

Biology 382 - Molecular Biology Lab Syllabus - Spring 2009

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Lecture: Tues/Thurs, 2:30-3:25 PM, ST128
Lab: Tues/Thurs 3:30-6:30 PM, ST429

Office Hours: Mon 1:30 - 3:00 PM, Tues 8:00 - 10:00 AM, Thurs 8:00 - 9:30 AM, or by appointment. Email is typically an excellent way to get a quick response to a question.

Course Home Page: home.sandiego.edu/~cloer/bio382.html

Goals of the Course

Students will learn principles and practice of basic bacterial culture techniques, transformation, agarose gel electrophoresis, nucleic acid purification (plasmid and genomic DNA, RNA), nucleic acid quantification, DNA restriction digestion and analysis, Southern hybridization, library construction, polymerase chain reaction (PCR), and basics of computer-based DNA sequence analysis and data acquisition over the internet. In addition, students will learn about the nature and selection of DNA cloning vectors, restriction enzymes, modifying enzymes, polymerases, and other reagents used in molecular biology. We will examine aspects of bioinformatics and genomics (comparative, functional, etc.), and newer molecular technologies such as next-generation sequencing and microarrays that are especially important in genomics. We will apply our newly learned molecular techniques toward solving real biological research questions.

Class periods will be used flexibly; in some cases we may meet in the lab beginning at 2:30, other times we will have a lecture may include any of the following: short lecture by instructor, pop quiz, demonstrations by instructor, or viewing of films or videos. It is *essential* that you review the next lab's procedures before showing up in lab as time may be short in the lab period. Be prepared and be on time. To provide additional motivation for reading and preparing lab material ahead of time, a short test ("pop quiz") may be given at the beginning of some labs. In some cases, a quiz may be given when a student has completed a given lab.

Work outside of the regular lab period

The practice of molecular biology cannot easily be restricted to a four-hour lab period (even twice per week). It is unavoidable that students will be required on a number of occasions either to come to the lab briefly the day before lab to set up something for the next day, or to return to the laboratory the day following a particular experiment to examine their results.

Lab Citizenship: Safety and Courtesy

Strictly following all safety rules is basic to good lab technique.

- ☞ No eating, drinking, smoking or application of cosmetics in the laboratory. Please do not bring any food or drink containers into the lab, or discard such items in trash containers inside the lab.
- ☞ Shoes that cover the feet must be worn at all times (no sandals).
- ☞ Long hair must be tied back.
- ☞ Lab coats should be worn at all times, except for computer-only labs.
- ☞ Protective eyewear must be worn as necessary.
- ☞ Contact lenses must not be worn in lab.
- ☞ No pipetting by mouth.
- ☞ No unauthorized experiments are to be performed; no unauthorized use of equipment.
- ☞ Follow carefully instructions for disposal of glass, bacterial cultures, wastes, etc.
- ☞ Wash hands during lab as necessary, and thoroughly following lab.

Deviation from safety policies will be taken seriously

In addition, please note that we may share the room with other biology laboratory sections. As a courtesy, we must diligently clean up after ourselves at the end of the lab period. Put away your experimental materials and other equipment as directed, and clean up as necessary. Don't leave it to someone else. Take care of your own mess! Furthermore, please do not disturb ongoing experiments of your classmates or those of other sections that may be in the room.

Books and Supplies

Laboratory DNA Science by Bloom, Freyer, and Micklos, ©1996 Benjamin-Cummings Publishing Co., Inc. - This is the main text for the lab, although there will be many modifications and additions to the protocols found here. Note: If you decide to buy a used copy of the manual, check the condition of the manual **very carefully** - the pages are perforated and therefore easily torn out.

Cold Spring Harbor Laboratory Research notebook, ©1999 Jones & Bartlett, Publishers

Principles of Gene Manipulation and Genomics by Primrose & Twyman, 7th edition, ©2006 Blackwell Publishing.

Optional: *Gene Cloning & DNA Analysis* by Brown, 5th edition, ©2001, Blackwell Publishing. This is a more basic text; much of the material you should already have had in Bio 225 & 300. You may want it if you feel you need more review and/or you no longer have the texts from those earlier classes.

Semi-log graph paper (print out the PDF or copy from the lab manual supplement handouts)

Sharpie extra-fine or ultrafine permanent markers (a few different colors may be useful), used for marking tubes and plates (they write on glass or plastic).

Watch with second hand or timer (although this year we may provide you with timers in lab).

Scientific calculator

Lab coat (You must bring this to every lab, excepting computer labs)

Attendance

Attendance at all classes (since most include lab) is **required**. If you have a legitimate conflict (e.g., college athletics, religious holiday) with a given lab session **please let the instructor know as soon as possible**. If you miss a lab unexpectedly for a legitimate reason (e.g., sickness) you may have the opportunity to make it up later; however, in some cases this may be impossible. Some other form of makeup may be arranged.

Lab Notebooks, Lab Reports

You are required to keep a lab notebook in which you will keep an accurate record of your observations and experiments. For some labs, it is appropriate to write out the procedures in the lab notebook prior to lab. Carbon copies from your notebooks may be collected and evaluated periodically. Thus, they should be interpretable to someone other than just yourself. We understand, however, that a lab notebook is a working document, so we don't expect them to be perfectly neat, just legible. Loose items such as photographs must be taped securely into your lab notebook immediately upon completion. Taking data on loose sheets is not acceptable.

For actual research projects, the top copy (original) from your lab notebook must be inserted in the project looseleaf including appropriate gel images each day of work in lab. You may print a 2nd copy of gels to keep with the back copy in your notebook. This looseleaf notebook should not leave the lab as it will be shared with your partners for your project.

For labs that require a report, you will typically be given specific instructions on the format. Since the kinds of labs vary through the course, formats may be somewhat

different for each report. The section of the lab manual for labs requiring a report should direct you to collect all the information you need for your lab report. Feel free to ask the instructor about these issues if you're not sure. Lab reports, unless otherwise specified, must be typed and neat. Lab reports that have many typographical errors or are unintelligible will be returned immediately for correction. Lab reports that are late will be reduced by 10%, with another 10% for each additional day late.

Provisional Lab Schedule - Spring 2009 - Molecular Biology

Lab numbers below indicate labs found in *Laboratory DNA Science*. Labs should be read completely prior to coming to lab, including "Results and Discussion" sections, sections in the supplemental manual, and any additional handouts given before lab. Readings in *Principles of Gene Manipulation and Genomics* are indicated as *PGMG*.

Date	Location	Topic / Activity
Tues. Jan. 27	ST128 ST429	Lecture: Plasmic biology, Plasmid vectors. Review of basic cloning. Lab: safety, pipetting, centrifugation, basic bacterial techniques: Labs 1 & 2A. Demonstrate Lab 2B. (Next day: check plates and tubes).
Thurs. Jan. 29	ST429 laptops	Lecture: Introduction to sequence databases, basic bioinformatics. Reading: <i>PGMG</i> Chapter 9 (157-166). Lab: Sequence retrieval, simple alignments, multiple alignments Basic sequence analysis 1 - due Feb. 3
Tues. Feb. 3	ST429	Lab 2B (<u>Evening before</u>) & 2C: Overnight and mid-log cultures Lab 8A: Classic procedure for making competent cells (also read pre-lab notes for Lab 5, pp. 73-80.) Calculations sheet and graph due at end of lab
Thurs. Feb. 5	ST429 laptops	Genomic sequence analysis: gene-finding, BLAST searching, genome annotation. <i>PGMG</i> Chapter 9 (166-176). Basic sequence analysis 2 - due Tues., Feb. 10
Tues. Feb. 10	ST429	Lab 5: Rapid Colony transformation of <i>E. coli</i> , Transformation with classical competent cells (supplement) (Next day: count colonies to assess transformation) Lab report due Tues., Feb. 17
Thurs. Feb. 12	ST128	Lecture: Agarose gel electrophoresis Lecture: Restriction endonucleases, other mol bio enzymes
Tues. Feb. 17	ST429	Lab 3: DNA Restriction and Electrophoresis Lab report due Tues., Feb. 24
Thurs. Feb. 19	ST128 ST429	Hourly Exam / Practical Exam (basic techniques to date)
Tues. Feb. 24	ST429 laptops	DNA sequence analysis II: Cloning strategies, computer-assisted restriction analysis. Cloning strategy report due Tues., Mar. 3
Thurs. Feb. 26	ST429	Lecture: Nucleic acid purification, quantification Lab 6: Purification and Characterization of Plasmid DNA (<u>Evening before</u> : set up overnight culture) Lab report due Mar. 5

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Date	Location	Topic / Activity
Tues. Mar. 3	ST128	Lecture: Polymerase Chain Reaction, other DNA amplification methods Lecture: Research Project 1 outlines, cloning genomic sequences by PCR, cloning techniques to be used (TOPO-TA, Gateway cloning systems).
Thurs. Mar. 5	ST128 ST429 laptops	Lecture: Genomics (whole genome sequencing), functional genomics (microarrays, KO mutants, RNA interference). Reading: PGMG Ch. 17, 19, supplements provided. Project 1: Lab: Retrieve genomic sequences, design PCR primers (Primer 3), order primers
March 9 - 13		SPRING BREAK
Tues. Mar. 17	ST429	Project 1: Purify genomic DNAs for PCR, set up reactions, run PCR.
Thurs. Mar. 19	ST429	Project 1: Run gels to analyze results of PCR. Clone bands from successful PCR (TOPO-TA method), transform cells and spread. (<u>Next day:</u> count colonies, respread. <u>Day after next:</u> examine and save respread plates)
Tues. Mar. 24	ST429	Project 1: Isolate plasmid DNAs by Qiaspin, analyze by digest and gel electrophoresis. Perform Gateway recombination reactions with good clones, transform. (<u>Evening before:</u> set up overnight cultures; <u>Next day:</u> respread colonies. <u>Day after next:</u>)
Thurs. Mar. 26	ST429	Lab 13 A & B: Electrophoresis and Southern Blotting of λ DNA digests (<u>Next day:</u> transfer 'pre-hyb' membranes to refrig.)
Tues. Mar. 31	ST429 laptops	Lab 13 C & D: Hybridization and Non-radioactive Probe Detection (<u>Evening before:</u> add hyb solution with probe.) Lab report due Tues. Apr. 7 Project 1: Examine and save respread plates.
Thurs. Apr. 2	ST429 laptops	Project 1: (<u>Evening before:</u> set up overnight cultures from respread plates) Isolate plasmid DNAs by Qiaspin, prepare and send DNAs for sequencing. Begin Research Project 2 planning: Analyze putative gene knockout mutants. Retrieve sequence, do computer restriction analysis, consider strategies for analyzing by Southern and/or PCR.
Tues. Apr. 7	ST429 laptops	Project 1: Analyze returned sequences of clones sent out Project 2: Present and discuss strategies for KO analyses, make final decisions, order reagents as needed (REs, PCR primers)
April 9 - 13		EASTER BREAK
Tues. Apr. 14	ST429	Project 2 (continued) – Activities depend on strategy.
Thurs. Apr. 16	ST128	Lecture: Comparative genomics. Reading: PGMG Chapter 18

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Date	Location	Topic / Activity
Thurs. Apr. 16 (continued)	ST429	Project 2 (continued)
Tues. Apr. 21	ST429	Project 2 (continued)
Thurs. Apr. 23	ST429	Lecture: Comparative genomics. Reading: PGMG Chapter 18 Project 2 (continued)
Tues. Apr. 28	ST429	Begin Research Project 3 (RT-PCR and cloning or site-directed mutagenesis): To be determined (TBD)
Thurs. Apr. 30	ST429	Lecture: TBD, related to Project 3 Project 3 (continued)
Tues. May 5	ST429	Project 3 (continued)
Thurs. May 7	ST429	Lecture: TBD Project 3 (continued)
Tues. May 12	ST429	Last day of classes, Project 3 ends
May 13-14		Study Days
May 15-21		FINAL EXAMS

Final project reports due by no later than end of finals. [Final exam? TBD.]

Some important notes on academic honesty and plagiarism in lab reports:

1. Although in some cases students may be using the same primary data to prepare reports, each student must write a separate report, and prepare separate figures, tables, graphs and data analysis. Students working together must not turn in identical figures, graphs or tables (except perhaps for some primary data, such as a copy of a gel photograph), as this calls into question whether one of the students has done the work assigned.

2. It is not appropriate to use direct quotations from a source without placing the material in quotation marks and attributing the source, even if the source is cited. This is plagiarism -- representing to the reader that another's writing is your own. Furthermore, in the context of a lab report, quotations should be used sparingly or not at all, and should never be more than a phrase or sentence long. A paragraph-long quotation means that you are not writing. Paraphrasing can be a bit trickier -- a little harder to draw the line on what is plagiarism -- but if the sentence or sentences is almost identical to the source with a few changed words, or slightly rearranged, then that is likely to be plagiarism as well. A good way to avoid paraphrasing or unambiguous plagiarism from sources is to write without having the source(s) in front of you. This way the writing can only be in your own words, synthesized from your reading.

3. Copying of any material from other Biology 382 students is plagiarism and will not be tolerated. Do your own work and demand that others do theirs. Take similar care in your use of material from the lab manuals and the textbook. All portions of lab reports and papers are expected to reflect **ONLY** your own work and your own writing. When working in groups, each member of the group is expected to synthesize the information, analyze data and

prepare an individual report. If you use literature in your report, cite it appropriately. [For example, copying sentences from the book, followed by a parenthetical citation (Primrose, 2006), is plagiarism.] **Be sure you understand what constitutes plagiarism.** If you have any questions about this, or any other item related to academic integrity, please ask. USD Academic Integrity policy will be strictly enforced.

4. USD subscribes to a service called **Turnitin.com**, which is a web-based application that compares the content of submitted papers to the Turnitin.com database and checks for textual similarities. All required papers for this course may be subject to submission to Turnitin.com for textual similarity review and to verify originality. (Remember to submit a regular paper copy for grading purposes.) All submitted papers will be included as source documents in the Turnitin.com reference database solely for the purpose of detecting textual similarities and verifying originality. Each student is responsible for submitting his or her papers in such a way that no identifying information about the student is included. A student may not have anyone else submit papers on the student's behalf to Turnitin.com. A student may request in writing that his or her papers not be submitted to Turnitin.com. If a student chooses this option, however, the student may be required to provide additional documentation in a form required by the faculty member to substantiate that the papers are the student's original work.

Electronic submissions to Turnit.com should be completed on the assignment due date.

Modifications to Labs

Minor to major modifications will be made to most of labs from the manual *Laboratory DNA Science*. Handouts detailing changes will be found in your lab supplements manual or will be handed out in class a week to a few days prior to the affected lab. You should read these and incorporate them in your preparations for lab. If you miss getting the handout, check with the instructor or on the class home page, in the announcements/ handouts section. It is a good idea to check there periodically for last minute updates, handouts and announcements for class. Occasionally, last minute changes must be made to labs because of events beyond the instructor's control.

Lab Grade Breakdown

Note that modifications to grading percentages may be necessary, depending on lab outcomes and alterations in assignments. Grading will follow the outlines below.

Reports:	78%
Exams:	10%
Quizzes:	8%
Participation:	4%