LABORATORY METHODS IN PLANT MOLECULAR BIOLOGY

SYLLABUS

I. Course and Instructor Information.

Course:	HOS 4313C	
Class Number:	14096	
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	
Instructors:	C. Eduardo Vallejos	Kevin Begcy
Office:	2243 Fifield Hall	1535 Fifield Hall
Phone:	273-4845	273-4528
e-mail:	vallejos@ufl.edu (Subject = "HOS4313C")	kbegcy.padilla@ufl.edu
Office hours:	M 1:00 - 2:00 pm, W 8:30 - 9:30 am, or by appointment	

COVID-19. The capacity of 184 CGRC is listed at 56 people, for this reason we have placed a registration cap of 12 students due to the current pandemic. The maximum occupancy of the lab will not exceed 25% including the two instructors. All register students will be required to wear face masks at all times. Gloves will also be provided as well as a surface disinfectant. Participants will be required to maintain safe distances as conditions allow it. Approximately 30% of the lab sessions will be conducted online as indicated in the Syllabus

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 characterize nucleic acid sequences and proteins. Instruction is based on a balanced combination of lectures, direct experimentation, and preparation of lab reports. Students will learn to measure plant genome sizes and use this information for a variety of applications in molecular biology and genomics. Students will also learn to: use current bioinformatics resources to identify specific DNA sequences, design primers for PCR amplification, and clone and sequence amplification products. Labs are designed for the isolation and characterization of DNA, RNA and proteins using standard technologies. 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 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 estimate the size of sequencing libraries
- 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.
- Extract high quality DNA, RNA and protein and carry out quantitative, qualitative and functional evaluations of these macromolecules.
- Design primers suitable for PCR amplification of specific DNA and RNA sequences.
- Clone DNA fragments into a suitable *Escherichia coli* host.
- Measure expression levels of specific sequences via RT-PCR analysis.
- Carry out stable transformation of Arabidopsis and transient transformation of diverse species.
- Feel confident about using the most basic plant molecular biology methods under different circumstances and be 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 instructors. 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. Supplemental material will be made available to the students in electronic format. Students are expected to read the pertinent sections of the manual before coming to class.

Recommended References

Green MR, J Sambrook, P MacCallum. 2012. Molecular Cloning: a Laboratory Manual. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, 4th Edition.
Health Science Library: QU 450 G744m 2012
Science Library: QH442.2.S26 2001

Ausubel F [et al.]. 2019. Current Protocols in Molecular Biology. New York: John Wiley & Sons. 128 volumes are available on line from the UF Library System.

VI. Lab Schedule: Online Lectures

Wk.	Tuesday		Thursday	
1	Sep-1	INTRODUCTION LECTURES *Flow Cytometry* *DNA Extraction* DNA Purification Restriction Enzymes Agarose Gel Electroph.	Sep-3	LECTURES *Bioinformatics* Sequence Select. & Anal. *PCR* LAB *Primer Design*
2	Sep-8	LAB *Flow Cytometry* *Floral Transformations*	Sep-10	LAB *DNA Extraction*
3	Sep-15	LAB *DNA Extraction* Finish Wash, dry & dissolve *PCR* Setup Reactions & Ampl.	Sep-17	LAB *DNA Extraction* Spectrophot. & Fluorom. Restriction Enzyme Digestion *PCR* Agarose Gel Electroph.
4	Sep-22	LECTURE *Cloning* *DNA Sequencing*	Sep-24	LAB *DNA Extractions* Agarose Gel Electroph. *Cloning* Ligate, Transform & Plate
5	Sep-29	LAB *Cloning*. Pick Colonies Inoculate LB PCR Screening	Oct-1	LAB *Cloning* Agarose Gel Electroph. *DNA Sequencing* Qiagen Plasmid Purif. Agarose Gel Electroph.
6	Oct-6	**REVIEW**	Oct-8	**First Mid-Term**
7	Oct-13	LECTURE *Gene Expression*	Oct-15	LAB *Gene Expression* RNA Extraction
8	Oct-20	LAB *Gene Expression* Finish RNA Extraction Measure RNA Concentration.	Oct-22	LAB *Gene Expression* RNA Denat. Gel Electroph
9	Oct-27	LAB *Gene Expression* RT-PCR	Oct-29	LECTURE *Protein Analysis* *Plant Transformation*

Wk.		Tuesday		Thursday
10	Nov-3	LAB *Protein Analysis* Taq Pol Extraction Dialysis	Nov-5	LAB *Protein Analysis* Concentrate *Taq* Pol Bradford Determination qPCR of Taq Pol
11	Nov-10	LAB *Protein Analysis* SDS-PAGE of Taq Pol *Plant Transformation* Plant Seeds in Agar Plates	Nov-12	LAB *Plant Transformation* Transient Transform
12	Nov-17	LAB *Plant Transformation* Eval. Transient Transform . Eval. Stable Transform.	Nov-19	**Review**
13	Nov-24	**Second Mid-Term**	Nov-26	**Thanksgiving**

VII. 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" (Official UF letterhead required).

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 about 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.

VIII. Student Evaluation.

Lab Preparation. Students are expected to read the lab manual section pertaining to the scheduled activities before arriving to each lab session. To demonstrate their preparedness, 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(s) of the experiment, *b)* An outline the technical principles of the technique, *c)* An outline of the biological processes addressed by this technique, and *d)* A brief summary of experimental results. **Part II** should contain 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 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 Lab Reports is a violation of Academic Honesty. Reports that show extensive similarities will be rejected. 20%

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 20%

well with your lab partner and others in the lab.

First 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%
Second Mid-Term Exam. This exam will emphasize the topics covered after the First Mid-Term, but it will be understood that students have mastered the topics of the first section.	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	

IX. Course evaluations – GatorEvals.

Students are expected to provide professional and respectful feedback on the quality of instruction in this course by completing course evaluations online via GatorEvals. Guidance on how to give feedback in a professional and respectful manner is available at https://gatorevals.aa.ufl.edu/students/. Students will be notified when the evaluation period opens, and can complete evaluations through the email they receive from GatorEvals, in their Canvas course menu under GatorEvals, or via https://ufl.bluera.com/ufl/. Summaries of course evaluation results are available to students at https://gatorevals.aa.ufl.edu/public-results/.

X. University Policies.

Academic Honesty

As a student at the University of Florida, you have committed yourself to uphold the Honor Code, which includes the following pledge: "We, the members of the University of Florida community, pledge to hold ourselves and our peers to the highest standards of honesty and integrity." You are expected to exhibit behavior consistent with this commitment to the UF

academic community, and on all work submitted for credit at the University of Florida, the following pledge is either required or implied: "On my honor, I have neither given nor received unauthorized aid in doing this assignment."

It is assumed that you will complete all work independently in each course unless the instructor provides explicit permission for you to collaborate on course tasks (e.g. assignments, papers, quizzes, exams). Furthermore, as part of your obligation to uphold the Honor Code, you should report any condition that facilitates academic misconduct to appropriate personnel. It is your individual responsibility to know and comply with all university policies and procedures regarding academic integrity and the Student Honor Code. Violations of the Honor Code at the University of Florida will not be tolerated. Violations will be reported to the Dean of Students Office for consideration of disciplinary action. For more information regarding the Student Honor Code, please see: http://www.dso.ufl.edu/sccr/process/student-conduct-honor-code.

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.

XI. 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
  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
- **International Student Services** 392-5323
  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.career.ufl.edu
- **Dean of Students Office**, A staff member is available to assist students. 392-1261
  P202 Peabody Hall
  Monday - Friday, 8:00am - 4:30pm
  http://www.dso.ufl.edu