them appropriately. Disposal is expensive.
  - Glass Waste Bins: glass only, not gloves, plastic, tissues, etc.
  - Biohazard Waste Bins: experimental plates only....
  - Ethidium Waste Bin: Gels only, not gloves and paper towels
- **Gloves**: always wear gloves in the lab except when using computer keyboards.
- **Wash Hands Always**: whenever you leave the lab.
- **Wear appropriate clothing**: Lab coats are available.
- **Goggles/Eyeglasses are recommended and available.**
  - Contact wearers must wear eye protection when handling hazardous materials.
- **No food or drink in the labs.**
- **No cell phones use in the lab (includes text).**
- **In the event of fire, evacuate the lab** – check and make sure your lab mate makes it out.
- **Be a conscientious citizen** – inform lab personnel of concerns or potential hazards, such as spills of unknown origin & do your part to keep the lab clean & organized.
- **Never use equipment without proper instruction**: particularly centrifuges.

Three Good Reasons for Proper Instrument Use & Care

1) You get **BETTER DATA** if you use our equipment properly, and this usually means a **BETTER GRADE**.
2) Proper use and care for our equipment is **SAFER**.
3) Our instruments are expensive so proper reduces costs and **lower lab fees**.

Centrifuge Safety

There are two general types of centrifuges in the Langley Teaching Labs:

- **Table Top centrifuges (the only ones your class uses):**
  - for quick spins of 2 ml or less which do not exceed 20,000 rpm
  - 12 Eppendorf 5418, one Hermle LabNet
  - potentially dangerous when tubes with hazardous substances break

- **Sorvall & Allegra Centrifuges:**
  - referred to as “Floor model” centrifuges that look like washing machines
  - handle large volume samples (greater than 2 ml to 250 ml)
  - exceed 20,000 rpm
  - potentially dangerous & very expensive - usage requires training, (see below)
Table Top Centrifuges: What you must do:

- **Balance Tubes:** When loading tubes into any centrifuge rotor, the mass **must** be distributed evenly. Unbalanced rotors become stressed, as does the spindle (think axle). Over time, this stress leads to failure of the metal rotor or spindle, which is potentially dangerous and costly. To balance correctly, see the strategies to balance an eight-position rotor diagramed below.

- **Keep it Clean:** If a tube leaks or breaks, or if any solution wets the rotor or the inside, notify lab personal. If you know the liquid spilled is not dangerous, clean it up and inform lab personnel. Solutions in rotors cause micro-etching of the metal, from which stress fractures occur leading to failure of the metal rotor.

Sorvalls Centrifuges: What you must do:

- **You must be trained in use the Sorvalls.** Do not use a Sorvall until you have been trained to do so.

- **You must fasten the lid of the rotor completely.** There is no wiggle room for this. Not fastening a rotor lid destroys the rotor.

- **Carefully balance your centrifuge bottles with their lids** on the “trip balance”, for which you will be trained.

- **Never overfill a centrifuge bottle.** At speed the liquid will assume a vertical position that will leak out of overfilled containers. A 500 ml bottle can only safely hold 250 ml.

- **Always stay with the Sorvall as it goes to speed.** If there is a problem such as an imbalance, they can be detected early and the machine **MUST** be shut off immediately to minimize the danger and the damage.

- **Keep it Clean:** If a tube leaks or breaks, or if any solution wets the rotor or the inside, attempt to clean it or notify lab personal. If you know the liquid spilled is not dangerous, attempt to clean it up and inform lab personnel

Common causes of centrifuge malfunctions include:

1) **OPERATOR ERROR**
   a. Failure to place the lid on the rotor. (Sorvall only)
   b. Improper balancing of centrifuge tubes.
   c. Failure to properly secure the rotor to the drive.
   d. Overloading the rotor's maximum mass.
2) **Metal Fatigue of the rotor.

→ → → IF A CENTRIFUGE MALFUNCTIONS ↔ ↔

- Turn off the centrifuge IMMEDIATELY & do open!
- Tell your instructor or lab staff.
Lab Layout Schematic:

Note the location of Exits and of Safety Equipment (EyeWash Stations, Emergency Showers, First Aid Kits). Other essential equipment is listed.