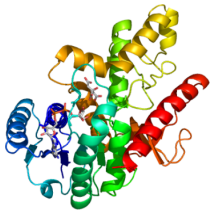
Techniques in Molecular Biology

BIOL/CHEM 330 Lect & Lab



Course Overview: 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 microarrays that are especially important in genomics, and discuss the burgeoning other '-omics' fields. Some primary literature articles illustrating standard techniques will be studied. We will apply our newly learned molecular techniques toward solving real biological research questions, and to carry out a basic DNA cloning project.

At the end of Biology/Chemistry 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 PCR and when to use them, and how to troubleshoot a PCR 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.

<u>Work outside of the regular lab period</u>

The practice of molecular biology cannot easily be restricted to a single four-hour lab period. 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.

<u>Books</u>

From Genes to Genomes. Concepts and Applications of DNA Technology 3rd Ed. Dale, Von Schantz and Plant. Wiley-Blackwell ISBN 978-0-470-68385-9

Tuesday Lab 8-12		Thursday Lecture/Lab	
		Sept 1	**Lect:** Intro, lab safety, notebooks, solutions, Lab math, intro to sterile and bacteriological tech
Sept 6	**Block 1:** Pipetting, bact culturing/media prep, autoclave training - *Lab Expt 1*	Sept 8	**Lect:** Discuss experiment, SOP/QA/QC, bacterial strains, antibiotics and transformation
Sept 13	**Block 1:** Transformation – *Lab Expt 2*, Bacterial Plasmids and Promoters.	Sept 15	**Lect:** Plasmid purification, DNA electrophoresis, DNA/RNA quantification
Sept 20	**Block 1:** DNA miniprep, quantification and gel electrophoresis – *Lab Expt 3*	Sept 22	**Lect:** Restriction digest, intro to cloning and subcloning.
Sept 27	**Block 1:** Restriction digest and gel analysis *Lab Expt 3 continued*.	Sept 29	**Lect:** Advanced Cloning/ Sub Cloning Stratagies pClone prep and primer design.
Oct 4	**Block 2:** Basic Subclone and sequence preparation – *Lab Expt 4*	Oct 6	**Lect:** DNA sequencing, sequencing primer design, next-generation sequencing
Oct 11	**Block 2:** pCloneRed Promoter - *Lab Expt 5*	Oct 13	**Block 2:** PCR, site directed mutagenesis, primer design, qPCR and pre-qPCR Prep
Oct 18	**Block 2:** RNA pep – qPCR part I *Lab Expt 6*	Oct 20	**Block 2:** RNA pep – qPCR part I *Lab Expt 6*
Oct 25	**Block 2:** RNA pep – qPCR part I I *Lab Expt 6*	Oct 27	**Block 3 Workshop**: Bioinformatics, genomic annotation - *Lab Expt 7*
Nov 1	**Block 3:** Bioinformatics and Genome Annotation – *Lab Expt 7*	Nov 3	**Block 4:** Cloning Project – *Lab Expt 8*
Nov 8	**Block 4:** Cloning Project – *Lab Expt 8*	Nov 10	**Block 4:** Cloning Project – *Lab Expt 8*
Nov 15	**Block 4:** Cloning Project – *Lab Expt 8*	Nov 17	**Lect:** shRNA, RNAi & Crispr Cas 9
Nov 22	**Block 4:** Cloning Project – *Lab Expt 8*	Nov 24	*Thanksgiving*
Nov 29	**Block 5:** CRSPR – *Lab Expt 9*	Dec 1	**Block 5:** CRSPR – *Lab Expt 9*
Dec 6	**Block 5:** CRSPR – *Lab Expt 9*	Dec 8	**Block 5:** CRSPR – *Lab Expt 9*
	Finals Opt 1 Thurs Dec 15 8-10		Finals Opt 2 Tues Dec 20 8-10

Deadlines - The timing in this document are guidelines. Some experiments may take more time, others less. Due dates for assignments will be provided in class. The web will have target dates for all assignments as well as a description for each homework assignment.

Each laboratory experiment - from simple DNA gel running to the more project oriented experiments MUST be written into the lab book following the instructed format. There are no other formal or informal write up. The discussion section should cover the salient points and make recommendations based on your results.

Grading: A 100-90%, B 80-89% C 70-79% D 60-69% F 59-0%

Points	**Description**
150	End of Semester Lab Practical
400	Take Home Exams (two at 200 points each)
150	Lab Notebook (50 points x three reviews)
300	Lab Results (25 pts x each Lab Expt 1-7 and 9 . Lab Expt 8 = 100 pts)
25	SOP
50	Unannounced Lab Notebook Checks 5 x 10 points

1075 total points