Biology 382 – Techniques in Molecular Biology – Syllabus, Spring 2013

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Lecture/Lab: Tues/Thurs, 8:00 AM -12:00 PM, SCST429

Office Hours: Mon 1–2 PM, Tues 3–5 PM, Wed 10-11 AM, Thurs 2-3 PM or by appointment. Email is usually an good way to get a quick response to a question – although it is no substitute for one-on-one help with discussion in my office.

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

Goals of the Course

This is a research projects-based course in which you will learn to apply molecular biology techniques (focused on nucleic acids) in the laboratory to ask scientific questions. 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, and newer/advanced molecular technologies such as next-generation sequencing and microarrays that are especially important in genomics, and discuss the burgeoning other '-omics' fields. We will apply our newly learned molecular techniques toward solving real biological research questions, and presenting results on a poster at USD's undergraduate research conference.

At the end of Biology 382, a student should be able to:

* List and explain safety issues and proper practices associated with standard molecular techniques, including bacterial culture, electrophoresis, and nucleic acid purification and detection chemistry.
* Explain, demonstrate and practice principles of bacterial culture, sterile technique, transformation, and DNA and RNA purification and quantification.
* Understand the nature of molecular biological hypothesis and testing – how molecular analysis answers scientific questions.
* Examine and incorporate primary literature in the formulation of molecular hypotheses
* Use and explain the application of various standard bioinformatic techniques to experimental planning and analysis, including sequence accessing and manipulation, BLAST, multiple sequence alignment, PCR primer design, etc.
* Explain and discuss the modern role of genomics (comparative, functional, etc.) and other '-omics' in molecular analysis.
* Understand and explain the many variations on PCR and when to use them, and how to troubleshoot a PCR protocol by selecting parameters to vary.
* Independently plan, execute and document a basic DNA cloning experiment involving PCR amplification, cloning into an appropriate DNA vector, transformation, plasmid DNA

isolation, followed by restriction enzyme analysis with agarose gel electrophoresis, DNA sequencing, and sequence analysis to evaluate success of the procedure.

Class periods will be used flexibly: class may begin with lecture or with lab work (and short lectures interspersed during the lab; at other times we will begin with any of the following: pop quiz, demonstrations by instructor, or viewing of films or videos. It is *essential* that you review the lab's procedures and background in detail before coming lab as time may be short to complete a procedure during the lab period. **Be *prepared* and *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 at the completion of 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 many 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

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

Recommended: *Gene Cloning & DNA Analysis* by Brown, 6th edition, ©2001, Blackwell Publishing. Much of the material here you should already have had in Bio 225 & 300. You may want it if you feel you need more review, you no longer have the texts from those earlier classes, or are taking Bio 300 currently.

Recommended: *Applied Molecular Genetics* by Miesfeld, ©1999 Wiley-Liss. Parts of this are more advanced than *GCDA*, and specfic to the material we will cover in class. We will use many illustrations from this book.

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.
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 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 research projects, the perforated copy from your lab notebook must be inserted in the project loose-leaf including appropriate gel images each day of work in lab. You may print a 2nd copy of gels to keep with in your own notebook. This loose-leaf notebook should generally stay in 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.

Conference Poster

An central part of the course is the preparation and presentation of a poster at USD's Undergraduate Research Conference (formerly known as 'Creative Collaborations.') To create a high quality takes a lot of work, and many drafts with revisions. An important part of your grade comes from meeting deadlines for poster drafts at an appropriate level of completion. Much of the work on the poster can be done before any results from your project are obtained. This includes explaining WHY you are doing the molecular biology – the background and experimental question and/or hypothesis (introduction) and HOW you plan to accomplish it (methods/techniques). As a presentation associated with a techniques class, your poster may have more focus on methods than a poster would normally have.

Provisional Lab Schedule - Spring 2013 - Molecular Biology

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.
GCDA = Gene Cloning & DNA Analysis; AMG = Applied Molecular Genetics

Date	**Topic / Activity**
Tues. Jan. 29	Lecture: History of molecular biology & techniques, genomics. Lab: safety, basic techniques: pipetting, sterile technique, bacterial culture. (Next day: check plates and tubes). Discuss research project selection.
Thurs. Jan. 31	Lecture: Review of DNA cloning techniques, plasmid biology, vectors, bacterial genetics related to DNA cloning methods. Lab: Sequence databases, basic bioinformatics: sequence retrieval, simple pairwise alignments, multiple alignments (laptops). **Basic sequence analysis 1 report - due Tues., Feb. 5**
Tues. Feb. 5	(**Evening before**) Set up overnight culture of DH5α. Lab: Mid-log suspension culture, classic competent cells preparation. **Calculations sheet and graph due at end of lab.**
Thurs. Feb. 7	Lecture: Questions and hypotheses using molecular biology. [Introduction to experimental systems.] Discussion: Lab project proposals, URC abstract preparation Lab: Genomic sequence analysis: gene-finding, BLAST searching, genome annotation. **Basic sequence analysis 2 report - due Tues., Feb. 12**
Tues. Feb. 12	Lab: Rapid colony & classical competent *E. coli* transformations (Next day: count colonies to assess transformation) **Transformation lab report due Tues., Feb. 19**
Thurs. Feb. 14	Lecture: Agarose gel electrophoresis safety, principles & practice Lecture: Restriction endonucleases, other molecular biology enzymes **Draft of URC Abstract due**
Tues. Feb. 19	Lab: DNA Restriction and Electrophoresis **Lab report due Tues., Feb. 26**
Thurs. Feb. 21	**Hourly Exam / Practical Exam (basic techniques to date)** Break **Discuss revisions of URC abstract**
Tues. Feb. 26	Lecture: Nucleic acid purification, quantification Lab: Purification and Characterization of Plasmid DNA (Evening before: set up overnight culture). **Lab report due Thurs., Mar. 7**

Date	**Topic / Activity**
Thurs. Feb. 28	Lecture: Polymerase Chain Reaction, other DNA amplification methods Lab: Discuss **Research Project 1**, cloning genomic sequences by PCR, cloning techniques to be used (TOPO-TA, Gateway cloning systems). **Final revisions of URC abstract due** (discuss as necessary)
Fri. Mar. 1	**USD Undergraduate Research Conference Abstract Submission Deadline** [see www.sandiego.edu/ ugresearch/urc/] (Abstract **must** be approved by instructor before submission) **Schedule poster printing time** (as close to conference as possible)
Tues. Mar. 5	Lab: **Project 1 begins**: Retrieve genomic sequences, design PCR primers (using 'Primer 3'), order primers (as needed)
Thurs. Mar. 7	Lecture: Genomics (whole genome sequencing), Gibson cloning, functional genomics (microarrays, KO mutants, RNA interference). Lab: **Project 1** - Purify genomic DNAs for PCR, plan PCRs
Tues. Mar. 12	**Project 1**: Prepare primers, Set up reactions, run PCR. **Work on URC Poster, introduction, Materials & Methods**
Thurs. Mar. 14	**Project 1**: Run gels to analyze results of PCR. Clone bands from successful PCR (TOPO-TA method), transform cells and spread. (Next day: count colonies, respread. Day after next: examine and save respread plates)
Tues. Mar. 19	**Project 1**: Isolate plasmid DNAs by Qiaspin, analyze by digest and gel electrophoresis. [Depending on project: Perform Gateway recombination reactions with good clones, transform. (Evening before: set up overnight cultures; Next day: respread colonies from any new transformations.)] **Lab report due Tues. Apr. 6**
Thurs. Mar. 21	**Project 1**: continued **Draft of URC Poster outline, introduction, M&M, results to date due**
Mar. 25 - April 1	**SPRING / EASTER BREAK**
Tues. Apr. 2	Lab: DNA sequence analysis - Cloning strategies, computer-assisted restriction analysis. **Cloning strategy report due Tues., Apr. 9.** **and Project 1** (continued**)**
Thurs. Apr. 4	**Project 1** (continued); help with cloning strategy exercise as needed Begin Research Project 2 planning (possible)
Tues. Apr. 9	**Project 1 and poster preparation** (continued)

Date	**Topic / Activity**
Thurs. Apr. 11	**Project 1 and poster preparation** (continued) **Final draft of URC poster due with results (or Fri. Apr 12)**
Tues. Apr. 16	**Project 1 and poster preparation** (continued) [Poster printing at scheduled time, final approval **required**.]
Thurs. Apr. 18 **UC Forum**	**Poster presentation at USD Undergraduate Research Conference (11:00 AM – 3:00 PM) Hahn University Center** **Project 2 proposal due in class (~ 1 page)**
Tues. Apr. 23	Lab: Electrophoresis and Southern Blotting of λ DNA digests (or other) **Project 2 individual conferences**
Thurs. Apr. 25 **It's DNA Day !**	Lab: Hybridization and Non-radioactive Probe Detection (or other) (Evening before: add hybridization solution with probe.) **Lab report due Thurs May 2**
Tues. Apr. 30	**Project 2**, **[Project 1 continuation as appropriate]**
Thurs. May 2	**Theory of Molecular Technique Exam #2** / **Project 2** (continued)
Tues. May 7	**Project 2** (continued)
Thurs. May 9	**Project 2 - Class presentations** [or May 21?]
May 14 - 15	Study Days
May 16 - 22	**FINAL EXAMS**

Note: our final period is **Tues, May 21, 8-10 AM** – reserve in case needed

Project lab books must be complete, all clones documented, and bacterial strains with DNAs frozen (as 'plasmid permanents') by no later than May 22.

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 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.

Grading Breakdown

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

Reports/Projects Notebook:	50%
Exams:	10%
Quizzes:	10%
URC Poster:	20%
Participation:	10%