AGR 5307: Molecular Genetics for Crop Improvement

Spring Semester 2020, 3 credits
Monday (3108 McCarty B) Period 4; Wednesday (3108 McCarty B) Period 4 and 5; Friday (3108 McCarty B) Period 4
Instructor: Fredy Altpeter
e-mail: altpeter@ufl.edu
Office-3085 McCarty B, Phone 273 3418
Contact Hours (in office): Period 5 after class and by appointment

Course Objectives:
Introduce into concepts and applied aspects of plant molecular and cellular biology that allow students to understand and discuss strategies for crop improvement through biotechnology.

Learning Outcomes:
After completion of this course the student will be able to:
1. Contrast and compare the organization, structure and control of prokaryote versus eukaryote genes.
2. Understand the details of gene expression control in prokaryotes and eukaryotes.
3. Describe eukaryotic posttranscriptional processing, initiation of translation and posttranslational modifications, subcellular targeting, stability and degradation of RNA and proteins.
4. Understand the fundamental concepts and techniques for the use of recombinant DNA technology, plant tissue culture, genetic engineering, gene expression, molecular characterization of plants and marker assisted breeding.
5. Understand the mechanisms, design and analysis of experiments, applications, regulatory and commercial issues of current and emerging biotechnologies including targeted regulation of (trans)gene expression, targeted gene silencing/RNAi, viral vectors, targeted genome editing (targeted mutagenesis, homology directed repair, base editing prime editing, alternative nucleases), intragenic, cisgenic biotechnologies for crop improvement.
6. Accomplishing the above course objectives will enable the students to apply these newly acquired skills in the design and analysis of experiments and critical evaluation of professional literature or scientific presentations in molecular genetics and biotechnology. The students will be able to evaluate these skills during the course while discussing selected recent plant biotechnology articles describing original research and during a case study.

65 % of the course will be lectures
15 % of the course will be laboratory demonstrations
15 % of the course will be analysis and discussion of molecular crop improvement papers
5 % of the course will be a case study on ethical aspects of transgenic crops
Lectures:

Introduction into gene expression in prokaryotes and plants (transcription, translation, protein sorting, regulation of gene expression).
Methodology from isolating a gene to its targeted expression in transgenic plants. (Isolation of nucleic acids, traditional and modular cloning, vector construction, PCR, sequencing, database analysis, plant tissue culture, gene transfer, characterization of transgenic plants, expression profiling).
Transgene silencing, viral vectors and application for crop improvement and functional genomics.
Crop Biotechnology: past, current, and future.
Barriers and paths to market for transgenic crops (regulatory and commercial aspects).
New Biotechnologies (intragenic, cisgenic, genome editing with zinc finger nuclease, TALEN, CRISPR/Cas9)
DNA repair pathways, Targeted mutagenesis, Precision nucleotide substitutions, Base editing, Prime editing.
Design and analysis of genome editing experiments
“Superweeds?” When and how to introduce containment factors into crops.

Laboratory Demonstrations of Methodology:

Isolation of nucleic acids, vector construction, preparation of culture media, plant tissue culture, gene transfer, selection and regeneration of transgenic tissues to plants, characterization of transgenic or genome edited plants for presence (PCR) and expression (qRT-PCR, ELISA) of transgenes or indels/nucleotide substitutions.

Papers: (each student will present one paper)

Recent original research articles describing molecular improvement of crops through biotechnology including transgenic and genome editing approaches.

Case study on ethical aspects of transgenic crops:

Students will read and discuss conceptual papers reflecting the view on ethical aspects of transgenic plants of a specific group and students will present this view (not their own) for further group discussion.

Prerequisites:

AGR 3303 or PCB 3063
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<thead>
<tr>
<th>Module</th>
<th>Title</th>
<th>Topics Covered</th>
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<tbody>
<tr>
<td>1</td>
<td>Genome organization</td>
<td>Genome organization in prokaryotes; genome organization in eukaryotes</td>
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<tr>
<td>2</td>
<td>Introduction to RNA and RNA polymerase</td>
<td>Central dogma of molecular biology; basal transcription and RNA polymerase</td>
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<td>3</td>
<td>Prokaryotic promoters and transcription cycle overview</td>
<td>Promoters and DNA binding proteins; prokaryotic transcription initiation, elongation and termination</td>
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<td>4</td>
<td>Introduction to prokaryotic transcriptional regulation and DNA binding proteins</td>
<td>Rationale for transcriptional regulation; DNA binding principles and motifs; transcription factors</td>
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<td>5</td>
<td>Prokaryotic transcriptional regulation and comparisons with eukaryotes</td>
<td>Examples of regulated transcription; differences between prokaryotes and eukaryotes</td>
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<td>6</td>
<td>Introduction to eukaryotic gene expression</td>
<td>Eukaryotic RNA polymerases; proteins involved in eukaryotic transcription; chromatin and eukaryotic regulation</td>
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<td>7</td>
<td>The eukaryotic promoter and basal transcription factors</td>
<td>Features of eukaryotic promoters; basal vs. activated transcription; basal transcription factors</td>
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<td>8</td>
<td>Sequence-specific DNA binding transcription factors</td>
<td>Transcription factor functional domains; conserved domains and transcription factor families; transcription factor regulation</td>
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<td>9</td>
<td>Processing of transcripts in eukaryotes – RNA Splicing</td>
<td>Messenger RNA (mRNA) molecular structure; 5' cap addition; biochemical mechanisms of splicing; alternative splicing</td>
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<td>10</td>
<td>Processing of transcripts in eukaryotes – polyadenylation and export</td>
<td>Polyadenylation; nuclear export of mRNA; RNA stability and degradation</td>
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<td>11</td>
<td>Eukaryotic translation I</td>
<td>Transfer RNA (tRNA); genetic code; ribosomes; translation initiation</td>
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<td>12</td>
<td>Eukaryotic translation II</td>
<td>Elongation and termination of the polypeptide chain; post-translational protein modifications; protein sorting; protein stability and degradation</td>
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<td>13</td>
<td>Molecular tools and techniques I</td>
<td>Isolation of nucleic acids</td>
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<td>14</td>
<td>Analysis of transgene integration</td>
<td>Polymerase chain reaction (PCR); agarose gel electrophoresis; Southern blot</td>
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<td>15</td>
<td>Restriction endonucleases and introduction to molecular cloning</td>
<td>Restriction sites and cleavage patterns; cloning vectors; traditional vs. Golden Gate cloning strategies</td>
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<td>16</td>
<td>Molecular cloning II</td>
<td>Host organisms and vector DNA; transformation methods; selection and screening methods</td>
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<td>17</td>
<td>DNA sequencing technologies</td>
<td>Sanger dyeoxy sequencing; NextGen sequencing platforms; transcriptome analysis with RNA seq; design and analysis of transcriptome experiments</td>
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<td>18</td>
<td>Introduction to real-time PCR (qPCR)</td>
<td>qPCR and its applications; basic principles; experimental design, controls and QC; quantification methods</td>
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<td>19</td>
<td>Introduction to droplet digital PCR (ddPCR)</td>
<td>basic principles and applications of ddPCR</td>
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<td>20</td>
<td>Analysis of transgenic protein expression</td>
<td>SDS-PAGE; Western blot; ELISA; chromatography</td>
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<td>21</td>
<td>Introduction to plant tissue culture</td>
<td>Tissue culture requirements; plant growth regulators; somatic embryogenesis</td>
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<td>22</td>
<td>Plant transformation I</td>
<td>Protoplast transformation; molecular steps in gene transfer by Agrobacterium</td>
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<td>23</td>
<td>Plant transformation II</td>
<td>Biolistic transformation; plastid transformation</td>
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<td>24</td>
<td>Gene silencing I</td>
<td>Transcriptional gene silencing; post-transcriptional gene silencing; RNA interference (RNAi)</td>
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<td>25</td>
<td>Gene silencing II</td>
<td>VIGS; applications of RNAi for functional genomics and crop improvement</td>
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<td>26</td>
<td>DNA repair pathways</td>
<td>non-homologous end joining (NHEJ); homology-directed repair (HDR); microhomology mediated end joining (MMEJ)</td>
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<td>27</td>
<td>New breeding technologies I</td>
<td>Cisgenics and intragenics; targeted genome editing</td>
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<td>28</td>
<td>New breeding technologies II</td>
<td>Alternative CRISPR nucleases; Base editing, Prime editing, transformation and segregation of transgenes and edits;</td>
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<td>29</td>
<td>New breeding technologies III</td>
<td>Design genome editing experiments and analysis of edits</td>
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<td>30</td>
<td>Biotech crops and sustainable agriculture</td>
<td>Commercial use of biotech crops and its importance for sustainable agriculture</td>
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<td>31-39</td>
<td>Paper discussion biotech for crop improvement</td>
<td>Metabolic engineering; RNAi; targeted mutagenesis with CRISPR/Cas9; Base editing; Prime Editing</td>
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<tr>
<td>40</td>
<td>Ethical case study</td>
<td>The Golden Rice Story from different view points</td>
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Suggested Text:
iGenetics – A Molecular Approach / P.J. Russell
Edition: 3rd
Published: San Francisco: Pearson, Benjamin Cummings, 2010.
ISBN-10: 0-321-56976-8

Molecular Cell Biology (MCB) / H. Lodish et al.
Edition: 8th

Plant Biotechnology and Genetics / C.N. Stewart JR.
Edition: 2nd
Published: John Wiley & Sons, 2016.
ISBN: 978-1-118-82012-4

Grading:
Grading will be based on two homework assignments (5% each) take home exam (15 %), in class exam (15 %), presentation of one assigned paper (20 %), presentation of case study paper (15%) and a final exam (25 %).

% of available marks   Grade
92-100                A
90-91.9               A-
88-89.9               B+
82-87.9               B
80-81.9               B-
78-79.9               C+
72-77.9               C
70-71.9               C-
68-69.9               D+
62-67.9               D
60-61.9               D-

Exam objectives:

Exam 2 (in class March 2nd, 2020): Concepts and techniques for the use of recombinant DNA technology, gene isolation, vector construction, plant tissue culture, genetic engineering, gene expression analysis, molecular characterization of plants, marker assisted breeding, mechanisms and applications of gene silencing for crop improvement, new biotechnologies.
Final exam (date to be announced): The final exam will evaluate the students’ ability to apply the acquired skills in the critical evaluation of professional literature. A scientific article in plant molecular genetics/biotechnology will be handed to students at least 1 week before the exam. During the exam questions will address molecular concepts, molecular techniques, results and conclusions associated with the article.

Expectations:

Students are expected to be prepared and participate in class discussions, ask questions and push for clarity. Students are also expected to arrive on time to class and to have cell phones turned off.

CLASS POLICIES

ATTENDANCE: Students are expected to attend all classes. Students are required to e-mail the course instructor if they need to be excused from any lecture or laboratory. Any student who makes an appointment for any review session is required to be present five (5) minutes before the beginning of the session.

MAKEUP EXAMS: Make-up exams will be accepted only by special permission of the course instructor. Permission to make up work will be granted on a case by case basis and not all requests will be approved.

Academic Honesty, Software Use, UF Counseling Services, Services for Students with Disabilities

In 1995 the UF student body enacted an honor code and voluntarily committed itself to the highest standards of honesty and integrity. When students enroll at the university, they commit themselves to the standard drafted and enacted by students.

In adopting this honor code, the students of the University of Florida recognize that academic honesty and integrity are fundamental values of the university community. Students who enroll at the university commit to holding themselves and their peers to the high standard of honor required by the honor code. Any individual who becomes aware of a violation of the honor code is bound by honor to take corrective action. The quality of a University of Florida education is dependent upon community acceptance and enforcement of the honor code.

The Honor 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.

On all work submitted for credit by students at the university, the following pledge is either required or implied: "On my honor, I have neither given nor received unauthorized aid in doing this assignment."

The university requires all members of its community to be honest in all endeavors. A fundamental principle is that the whole process of learning and pursuit of knowledge is
diminished by cheating, plagiarism and other acts of academic dishonesty. In addition, every dishonest act in the academic environment affects other students adversely, from the skewing of the grading curve to giving unfair advantage for honors or for professional or graduate school admission. Therefore, the university will take severe action against dishonest students. Similarly, measures will be taken against faculty, staff and administrators who practice dishonest or demeaning behavior.

Students should report any condition that facilitates dishonesty to the instructor, department chair, college dean, Student Honor Council, or Student Conduct and Conflict Resolution in the Dean of Students Office.

(Source: 2010-2011 Undergraduate Catalog)

It is assumed all work will be completed independently unless the assignment is defined as a group project, in writing by the instructor.

This policy will be vigorously upheld at all times in this course.

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.

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 & Wellness Center, 3190 Radio Road, 352-392-1575, www.counseling.ufl.edu/cwc/
- Counseling Services
- Groups and Workshops
- Outreach and Consultation
- Self-Help Library
- Training Programs
- Community Provider Database

- Career Resource Center, First Floor JWRU, 392-1601, www.crc.ufl.edu/
Students with Disabilities

Students with disabilities requesting accommodations should first register with the Disability Resource Center (352-392-8565, www.dso.ufl.edu/drc/) by providing appropriate documentation. Once registered, students will receive an accommodation letter which must be presented to the instructor when requesting accommodations. Students with disabilities should follow this procedure as early as possible in the semester.

Instructor and Course Evaluation

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/.”