

LABORATORY METHODS IN PLANT MOLECULAR BIOLOGY

SYLLABUS

I. Course and Instructor Information.

Course:	HOS 4313C/HOS 6932
Section:	041B
Credit Hours:	2
Period 6-9:	Tu & Th 1:30 - 4:30 pm
Room:	184 CGRC
Pre-requisites:	AGR 3303 or PCB 3063 - Genetics, and HOS 3305 - Introduction to Plant Molecular Biology, or Permission from Instructor
Instructor:	C. Eduardo Vallejos
Office:	2243 Fifield Hall
Phone:	273-4845
e-mail:	vallejos@ufl.edu (Subject must be "HOS4313C")
Office hours:	M 1:00 - 2:00 pm, W 8:30 - 9:30 am, or by appointment

II. Course Description.

Molecular Biology is the branch of biology that studies the structure and function of macro molecules that encode and regulate the flow of genetic information used by living organisms. This course will provide students with hands-on experience in the most basic laboratory methods used to isolate, clone and analyze nucleic acid sequences. Through a balanced combination of lectures, direct experimentation, and preparation of lab reports students will learn how to use current bioinformatics resources to identify specific DNA sequences, design primers for PCR amplification of these sequences. Amplification products will be cloned, and sequenced. Students will carry out a basic structural analysis of the cloned sequences and analyze pattern of expression in response to virus inoculations using RT-PCR. In addition, the class will carry out transient and stable plant transformation experiments.

III. Course Goals. This laboratory course aims to:

- Provide students with a thorough understanding of the physical-chemical and biological principles underlying the most basic techniques and procedures used in molecular biology.
- Foster the development of laboratory technique and organizational skills at the bench.
- Acquaint students with the exploration and utilization of bioinformatics resources.
- Help students develop critical thinking skills in the interpretation and reporting of scientific data.

IV. Learning Objectives. After taking this course students will be able to:

- Apply their knowledge of genome size to successfully detect specific sequences by DNA blot and/or PCR analysis, screen genomic libraries, or plan the sequencing of a genome.
- Extract high quality DNA and RNA from plant tissues.
- Use the various tools of the *Entrez* database to search and find specific DNA and protein sequences, and all the available information associated with those sequences.
- Design primers suitable for PCR amplification of specific DNA sequences.
- Clone DNA fragments into a suitable *Escherichia coli* host.
- Measure expression levels of specific sequences via RT-PCR analysis.
- Feel confident about using the most basic plant molecular methods under different circumstances and being able to troubleshoot problems that may arise.

V. Reading Material.

There is no textbook selected for this course. However, a laboratory manual will be provided by the instructor. Each unit in the manual has background information including some key citations, a list of reagents and solutions, and a detailed protocol for the experimental procedures. Copies of the supplemental material will be made available to the students in the form of a photocopy or an electronic file. Students are expected to read the pertinent sections of the manual before coming to class.

Reference Books

Sambrook J, DW Russell, T Maniatis. 2001. *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, c2001. 3rd Edition. 3 vol.
Health Science Library: QH442.M2781m 2001
Science Library: QH442.2.S26 2001

Ausubel F [et al.]. 2001. *Current Protocols in Molecular Biology*. New York: John Wiley & Sons. 5 volumes. (Loose-leaf)
Health Science Center Library Reference: QH 506 C976
Health Science Center Library: Electronic Resource

VI. Lab Schedule

Wk.		Tuesday		Thursday
1			Aug-31	INTRODUCTION LECTURES Light Absorption Flow Cytometry DNA Extraction
2	Sep-5	LAB Flow Cytometry LECTURES Restriction Enzymes Agarose Gel Electrophoresis	Sep-7	
3	Sep-12	LAB DNA Extraction LECTURE Bioinformatics (Assignments)	Sep-14	LAB DNA Finish DNA Extraction LAB Bioinformatics – Seq. Retrieval LECTURE PCR LAB Primer Design
4	Sep-19	LAB DNA EXTRACTION Spectrophotometry & Fluorometry Restriction Enzyme Digestion Agarose Gel Electrophoresis	Sep-21	LAB PCR Reactions Agarose Gel Electrophoresis LAB Floral Transformations LECTURE Cloning
5	Sep-26	LAB Cloning, Ligation, Transform. LECTURE DNA Sequencing	Sep-28	LAB Cloning, Pick Colonies LECTURE Southern Hybridization
6	Oct-3	LAB Colony PCR Screen Agarose Gel Electrophoresis Plasmid purification REVIEW	Oct-5	Mid-Term
7	Oct-10	LAB Measure pDNA concentration Agarose gel electrophoresis	Oct-12	LAB Southern Procedure Agarose Gel Electrophoresis Blotting
8	Oct-17	LAB Probe Labeling Hybridization	Oct-19	LAB Southern Blot Washing and Visualization

Wk.		Tuesday		Thursday
9	Oct-24	LAB RNA Extraction LECTURE Gene Expression	Oct-26	LAB Finish RNA Extraction Measure RNA Concentration
10	Oct-31	LAB Gene Expression RNA Denaturing Gel Electroph.	Nov-2	LAB Gene Expression RT-PCR
11	Nov-7	LECTURE Plant Transformation LAB Transformation Plant Seeds in Agar Plates	Nov-9	LAB Transformation Transient Transformation
12	Nov-14	LAB Evaluate Transformants	Nov-16	REVIEW
13	Nov-21	FINAL EXAM, or	Nov-23	Thanksgiving
	Dec-13	FINAL EXAM		

VII. Student Evaluation.

Lab Preparation. Students are expected to arrive to each lab session fully prepared. This means that they should have read the lab manual section pertaining to the scheduled activities. To demonstrate their preparation, they must arrive at the lab with two copies of a Flow Chart for the planned activities; one of these copies must be turned in at the beginning of the lab session. A flowchart example will be provided for the first lab. Re-writes of the protocol in a flowchart format will not be accepted. *Student's charts will be checked and graded.* In addition, student will be asked about the steps they will be working on. 20%

Lab Reports. Students will be required to turn in a Lab Report one week after the conclusion of each experiment. *Due dates will be announced in class.* **Part I** should be no more than a page long and **must** include: *a)* the main objective of the experiment, *b)* the most important findings, *c)* the significance of the results, and *d)* major conclusions. **Part II** should contain a brief summary of the results obtained in the lab. The format for this section will depend on the nature of the experimental results and may include one or more of the following: tables, diagrams, photos, and a description of the outcome of the experiment. In some cases, students will 20%

be asked to include results obtained by the entire class, and in others just those obtained by the group. **Part III** should include answers to the questions listed in the lab manual. Keep in mind that most of the experiments will be carried out over more than one lab session. For this reason, a report will not be required for every lab session, just after the completion of each experiment.

IMPORTANT: Sharing of Lab Reports among students is a violation of Academic Honesty. Reports that show extensive similarities will be rejected.

Lab Technique. This criterion relates to organizational skills, the ability to clean after yourself and avoid clutter on your bench, and your ability to work well with your lab partner and others in the lab.	20%
Mid-Term Exam. Students will be tested on the principles of the techniques and procedures used in the previous lab sessions. Topics to be addressed in the exam will be clearly pointed out during lectures and lab sessions. Some questions in the exam will also be derived from the content of lab reports.	20%
Final Exam. This exam will be comprehensive because the experiments in most of the lab sessions are interconnected making it difficult to separate the topics. However, emphasis will be placed on the topics covered after the Mid-Term.	20%

Grade allowance.

Students will be allowed to remove one grade from the final computation for each of the first three evaluation criteria. Absences without appropriate excuse or reports that have not been turned in will be excluded from the allowance.

Make-up exams.

Students who are unable to take scheduled exams in this course due to scheduling conflicts with other courses, or with religious holidays, should contact the instructor for alternate arrangements.

Grading Scale

100 ≥ A > 90	86 ≥ B+ > 82	74 ≥ C+ > 70	62 ≥ D+ > 58	E ≤ 50
90 ≥ A- > 86	82 ≥ B > 78	70 ≥ C > 66	58 ≥ D > 54	
	78 ≥ B- > 74	66 ≥ C- > 62	54 ≥ D- > 50	

VIII. Lab Policies.

Attendance

ATTENDANCE IS ABSOLUTELY MANDATORY. *“After due warning, professors can prohibit further attendance and subsequently assign a failing grade for excessive absences.”* In general, acceptable reasons for absence from class include illness, serious family emergencies, special curricular requirements (e.g., judging trips, field trips, professional conferences), military obligation, severe weather conditions, religious holidays and court-imposed legal obligations (e.g., jury duty or subpoena). When appropriate, supporting documentation will be required.

A student may request a Verification of Visit form (ACL-012) which will display the date and time the student was seen at the SHCC. However, if an official Excuse Note is required, it must be requested as stated above.

"The Student Health Care Center will only write excuse notes for illnesses or injuries that have resulted, or will result, in absence of three or more days of class. Any shorter term absences will not receive notes. If an instructor requires a note for an absence of fewer than three days, one can be written upon the written request of the instructor." This request must be on official UF letterhead.

Laboratory Safety

Several rules and procedures must be followed during laboratory sessions to ensure the safety of people in the lab. The first of these is that food or beverages are not allowed in the lab. Students must wear closed-toe shoes, those who do not comply will not be allowed in the lab. Handling hazardous chemicals (propidium iodide, ethidium bromide, acids, bases, phenol, etc.) requires wearing protective gloves. Disposable gloves will be provided in the lab. Students will be expected to keep their working area clean and uncluttered. **CELL PHONES SHOULD BE TURNED OFF.** Finally, one of the most important rules in this lab is that no question will be considered dumb, so when in doubt you should ask. This is particularly important if you are going to operate some equipment or instrument and you are not completely sure of what you are doing. You are here to learn. One way to do this is to ask questions.

Pre-Lab Preparation

Success in the lab depends to a great extent on advance preparation. For this reason, students will be required to thoroughly read and understand the experimental procedures that will be carried out during the lab session. To ensure that students have read the manual, they will be required to bring to class a **Flow Chart** for the programmed activities. Any questions regarding this pre-lab assignment should be brought to the attention of the Lab Instructor or the Teaching Assistant.

IX. University Policies.

Academic Honesty

Every student has signed the following statement after completion of the registration form at the University of Florida:

“I understand that the University of Florida expects its students to be honest in all their academic work. I agree to adhere to this commitment to academic honesty and understand that my failure to comply with this commitment may result in disciplinary action up to and including expulsion from the University.”

Software Use

All faculty, staff and students of the University are required and expected to obey the laws and legal agreements governing software use. Failure to do so can lead to monetary damages and/or criminal penalties for the individual violator. Because such violations are also against University policies and rules, disciplinary action will be taken as appropriate.

Students with Disabilities.

Students requesting classroom accommodation must first register with the Dean of Students Office. The Dean of Students Office will provide documentation to the student who must then provide this documentation to the Instructor when requesting accommodation.

X. Student Services.

The University and Gainesville Community offer a number of personal counseling services for students at the University of Florida. Contact the appropriate agency listed below:

- Student Health Services 392- 1161
 Student Health Care Center (1 Fletcher Driver)
 Monday - Friday, 8:00am - 4:30pm
<http://www.shcc.ufl.edu>

- University Counseling & Wellness Center 392-1575
 A counselor is available to assist students to work through personal issues.
 P301 Peabody Hall
 Monday - Friday, 8:00am - 5:00pm
<http://www.counseling.ufl.edu/cwc/>

- International Student Services 392-5323, ext. 600
 Assistance is provided for International students at the University.
 123 Grinter Hall
 Monday - Friday, 8:00am - 4:30pm
<http://www.ufic.ufl.edu>

- Career Development Assistance and Counseling 392-1601
 Career Resource Center M-F; 8:00am - 4:30pm
<http://www.crc.ufl.edu>

- Dean of Students Office, 392-1261

A staff member is available to assist students.
P202 Peabody Hall
Monday - Friday, 8:00am - 4:30pm
<http://www.dso.ufl.edu>