Biology / Chemistry 330 – Techniques in Molecular Biology

Dr. Curtis Loer
Office: SCST437, 619-260-4129
Office Hours: Mon 3:30-5:00 PM, Tues 9:30-11:00 AM, Thurs 11:00 – 12 noon or by appointment. I will be present in my office in person but can also meet by Zoom. Please email or call if you would like for me to open a Zoom room to meet during office hours.
Email: cloer@sandiego.edu

Lecture: Monday, Wednesday 11:15 – 12:10 PM, SCST 261
Lab: Wednesday 2:30 – 6:30 PM, SCST 429

Websites: Canvas, LabArchives (ELN), and occasionally the legacy course web page: www.sandiego.edu/~cloer/bio&chem330.html (mostly superseded by the Canvas site).

Goals of the Course

Students will learn and apply molecular biology techniques (focused on nucleic acids) in the laboratory. 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, polymerase chain reaction (PCR), and basics of computer-based DNA sequence analysis and planning of cloning strategies. In addition, students will learn about the nature and selection of DNA cloning vectors (bacterial & eukaryotic), 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 CRISPR-Cas9 genome editing. Some primary literature articles illustrating standard techniques may be studied. We will apply our newly acquired molecular techniques toward examining real biological research questions, and to perform a basic DNA cloning project.

At the end of Biology 330, 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, detection, and quantification.
* Explain, demonstrate, and practice principles of sterile technique, bacterial culture, transformation, and DNA and RNA purification and quantification.
* Understand the nature of molecular biological hypothesis and testing – how molecular (genetic) analysis answers scientific questions.
* 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, cloning strategies, 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 polymerase chain reaction (PCR) and when to use them, and how to troubleshoot a PCR or other nucleic acid synthesis protocol by selecting parameters to vary.
* Plan, execute and document a basic DNA cloning project 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.

* Plan (and execute, time permitting) one or more advanced cloning projects using RNA isolation and RT-PCR *or* cloning techniques such as Golden Gate, Gibson assembly, SLIC, Oligonucleotide stitching, etc. *or* designing a CRISPR guide RNA (sgRNA) and testing its function *in vitro*.

There is no required textbook for the class. A possible text for theory if you want one – *From Genes to Genomes,* 3rd Ed., by Dale, Schantz & Plant. ©2012 Wiley. (Your Bio 240/242 and 300 texts will also be useful.) We will also use eBooks at Addgene.org such as 'Plasmids101.'

Electronic Lab Notebook (required) – Lab Archives – https://mynotebook.labarchives.com, specific notebook setup: 330s24 Lab Notebook

Small composition book (useful for in-lab notes)

Other required items: lab coat

Grading Breakdown

Note that adjustments to grading percentages may be necessary based on final disposition of all assignments and cloning projects.

Pre-labs:	10%
Weekly post-lab write-ups (in ELN)	40%
Short assignments / quizzes (may be online):	10%
During semester quizzes / short exams	20%
End of semester final exam:	15%
Citizenship / Participation / Attendance:	5%

There will be four short exams/quizzes during the semester including both theory and practice, and a final theory exam at the end of the semester (see calendar/schedule below).

Class periods will be used flexibly: class may begin with lecture or lab work (and short lectures interspersed during lab); as we progress later in the semester some lecture periods may be given over to additional lab time. It is *essential* that you review each lab's procedures and background in detail before coming lab and write up protocols in your lab notebook in advance since time may be short to complete a procedure during the lab period. **Be *prepared* and *on time*.** Occasionally, a short test ('pop quiz') or lab notebook check may occur at the beginning of some classes.

Attendance at all lab sessions in your registered section is **required**. If you have a legitimate conflict (e.g., college athletics, religious holiday) **please let the instructor know as soon as possible**. Ideally you should contact the instructor prior to the lab; otherwise as soon as possible (i.e., within 24 hrs). Many lecture periods involve exercises and active participation, so attendance there is needed to get credit for such activities. Lectures may also include important preparation for upcoming labs.

Deadlines - The timing and due dates of assignments in this document are provisional and may change during the semester. Ultimate due dates for assignments will be provided in class. The course web page will also provide dates for all assignments as well as a description for each assignment.

Each laboratory experiment, from simple DNA gel running to the more project-oriented experiments - MUST be recorded into the lab notebook following the prescribed format - the electronic lab notebook (ELN) is central to your evaluation. See the more detailed lab notebook instructions and evaluation rubric.

Work outside of the regular lab period - The practice of molecular biology cannot easily be restricted to a four-hour lab period. It is unavoidable that students will be required on many occasions to work in the lab outside of scheduled lab times to prepare cultures, set up experiments and score results of experiments, particularly once students are working on their own cloning projects. Students will have access to the lab and associated rooms 7 days a week, 7 AM – midnight (maximum authorized undergraduate student access), along with the required building access.

Barriers

If you have issues participating in class for personal or technological reasons (or anticipate them), it's ***really important*** that you let me know about these. I can't help you or provide accommodations if I am unaware of problems. Contact me as soon as possible about concerns. As always, reasonable accommodations are also available for students with a documented disability through USD's Disability and Learning Differences Resource Center (260-4655) http://www.sandiego.edu/disability/.

Wellness

During this time of continuing COVID-19 pandemic issues, you may experience a range of issues that can cause barriers to learning, such as strained relationships, increased anxiety, trauma, alcohol and other drug concerns, difficulty concentrating, and/or lack of motivation.

USD has many resources that are free to you, including:

- **Counseling Center** 24/7 access to a counselor: (619) 260-4655, press 1 for urgent concerns
- **Disability and Learning Difference Resource Center** disabilityservices@sandiego.edu
- **C.A.R.E. Advocate** 24/7 through Public Safety Dispatch (619) 260-7777
- **Student Health Center** MyWellness Portal https://mywellness.sandiego.edu/
    - Non-urgent email usdhealthcenter@sandiego.edu

Academic Integrity - Copying of material from other students or sources is plagiarism and will not be tolerated. Do your own work and demand that others do theirs. Take care in your use of material from class handouts, textbooks, and the internet. All assignments turned in are expected to reflect ONLY your own work and your writing. When working in groups, each member of the group is expected to be able to write an individual analysis of results, even with shared data. If you use literature, cite it appropriately. **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.

Lab Citizenship: Safety and Courtesy

Strictly following all safety rules is basic to good lab technique. Minimally, note the following:

- ☞ No eating, drinking, smoking or application of cosmetics in the laboratory. Please do not bring any food or drink containers into the lab or throw food trash away in lab.
- ☞ Lab coats, protective eyewear (goggles) and/or gloves must be worn at all appropriate times (as instructed). [A lab coat will be standard wear for Molec Tech lab, required for all labs except bioinformatics only labs.]

- ☞ Close-toed shoes that fully cover the feet must be worn at all times (i.e., no sandals or flip-flops). [You may wish to keep a lab coat and pair of shoes in your lab drawer.]
- ☞ Contact lenses must not be worn in lab.
- ☞ No pipetting by mouth.
- ☞ No unauthorized experiments are to be performed, or unauthorized use of equipment.
- ☞ Follow carefully instructions for disposal of glass, hazardous materials, etc.
- ☞ Wash hands during lab as necessary, and thoroughly following lab.

Lab safety training will occur at the first lab (or as soon as in-person), and experiment-specific safety issues will be addressed throughout the semester. Deviation from safety policies will be taken seriously.

In addition, please note that we share the room with another laboratory class. As a courtesy to others, we must diligently clean up after ourselves and put away our equipment & materials at the end of the lab period. 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. Be a good lab citizen.

When you come to the lab outside of regular lab times, ask permission of the instructor if there is another lab class in session. Avoid using the main lab bench areas where the class is working. Try to avoid the first hour of class when the instructor may be lecturing or demonstrating.

Lab Citizenship: Electronic devices and communications

Your focus while in lab must be on studying molecular techniques, monitoring procedures and experiments, and listening to instruction about the lab. Students are not to use cell phones, smart phones, tablets, laptops, etc. in lab for *any purpose other than class-related activities*, including texting, web-surfing, etc. This is distracting to you and your classmates. If you must communicate during lab time, do so **outside** the room, and only when you are working on your own time (usually only after the first 1-2 hours of lab).

Provisional Schedule - Molecular Biology Techniques Topics

		Lecture Topics (Mon, Wed)	Lab Topic (Wednesday)
BLOCK 1 (Weeks 1 - 4) – BASIC SKILLS			
Week 1	Jan 29 Jan 31	Introduction; history, basic cloning methods, vectors, lab notebooks	Lab safety, pipetting, bacterial culture, sterile technique, making media, antibiotics, autoclaving media & glassware. **[Next day: check plates]**
Week 2	Feb 5 Feb 7 (Add deadline)	Bacterial growth and transformation **[Set up overnight cultures evening before lab - Tues]**	Liquid bacterial culture (and monitoring); make competent cells, transformations **[Next day: count transformants]**
Week 3	Feb 12 Feb 14	Agarose gel electrophoresis, REs, modifying enzymes (theory)	Lambda restriction digests, agarose gels, includes solving 'unknown' digest
Week 4	Feb 19 - **Q** Feb 21	PCR, polymerases (theory)	PCR practical issues, primer design (and ordering) for Cloning Project 1
BLOCK 2 (Weeks 5 - 9) – PCR & CLONING PROJECT 1			
Week 5	Feb 26 Feb 28	Nucleic acids & proteins, basic bioinformatics	Bioinformatics exercises 1
Week 6	Mar 4 Mar 6	TOPO-TA Cloning; 'PCR Math'	Cloning Project 1: Primers prep, PCR setup and run
Week 7	Mar 11 Mar 13	More Lab Math: review to date	Cloning Project 1: Gel analysis of PCRs, cloning & transformations **[Next day: score plates, restreak several transformants]**
Week 8	Mar 20 - **Q** Mar 22	DNA purification, quantification. **[Set up overnight cultures evening before lab – Tues]**	Cloning Project 1: Miniprep DNA purification, quantification, gel analysis, prep DNA for sequencing
Week 9	**Mar 25 - Apr 1**	**SPRING / EASTER BREAK - No lectures (including Mon., Week 10)**	**SPRING / EASTER BREAK - No lab**
BLOCK 3 (Weeks 10 - 15) – CLONING PROJECT 2			
Week 10	**Apr 1** Apr 3	**No lecture - Monday** Sanger DNA Sequencing (theory)	Sequence analysis of Project 1 clones; Planning / ordering for Cloning Project 2
Week 11	Apr 8 Apr 10	Next generation sequencing or TBA	Bioinformatics exercises 2
Week 12	Apr 15 Apr 17	Introduction to CRISPR	Cloning Project 2
Week 13	Apr 22 - **Q** Apr 24	To be announced [Apr 25 - Celebrate **DNA Day**!]	Cloning Project 2 (cont.)
Week 14	Apr 29 May 1	To be announced	Cloning Project 2 (cont.)
Week 15	May 6 May 8	Flexible use of lecture and lab time to wrap-up Cloning Project 2; lab clean-up	
Week 16	May 13 (M)	Review for final, etc.	
	May 14-15	**STUDY DAYS**	
	May 16-22	**FINALS - Final Exam – Fri, May 17, 11 AM - 1 PM**	