HOS 4313C - LABORATORY METHODS IN PLANT MOLECULAR BIOLOGY
SYLLABUS

I. Course and Instructor Information

Course:	HOS4313C
Section:	041B
Credit Hours:	2
Period 7-9:	Tu & Th 1:55 – 4:55 pm
Room:	Meet in 2318 Fifield – Lab in 2219, 2225, 2231 Fifield
Pre-requisites:	AGR 3303 or PCB 3063 – Genetics ***and*** HOS 3305 - Introduction to Plant Molecular Biology ***or*** Permission from Instructor
Instructor:	Christine Chase
Office:	2215 Fifield Hall Phone: 273-4862
e-mail:	cdchase@ufl.edu (Subject must be "HOS4313C")
Office hours:	by appointment

II. Course Description

This course will provide students with hands-on experience in the basic laboratory methods and bioinformatics tools used to isolate, manipulate and analyze nucleic acid sequences. Through a balanced combination of lectures, direct experimentation, and computer exercises, students will identify and amplify specific genomic and cDNA sequences. Amplification products will be cloned, and sequenced. Students will carry out a basic structural analysis of the cloned sequences and analyze patterns of gene expression in maize development using RT-PCR. In addition, the class will carry out transient plant transformation experiments.

III. Course Objectives

The objectives of HOS 4313C are to strengthen students' technical, organizational and record keeping skills; acquaint students with the exploration and utilization of bioinformatics resources; and develop critical thinking skills in the interpretation and reporting of scientific data.

IV. Learning Objectives

Upon successful completion of this course students will be able to:

1 - Work comfortably and safely in a molecular biology laboratory environment, successfully executing:

- Extraction and quantification of nucleic acids
- PCR amplification of genomic and cDNA sequences
- Analysis of amplification products by gel electrophoresis
- Molecular cloning for the incorporation of amplification products into plant transformation constructs

2 – Apply and trouble shoot these basic techniques in different experimental organisms or systems

3 – Use current web-based bioinformatics tools for the analysis of nucleic acid sequences including:

- Recovery of specific genomic, cDNA and protein sequences through use of genome browsers and sequence databases
- Identification of orthologs and paralogs for genes of interest
- Design of primers for the PCR amplification of specific genomic and cDNA sequences
- Prediction of protein functions and locations
- Design of plant transformation constructs
- Confirmation of correctly cloned construct sequences

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 that students might find useful for future work:
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. Student Evaluation

Final grades will be assigned on the basis of 500 total possible points according to the criteria and scale below

1- Lab Safety – 100 points. Students must complete two on-line lab safety modules before working in the lab. These are assigned a value of 50 points each, but 10 points will be deducted for each violation of basic lab safety rules throughout the semester – failing to wear correct footwear or personal protective equipment (PPE), improper disposal of chemical or biowastes, bringing food into the lab, etc.

2 - Lab Reports – 300 points. Students will be required to turn in three lab reports (100 points per report). The due dates are listed on the class schedule. The lab manual contains a template for each report. The lab reports include experimental objectives; protocols; data; calculations; conclusions; questions related to protocols; data & interpretation; and a bioinformatics component. 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: Students may consult notes, printed and on-line resources, but ***not*** other individuals in the preparation of lab reports. Lab reports must represent be the student's own work. Sharing of Lab Reports among students is a violation of Academic Honesty. Reports that show extensive similarities will be rejected.

3 – Research Power Point Presentation – 100 points. Each student will work with a different maize gene throughout the semester and, at the end of the semester, give a 10 minute presentation focused on their experimental and informatics findings about the gene, its transcripts, predicted protein products and functions.

4 – Grading Scale

- A 450-500 points
- B+ 425-449 points
- B 400-424 points
- C+ 375-399 points
- C 350-374 points
- D+ 325-349 points
- D 300-324 points
- F <300 points

VII. Course Policies

1 - Attendance

Since this is a lab class, ***attendance is absolutely mandatory!*** From the University of Florida Undergraduate Catalog: "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. "After due warning, professors can prohibit further attendance and subsequently assign a failing grade for excessive absences."

2 - 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. It is also required that students wear closed toe shoes, safety glasses, lab coat and gloves whenever working in the lab. Labcoats, safety glasses or side shields for prescription glasses and disposable gloves will be provided. Students are expected to keep their working area clean and uncluttered. ***Cell phones should be turned off.*** Finally, one of the most important safety rules in this lab is: ***when in doubt, always 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.

3 - Pre-Lab Preparation
All classes will begin with a brief pre-lab meeting and overview in classroom 2316 Fifield. Success in the lab depends to a great extent on advance preparation. For this reason, students will be required to thoroughly read the experimental procedures and other assigned reading ***before*** coming to class. Students are required to bring to class a hard copy of the scheduled protocols for use as a worksheet during the lab. Any questions regarding the protocol can be asked at the pre-lab meeting.

X. University Policies and resources
1 - Online Course Evaluation Process: Student assessment of instruction is an important part of efforts to improve teaching and learning. At the end of the semester, students are expected to provide feedback on the quality of instruction in this course using a standard set of university and college criteria. These evaluations are conducted online at: https://evaluations.ufl.edu Evaluations are typically open for students to complete during the last two or three weeks of the semester; students will be notified of the specific times when they are open. Summary results of these assessments are available to students at: https://evaluations.ufl.edu/results

2 - 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: https://www.dso.ufl.edu/sccr/process/student-conduct-honor-code/

3-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.

4-Services for Students with Disabilities: The Disability Resource Center coordinates the needed accommodations of students with disabilities. This includes registering disabilities, recommending academic accommodations within the classroom, accessing special adaptive computer equipment, providing interpretation services and mediating faculty-student disability

related issues. 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. 0001 Reid Hall, 352-392-8565, www.dso.ufl.edu/drc

5-Campus Helping Resources: Students experiencing crises or personal problems that interfere with their general well - being are encouraged to utilize the university's counseling resources. The Counseling & Wellness Center provides confidential counseling services at no cost for currently enrolled students. Resources are available on campus for students having personal problems or lacking clear career or academic goals, which interfere with their academic performance.

University Counseling and Wellness Center, 3190 Radio Road, 352-392-1575, www.counseling.ufl.edu/cwc/ The center offers counseling services, groups and workshops, outreach and consultation, a self help library, training programs, and a community provider database.

Career Resource Center, First Floor JWRU, 392-1601, www.crc.ufl.edu/
- Friday, 8:00am - 4:30pm http://www.dso.ufl.edu

SCHEDULE - HOS 4313C Fall 2016 LABORATORY METHODS IN PLANT MOLECULAR BIOLOGY

Date	**Classroom topics**	**Lab**	**Weekly reading required *before* Tues class**	**Computer**
Tues 8/23	Course Introduction	PPE, Lab orientation, Lab safety, Pipetting technique – group 1		Lab Safety & Chemical Hygiene online training – group 2
Thurs 8/25	Project introduction	PPE, Lab orientation, Lab safety, Pipetting technique – group 2	Project Proposal: Mito-chondrial functions in plant reproduction, pp. 1-8, 13-14	Lab Safety & Chemical Hygiene online training – group 1
Tues 8/30	DNA extraction Introduction to maize genome browsers, PCR & PCR primer design	DNA extraction part I – group 1	Integrated DNA Technologies: IDTutorial: The Polymerase Chain Reaction MP Miomedicals: Instruction Manual FastPrep®-24 Tissue and Cell Homogenizer, pp. 5, 7-14, 21-23 DNA extraction lab protocol	Maize genome browser – locate genomic sequence & *Mutator* (*Mu*) transposon insertion site PrimerQuest & Primer-Blast - Design PCR primers flanking *Mu* insertion site – group 2
Thurs 9/1		DNA extraction part I – group 2		Maize genome browser – locate your gene & *Mutator* (*Mu*) transposon insertion site PrimerQuest & Primer-Blast - Design PCR primers flanking *Mu* insertion site – group 1
Tues 9/6	DNA quantification Paralogs & sequence alignment tools	DNA precipitation and quantification – group 1	DeNOVIX: Absorbance and Fluorescence Quantification Bio-Rad: SmartSpec Plus User Manual: Sections 4.1, 4.5, 5, 6, 7.1.1, 7.1.2A	Maize genome browser – Locate cDNA sequences & paralog cDNA sequences SDSC Biology Workbench & Clustal W - Align cDNA sequences – group 2

Date	Classroom topics	Lab	Weekly reading required *before* Tues class	Computer
Thurs 9/8		DNA precipitation and quantification – group 2		Maize genome browser – Locate cDNA sequences & paralog cDNA sequences SDSC Biology Workbench & ClustalW - Align cDNA sequences – group 1
Tues 9/13	PCR	DNA dilution & PCR – group 1	PCR lab protocol	PrimerQuest and Primer-Blast - Design gene-specific primers for cDNA amplification – group 2
Thurs 9/15		DNA dilution & PCR – group 2		PrimerQuest and Primer-Blast - Design gene-specific primers for cDNA amplification – group 1
Tues 9/20	Electrophoresis	Gel electrophoresis of PCR products – group 1	Lee & Bahaman (2012) Discriminatory Power of Agarose Gel Electrophoresis iNtRON Biotechnology: RedSafeTM Nucleic Acid Staining Solution Electrophoresis lab protocol	Translate cDNA sequences TargetP & Psort – targeting predictions – group 2
Thurs 9/22	Orthologs & translation tools	Gel electrophoresis of PCR products – group 2		Translate cDNA sequences TargetP & Psort – targeting predictions – group 1
Tues 9/27 **Due: Report 1**	Reverse transcription PCR (RT-PCR) Protein targeting prediction tools	RT of pollen and seed RNAs – group 1	Roche: Working with RNA In Vitrogen: SuperScript® III First-Strand Synthesis System for RT-PCR RT-PCR lab protocol	NCBI BLAST & Multalin – Find & align orthologs; predict targeting leader – group 2
Thurs 9/29		RT of pollen and seed RNAs – group 2		NCBI BLAST & Multalin – Find & align orthologs; predict targeting leader – group 1

Date	Classroom topics	Lab	Weekly reading required *before* Tues class	Computer
Tues 10/4	Plant transformation vectors Cloning strategies	PCR of pollen and seed cDNA – group 1	Biotechnology and Biological Sciences Research Council: 30 years of plant transformation ClonTech: In-Fusion™ Liquid PCR Cloning Kit User Manual CAMBIA: pCAMBIA vectors, T-DNA regions [see pCAMBIA 1302 & p. 5]	Design cloning strategy & primers for in-frame protein fusion with GFP – group 2
Thurs 10/6		PCR of pollen and seed cDNA – group 2		Design cloning strategy & primers for in-frame protein fusion with GFP – group 1
Tues 10/11	Gene expression tools	Gel electrophoresis of cDNAs; PCR amplify targeting leader for In-Fusion™ cloning- group 1	Sekhon et al. Genome wide atlas of transcription during maize development Plant J. 66:553–563	In silico gene expression, work on lab report 2 - group 2
Thurs 10/13		Gel electrophoresis of cDNAs; PCR amplify targeting leader for In-Fusion™ cloning – group 2		In silico gene expression, work on lab report 2 - group 1
Tues 10/18 **Due: Report 2**	Biosafety PubMed & Web of Science	DNA joining - group 1	CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Ed, Section IV - Laboratory Biosafety Level 1 DNA joining lab protocol	PubMed & Web of Science- search for gene/protein information – group 2
Thurs 10/20		DNA joining - group 2		PubMed & Web of Science- search for gene/protein information – group 1
Tues 10/25	*E. coli* transformation PowerPoint guidelines	*E. coli* transformation – group 1	In Vitrogen: One Shot® Stbl3™ Chemically Competent *E. coli* Transformation lab protocol	Work on Power Point presentations
Thurs 10/27		*E. coli* transformation – group 2		Work on Power Point presentations
Tues 11/1	Direct PCR	Colony pick & PCR – group 1	ClonTech: Terra™ PCR Direct Polymerase Mix User Manual Direct PCR Lab protocol	Work on Power Point presentations
Thurs 11/3		Colony pick & PCR – group 2		Work on Power Point presentations

Date	Classroom topics	Lab	Weekly reading required *before* Tues class	Computer
Tues 11/8	DNA sequencing Sanger sequencing video	Gel electrophoresis of colony PCRs, ID colonies for DNA sequencing – group 1	ICBR Sanger Sequencing Core Lab protocols for sequence template preparation	Work on Power Point presentations - group 2
Thurs 11/10		Gel electrophoresis of colony PCRs, ID colonies for DNA sequencing – group 2		Work on Power Point presentations – group 1
Tues 11/15	Give Power Point – group 2	Lab catch up – group 1		
Thurs 11/17	Give Power Point group 1	Lab catch up – group 2		
Tues 11/22	Catch up lab day			
Thurs 11/24	HOLIDAY	HOLIDAY	HOLIDAY	HOLIDAY
Tues 11/29	Plant transformation and transient expression	Demonstration - infiltration & microscopy of petunia petals with Agrobacterium carrying gfp fusion construct – group 1	I Sparks et al. Rapid, transient expression of fluorescent fusion proteins in tobacco plants. Nature Protocols 1:2019	Translate DNA sequences to check for successful in-frame gfp fusion clones – group 2
Thurs 12/1		Demonstration - infiltration & microscopy of petunia petals with Agrobacterium carrying gfp fusion construct – group 2		Translate DNA sequences to check for successful in-frame gfp fusion clones – group 1
Tues 12/6 **Due: report 3**	DNA sequencing core lab tour			

HOS 4313C Laboratory Methods in Plant Molecular Biology Fall 2016 Reading and Web Resources

Reading (In Reading Order):

Research Project Proposal – Mitochondrial functions in plant reproduction, pp. 1-8, 13-14

Integrated DNA Technologies: IDTutorial: The Polymerase Chain Reaction

DNA extraction lab protocol

MP Miomedicals: Instruction Manual FastPrep®-24 Tissue and Cell Homogenizer, pp. 5, 7-14, 21-23

DeNOVIX: Absorbance and Fluorescence Quantification

Bio-Rad: SmartSpec Plus User Manual, Sections 4.1, 4.5, 5, 6, 7.1.1, 7.1.2A

Lee, SV and AR Bahaman (2012) Discriminatory Power of Agarose Gel Electrophoresis In DNA Fragments Analysis, Gel Electrophoresis - Principles and Basics, S Magdeldin (Ed.), ISBN: 978-953-51-0458-2, InTech, Available from: http://www.intechopen.com/books/gel-electrophoresis-principles-andbasics/
discriminatory-power-of-agarose-gel-electrophoresis-in-dna-fragment-analysis

PCR lab protocol

Agarose Gel Electrophoresis lab protocol

iNtRON Biotechnology: RedSafeTM Nucleic Acid Staining Solution

Roche: Working with RNA

In Vitrogen: SuperScript® III First-Strand Synthesis System for RT-PCR

RT-PCR lab protocol

Centers for Disease Control: Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition - Section IV - Laboratory Biosafety Level 1 Criteria

Biotechnology and Biological Sciences Research Council: 30 years of plant transformation
http://www.bbsrc.ac.uk/research/impact/thirty-years-plant-transformation/

CAMBIA: pCAMBIA vectors, T-DNA regions

MarkerGene: pCAMBIA 1302 expression vector

Sekhon et al. (2011) Genome-wide atlas of transcription during maize development. Plant J. 66:553–563

QIAGEN: QIAquick® Spin Handbook pp. 1-19

PCR clean up lab protocol

ClonTech: In-Fusion™ HD Cloning Kit User Manual

DNA joining lab protocol

In Vitrogen: One Shot® Stbl3TM Chemically Competent E. coli

Transformation lab protocol

ClonTech: Terra™ PCR Direct Polymerase Mix User Manual
Direct PCR Lab protocol

Sparks, I J Runions, A Kearns, C Hawes (2006) Rapid, transient expression of fluorescent fusion proteins in tobacco plants. Nature Protocols 1:2019-2024

Web resources:

Lab safety training:
log into myufl.edu and select >myselfservice>Training and Development>my training.
Search for UF_EHS862_OLT Lab Safety Actions and Reactions to take the online training
and for UF_EHS809_OLT Hazardous Waste Management to take the online training

Maize genome databases:
http://www.maizegdb.org/#
http://ensembl.gramene.org/Zea_mays/Info/Index

Sequence formatting and re-formatting:
http://www.attotron.com/cybertory/analysis/seqMassager.htm

Primer design:
http://www.idtdna.com/PrimerQuest
http://www.ncbi.nlm.nih.gov/tools/primer-blast/

Sequence storage and manipulations:
http://workbench.sdsc.edu/

(Slow but very convenient for storage and access to multiple tools)

Other sequence alignment sites and tools:
http://multalin.toulouse.inra.fr/multalin/
http://www.ebi.ac.uk/Tools/msa/
http://www.ch.embnet.org/software/ClustalW.html

Other translation tools:
http://web.expasy.org/translate/
http://www.ebi.ac.uk/Tools/st/

Protein targeting prediction tools:
http://www.cbs.dtu.dk/services/TargetP/
http://psort.hgc.jp/
http://www.genscript.com/wolf-psort.html

Gene expression tools:
http://www.maizegdb.org/#
http://bar.utoronto.ca/

Sanger DNA Sequencing Video:
http://media.invitrogen.com.edgesuite.net/ab/applications-technologies/pharma-biotherapeutics/DNA_sequencing.swf

Geospiza Finch TV for viewing DNA sequence chromatograms
http://www.geospiza.com/ftvdlinfo.html