

**Spring 2022, 3 credits**

**AGR 5307: Molecular Genetics for Crop Improvement**

**100% online – asynchronous** (except office hours & paper presentations)

**Instructor:** Fredy Altpeter

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**Meeting times and Location:**

**Online asynchronous** (except course review & paper presentation sessions on Wednesday 4<sup>th</sup> and 5<sup>th</sup> period synchronous online or in person in 426 McCarty C).

Contact Hours (in office or online) by appointment [altpeter@ufl.edu](mailto:altpeter@ufl.edu)

**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.
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 (DNA repair pathways, targeted mutagenesis, gene targeting, 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.

**65 %** of the course will be **lectures**

**15 %** of the course will be **laboratory demonstrations**

**20 %** of the course will be analysis and discussion of molecular crop improvement **papers**

***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 nucleases, 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) of transgenes or indels/nucleotide substitutions.

***Papers: (each student will present two papers)***

Recent original research articles describing molecular improvement of crops through biotechnology including transgenic and genome editing approaches. Videos with recorded student presentations of review articles and original research articles discussing or describing molecular improvement of crops through various biotechnology approaches will also be provided.

**Prerequisites:**

AGR 3303 or PCB 3063

Week	Module	Title	Topics Covered	Suggested Readings
1 01/05-01/07	1	Genome organization	Genome organization in prokaryotes; genome organization in eukaryotes	iGenetics 15-35; 326-329 <a href="#">Lumenlearninbg.com Structure-and-function-of-cellular-genomes</a> <a href="#">Brown 2018 Eukaryotic nuclear genomes</a>
	2	Introduction to RNA and RNA polymerase	The new central dogma of molecular biology; basal transcription and RNA polymerase	<a href="#">Tan and Anderson New Central Dogma</a> <a href="#">Dornell 2021 RNA polymerase function</a>
	3	Prokaryotic promoters and transcription cycle overview	Promoters and DNA binding proteins; prokaryotic transcription initiation, elongation and termination	iGenetics 81-86 <a href="#">Liu et al. 2020 Prediction and analysis of prokaryotic promoters</a>
2 01/10-01/14	4	Introduction to prokaryotic transcriptional regulation and DNA binding proteins	Rationale for transcriptional regulation; DNA binding principles and motifs; transcription factors	<a href="#">Garvie and Wolberger 2001 Recognition of specific DNA sequences</a>
	5	Prokaryotic transcriptional regulation and comparisons with eukaryotes	Examples of regulated transcription; differences between prokaryotes and eukaryotes	iGenetics 491-507; 87-90 <a href="#">Santillan &amp; Mackey 2004 Lac operon</a>
	6	Introduction to eukaryotic gene expression	Eukaryotic RNA polymerases; proteins involved in eukaryotic transcription; chromatin and eukaryotic regulation	iGenetics; 518-531 <a href="#">Benner 2018 Epigenetic regulation of gene activity</a>
	7	The eukaryotic promoter and basal transcription factors	Features of eukaryotic promoters; basal vs. activated transcription; basal transcription factors	<a href="#">Haberle &amp; Stark 2018 Eukaryotic core promoters</a> <a href="#">Jores et al. 2021 Plant core promoter analysis</a>
	8	Sequence-specific DNA binding transcription factors	Transcription factor functional domains; conserved domains and transcription factor families; transcription factor regulation	<a href="#">Hong 2016 Plant TF Families</a> <a href="#">Feng et al. 2020 AP2/ERF TF in plants</a>
<b>Homework1</b> available January 13 <sup>th</sup> 8:00 am due January 14 <sup>th</sup> 11:59pm				
3 01/18-01/21	9	Processing of transcripts in eukaryotes – RNA Splicing	Messenger RNA (mRNA) molecular structure; 5' cap addition; biochemical mechanisms of splicing; alternative splicing	iGenetics 90-97 <a href="#">Lee &amp; Rio 2015 Mechanisms and Regulation of Alternative pre-mRNA splicing</a> <a href="#">Ehmsberger et al. 2019 mRNA transport in plants: Export factors and their influence on plant development</a>
	10	Processing of transcripts in eukaryotes – polyadenylation and export	Polyadenylation; nuclear export of mRNA; RNA stability and degradation	<a href="#">Yang et al. 2021 Co-transcriptional RNA processing &amp; Alternative Polyadenylation</a>
	11	Eukaryotic translation I	Transfer RNA (tRNA); genetic code; ribosomes; translation initiation	iGenetics 102-117 <a href="#">Castellano and Merchante 2021 Regulation of Translation initiation in plants</a>
	12	Eukaryotic translation II	Elongation and termination of the polypeptide chain; post-translational protein modifications; protein sorting; protein stability and degradation	iGenetics 117-124 <a href="#">Wang et al. 2021 Post-translational modifications: Regulation of nitrogen utilization and signaling</a>
<b>Take Home Exam</b> available January 21 <sup>st</sup> 8:00 am due February 4 <sup>th</sup> 11:59 pm				
4 01/24-01/28	13 & 13LDV	Molecular tools and techniques I	Isolation of nucleic acids	iGenetics 171-183; 248-255; 261-263 <a href="#">Abdel-Latif &amp; Osman 2017 Comparison of three plant genomic DNA extraction methods</a>
	14 & 14LDV	Analysis of transgene integration	Polymerase chain reaction (PCR); agarose gel electrophoresis; Southern blot	<a href="#">Plant Biotechnology and Genetics 181-205</a> <a href="#">TU Eindhoven - The cloning guide</a>
	15 & 15LDV	Restriction endonucleases and introduction to molecular cloning	Restriction sites and cleavage patterns; cloning vectors; traditional vs. Golden Gate cloning strategies	<a href="#">Marillonnet &amp; Gruetzner 2020 Synthetic DNA Assembly Using Golden Gate Cloning</a> <a href="#">Bajpai 2014 High Capacity Vectors</a>
	16	Molecular cloning II	Host organisms and vector DNA; transformation methods; selection and screening methods	
<b>Homework 2</b> available January 28 <sup>th</sup> 8am due February 11 <sup>th</sup> 11:59 pm				

Week	Module	Title	Topics Covered	Suggested Readings TBD
5 01/31 - 02/04	17	Sequencing technologies	Sanger dideoxy sequencing; NextGen sequencing platforms; transcriptome analysis with RNA seq; design and analysis of transcriptome experiments	<a href="#">Hu et al. 2021 Next Generation Sequencing: An overview</a> <a href="#">Van den Berge et al. 2019 RNAseq</a>
	18 & 18LDV	Introduction to real-time PCR (qPCR)	qPCR and its applications; basic principles; experimental design, controls and QC; quantification methods	<a href="#">qRT-PCR application guide</a>
6 02/07 - 02/11	19	Introduction to droplet digital PCR (ddPCR)	Basic principles and applications of ddPCR	<a href="#">Morcia et al. 2020 Digital PCR: What Relevance to Plant Studies?</a>
	20	Analysis of transgenic protein expression	SDS-PAGE; Western blot; ELISA; chromatography	<a href="#">iGenetics 181-182; 259; 261-263</a> <a href="#">Galagher 2012 SDS-PAGE</a> ; <a href="#">Hornbeck 2015 ELISA</a> ; <a href="#">Lough 1998 Western of transgenic plants</a> <a href="#">Caskun 2016 Chromatography</a>
7 02/14 - 02/18	21 & 21LDV	Introduction to plant tissue culture	Tissue culture requirements; plant growth regulators; somatic embryogenesis	<a href="#">Phillips &amp; Garda 2019 Plant tissue culture media and practices: an overview</a>
	22	Plant transformation I	Protoplast transformation; molecular steps in gene transfer by <i>Agrobacterium</i>	<a href="#">Altpeter et al. 2016 Advancing crop transformation</a> <a href="#">Ghogare et al. 2021 Genome editing reagents delivery in plants</a>
	23 & 23LDV	Plant transformation II	Biolistic transformation; plastid transformation	<a href="#">Rascon Cruz et al. 2021 Plastid transformation Plant Biotechnology &amp; Genetics 107-125; 262-284</a>
8 02/21 - 02/25	24	Gene silencing & RNAi I	Transcriptional gene silencing; post-transcriptional gene silencing; RNA interference (RNAi); micro RNA	<a href="#">Guo et al. 2016 RNAi silencing in plants</a> <a href="#">Samad et al. 2017 MicroRNA and transcription factors in plant regulatory networks</a>
	25	Gene silencing & RNAi II	VIGS; applications of RNAi for functional genomics and crop improvement	<a href="#">Dubrovina &amp; Kiselev 2019 Exogenous RNAi</a> <a href="#">Zhou et al. 2022 VIGS vectors for plants</a>
	26	DNA repair pathways	non-homologous end joining (NHEJ); homology-directed repair (HDR); microhomology mediated end joining (MMEJ)	<a href="#">Que et al. 2019 Plant DNA repair pathways and their applications in genome engineering Transgenic Plants 237-266</a>
9 02/28 - 03/04	27	New breeding technologies I	Cisgenics and intragenics; targeted genome editing	<a href="#">Holme et al. 2013 Intragenesis and cisgenesis</a> <a href="#">Zhan et al. 2021 Genome editing for plant research</a>
	28	New breeding technologies II	Alternative & Engineered Cas nucleases; Base editing, Prime editing, Epigenetic Editing;	<a href="#">Gao 2021 Genome engineering for crop improvement and future agriculture</a>
	29	New breeding technologies III	Segregation of transgenic and edited loci for production of transgene free events	<a href="#">Molla et al. 2021 Precise plant genome editing using base editors and prime editors</a> <a href="#">He et al. 2020 Generation of transgene-free gene edited plants</a>
<b>Spring Break March 5<sup>th</sup> to March 12<sup>th</sup></b>				
10 03/14 - 03/18	30	Design and Analysis of Gene Editing Experiments	Design considerations and tools for gene editing. Pipeline for analysis of gene editing events	<a href="#">Hassan et al. 2021 Construct design for genome editing in plants</a> <a href="#">Peng et al. 2020 ddPCR for gene editing analysis</a> <a href="#">Germini et al. 2018 Analysis of gene editing events</a>
	31	Commercial Use of Biotech Crops	Commercial use of biotech crops and its importance for sustainable agriculture	<a href="#">ISSA 2018 Facts about biotech crops</a> <a href="#">Brookes 2020 Environmental impacts of GM crops</a>
	32	Risks Benefits & Risk Management of Biotech Crops	Risks and benefits associated with transgenic crops, Risk management and regulatory approval	<a href="#">Zannoni 2019 Evolving Regulatory Landscape for Genome-Edited Plants</a>
<b>Exam 2 available March 18<sup>th</sup> 8am due March 18<sup>th</sup> 6pm</b>				

LDV: Lab demonstration video(s)

Week	Paper Presentation & Discussion	Paper Reference & Presenter	Optional Videos of Paper Presentations & Discussions
11 03/21 - 03/25	03/23; 10:40am - 12:35pm	See details in "Research paper presentation schedule" list on next page	See details in "Optional video list" below. Links to videos will be provided in CANVAS
12 03/28 - 04/01	03/30; 10:40am - 12:35pm		
13 04/04 - 04/08	04/06; 10:40am - 12:35pm		
14 04/11 - 03/16	04/13; 10:40am - 12:35pm		
<b>Exam 3</b> available April 20 <sup>th</sup> 10:40 am due April 20 <sup>th</sup> 2:10 pm			

### Paper Presentation and Discussion Overview,

#### Instructions for paper presentations and discussions:

Papers and presentation dates are assigned randomly to students. Prepare 20 min presentation, 20 slides max, 20 min max, including introduction, main methods, results and discussion and summary. Use font size of at least 22. Send presentation 3 days before presentation to the instructor [altpeter@ufl.edu](mailto:altpeter@ufl.edu) (if you want feedback). Send presentation to all classmates 1 day before presentation as small size ppt or pdf file. You are encouraged to make an appointment with the instructor ([altpeter@ufl.edu](mailto:altpeter@ufl.edu)) for a zoom meeting to go over any questions you may have. If only few questions you can ask them by email. Make sure you can attend all synchronously scheduled paper presentations and come prepared with questions (read paper before presentation) since your contribution to the discussion is a high proportion of the grade.

#### Grading of paper presentation and discussion:

30% of the grade for this activity is your contribution to the discussion of all presented papers; 30% of the grade for this activity is for presentation at audience level (information flow, correct interpretation of results, correct terminology, including sufficient detail, explain topic and results so that others can follow); 10% of the grade for this activity is for presentation delivery (pace, voice volume, poise and confidence, professionalism); 10% of the grade for this activity is for organization of the slides, quality of visual aids, font size (at least 22); 10% of the grade for this activity is for response of the presenting student to questions from others; 10% of the grade for this activity is for staying on time (20 min max, 20 slides max).

**Research Paper Presentation Schedule** (synchronous 10:40am – 12:35pm, except where noted)

<b>Presenter</b>	<b>Presentation Date</b>	<b>Reference</b>	<b>Title</b>
<b>Manuel</b>	3/23/22	Feng et al. 2021 Plant Biotechnology Journal 19, 212-214 + supplement <a href="https://onlinelibrary.wiley.com/doi/full/10.1111/pbi.13459">https://onlinelibrary.wiley.com/doi/full/10.1111/pbi.13459</a>	Development of marker-free rice with stable and high resistance to rice black-streaked dwarf virus disease through RNA interference
<b>Owen</b>	3/23/22	Miroshnichenko et al. 2020 Plant Cell, Tissue and Organ Culture (PCTOC) 140, 691–705 + supplement <a href="https://link.springer.com/article/10.1007/s11240-019-01746-9">https://link.springer.com/article/10.1007/s11240-019-01746-9</a>	Enhancement of resistance to PVY in intragenic marker-free potato plants by RNAi-mediated silencing of eIF4E translation initiation factors
<b>Katie</b>	3/23/22	Burke et al. 2019, Frontiers in Plant Science 10, 313 + supplement <a href="https://www.frontiersin.org/articles/10.3389/fpls.2019.00313/full">https://www.frontiersin.org/articles/10.3389/fpls.2019.00313/full</a>	RNA Interference in the Tobacco Hornworm, <i>Manduca sexta</i> , Using Plastid-Encoded Long Double-Stranded RNA
<b>Marianna</b>	3/30/22	Lorenzo et al 2019. Plant Biotechnology Journal ,18: 944-954 + supporting information <a href="https://onlinelibrary.wiley.com/doi/10.1111/pbi.13258">https://onlinelibrary.wiley.com/doi/10.1111/pbi.13258</a>	Improvement of alfalfa forage quality and management through the down-regulation of MsFTa1
<b>Janam</b>	3/30/22	Pandeya et al. 2018 PNAS 115, E6946-E6955 + supplement <a href="https://www.pnas.org/content/115/29/E6946">https://www.pnas.org/content/115/29/E6946</a>	Selective fertilization with phosphite allows unhindered growth of cotton plants expressing the ptxD gene while suppressing weeds
<b>Ian</b>	3/30/22	Aregawi et al. 2022 Plant Biotechnology Journal online first. <a href="https://onlinelibrary.wiley.com/doi/10.1111/pbi.13754">https://onlinelibrary.wiley.com/doi/10.1111/pbi.13754</a>	Morphogene-assisted transformation of Sorghum bicolor allows more efficient genome editing
<b>Maria</b>	3/30/22 12:35-1:05pm	Liu et al. 2021 Plant Biotechnology Journal 19, 1812-1823 + supplement <a href="https://doi.org/10.1111/pbi.13593">https://doi.org/10.1111/pbi.13593</a>	Metabolic engineering of astaxanthin-rich maize and its use in the production of biofortified eggs
<b>Manuel</b>	4/6/22	Bai et al. 2020 Plant Biotechnology Journal 18, 721-731 + supplement <a href="https://onlinelibrary.wiley.com/doi/10.1111/pbi.13239">https://onlinelibrary.wiley.com/doi/10.1111/pbi.13239</a>	Generation of a multiplex mutagenesis population via pooled CRISPR-Cas9 in soya bean
<b>Katie</b>	4/6/22	Liu et al. 2021 Nature Plants, 7, pages287–294 + supporting information <a href="https://www.nature.com/articles/s41477-021-00858-5">https://www.nature.com/articles/s41477-021-00858-5</a>	Enhancing grain-yield-related traits by CRISPR–Cas9 promoter editing of maize CLE genes
<b>Owen</b>	4/6/22	Mei et al. 2019 Molecular Plant 9, 628-631 + supplement <a href="https://onlinelibrary.wiley.com/doi/full/10.1002/pld3.181">https://onlinelibrary.wiley.com/doi/full/10.1002/pld3.181</a>	Protein expression and gene editing in monocots using foxtail mosaic virus vectors
<b>Janam</b>	4/13/22	Dong et al. 2020 Nature Communications 11, 1178 + supporting information <a href="https://www.nature.com/articles/s41467-020-14981-y">https://www.nature.com/articles/s41467-020-14981-y</a>	Marker-free carotenoid-enriched rice generated through targeted gene insertion using CRISPR-Cas9
<b>Ian</b>	4/13/22	Xu et al. 2020 Plant Biotechnology Journal 19,11-13 + supplement <a href="https://onlinelibrary.wiley.com/doi/full/10.1111/pbi.12603">https://onlinelibrary.wiley.com/doi/full/10.1111/pbi.12603</a>	Fine-tuning the amylose content of rice by precise base editing of the Wx gene
<b>Marianna</b>	4/13/22	Lin et al. 2021 Nature Biotechnology 39, 923-927 + supplement <a href="https://www.nature.com/articles/s41587-021-00868-w">https://www.nature.com/articles/s41587-021-00868-w</a>	High-efficiency prime editing with optimized, paired pegRNAs in plants
<b>Maria</b>	4/13/22 12:35p-1:05pm	Selma et al. 2019 Plant Biotechnology Journal 17, 1703-1705 + supplement <a href="https://doi.org/10.1111/pbi.13138">https://doi.org/10.1111/pbi.13138</a>	Strong gene activation in plants with genome-wide specificity using a new orthogonal CRISPR/Cas9-based programmable transcriptional activator

## Optional Videos of Paper Presentations & Discussions

Week	Topics	#	Links to the Papers for which Video Presentations & Discussions will be Provided in CANVAS
11 03/21 - 03/25	Bottlenecks for efficient plant transformation, genome editing and tissue culture response. Impact of regulatory networks and ectopic expression of morphogenic genes on tissue culture response	1	Advancing Crop Transformation in the Era of Genome Editing <a href="https://doi.org/10.1105/tpc.16.00196">https://doi.org/10.1105/tpc.16.00196</a>
		2	Signaling Overview of Plant Somatic Embryogenesis <a href="https://doi.org/10.3389/fpls.2019.00077">https://doi.org/10.3389/fpls.2019.00077</a>
		3	Use of non-integrating Zm-Wus2 vectors to enhance maize transformation <a href="https://doi.org/10.1007/s11627-019-10042-2">https://doi.org/10.1007/s11627-019-10042-2</a>
	Gene transfer technologies for genetic transformation and genome editing: Protoplast, agrobacterium-mediated, biolistic or viral delivery of transgenes	4	Genome editing reagent delivery in plants <a href="https://doi.org/10.1007/s11248-021-00239-w">https://doi.org/10.1007/s11248-021-00239-w</a>
		5	Agrobacterium tumefaciens: A Bacterium Primed for Synthetic Biology <a href="https://doi.org/10.34133/2020/8189219">https://doi.org/10.34133/2020/8189219</a>
12 03/28 - 04/01		6	An improved ternary vector system for Agrobacterium-mediated rapid maize transformation <a href="https://doi.org/10.1007/s11103-018-0732-y">https://doi.org/10.1007/s11103-018-0732-y</a>
		7	A biolistic method for high-throughput production of transgenic wheat plants with single gene insertions <a href="https://doi.org/10.1186/s12870-018-1326-1">https://doi.org/10.1186/s12870-018-1326-1</a>
		8	A sugarcane mosaic virus vector for gene expression in maize <a href="https://doi.org/10.1002/pld3.158">https://doi.org/10.1002/pld3.158</a>
	RNAi mediated gene silencing for crop improvement	9	New wind in the sails: improving the agronomic value of crop plants through RNAi-mediated gene silencing <a href="https://doi.org/10.1111/pbi.12226">https://doi.org/10.1111/pbi.12226</a>
		10	Ultra-low gossypol cottonseed: generational stability of the seed-specific, RNAi-mediated phenotype and resumption of terpenoid profile following seed germination <a href="https://doi.org/10.1111/j.1467-7652.2011.00652.x">https://doi.org/10.1111/j.1467-7652.2011.00652.x</a>
13 04/04 - 04/08	Genome editing for crop improvement, including targeted mutagenesis, prime editing, base editing chromosome engineering	11	Genome Editing Technologies for Rice Improvement: Progress, Prospects, and Safety Concerns <a href="https://doi.org/10.3389/fgeed.2020.00005">https://doi.org/10.3389/fgeed.2020.00005</a>
		12	Sequence modification on demand: search and replace tools for precise gene editing in plants <a href="https://doi.org/10.1007/s11248-021-00253-y">https://doi.org/10.1007/s11248-021-00253-y</a>
		13	CRISPR/Cas9 directed editing of lycopene epsilon-cyclase modulates metabolic flux for $\beta$ -carotene biosynthesis in banana fruit <a href="https://doi.org/10.1016/j.ymben.2020.01.008">https://doi.org/10.1016/j.ymben.2020.01.008</a>
		14	Novel CRISPR/Cas applications in plants: from prime editing to chromosome engineering <a href="https://doi.org/10.1007/s11248-021-00238-x">https://doi.org/10.1007/s11248-021-00238-x</a>
		15	Base-Editing-Mediated Artificial Evolution of OsALS1 In Planta to Develop Novel Herbicide-Tolerant Rice Germplasms <a href="https://doi.org/10.1016/j.molp.2020.01.010">https://doi.org/10.1016/j.molp.2020.01.010</a>
14 04/11 - 03/16	Gene targeting	16	CRISPR/Cas9-Mediated Multi-Allelic Gene Targeting in Sugarcane Confers Herbicide Tolerance <a href="https://www.frontiersin.org/articles/10.3389/fgeed.2021.673566/full">https://www.frontiersin.org/articles/10.3389/fgeed.2021.673566/full</a>
	Prime editing	17	Prime editing efficiently generates W542L and S621I double mutations in two ALS genes in maize <a href="https://doi.org/10.1186/s13059-020-02170-5">https://doi.org/10.1186/s13059-020-02170-5</a>
	Epigenetic editing review	18	Perspectives for epigenetic editing in crops <a href="https://doi.org/10.1007/s11248-021-00252-z">https://doi.org/10.1007/s11248-021-00252-z</a>
	Epigenetic editing: Synthetic transcription activation	19	CRISPR-Act3.0 for highly efficient multiplexed gene activation in plants <a href="https://doi.org/10.1038/s41477-021-00953-7">https://doi.org/10.1038/s41477-021-00953-7</a>
	Improving editing constructs to enhance precision genome editing (targeted nucleotide substitutions) by homology directed repair	20	Improving CRISPR-Cas9 Genome Editing Efficiency by Fusion with Chromatin-Modulating Peptides <a href="https://www.liebertpub.com/doi/10.1089/CRISPR.2018.0036">https://www.liebertpub.com/doi/10.1089/CRISPR.2018.0036</a>
	21	Increasing Cas9-mediated homology-directed repair efficiency through covalent tethering of DNA repair template <a href="https://www.nature.com/articles/s42003-018-0054-2">https://www.nature.com/articles/s42003-018-0054-2</a>	

**Suggested Text:**

iGenetics – A Molecular Approach / P.J. Russell

**Edition:** 3<sup>rd</sup>**Published:** San Francisco: Pearson, Benjamin Cummings, 2010.**ISBN-10:** 0-321-56976-8Molecular Cell Biology (**MCB**) / H. Lodish et al.**Edition:** 9th**Published:** New York: Freeman and Company, 2021.**ISBN-13:** 978-1319208523

Plant Biotechnology and Genetics / C.N. Stewart JR.

**Edition:** 2<sup>nd</sup>**Published:** John Wiley & Sons, 2016.**ISBN:** 978-1-118-82012-4**Grading:**

Grading will be based on two homework assignments (5% and 6% respectively) take home exam (14 %), in class exam (20 %), presentation of assigned paper on transgenic technologies for crop improvement (15 %), presentation of assigned paper on genome editing technologies for crop improvement (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-

**Grades and Grade Points Effective May 11, 2009 - Summer A**<https://catalog.ufl.edu/ugrad/current/regulations/info/grades.aspx>

Passing Grade	A	B+	B	C+	C	D+	D	S
Grade Points	4.0	3.3	3.0	2.3	2.0	1.3	1	0

**Course website:**

E-Learning system, Canvas to <http://elearning.ufl.edu> is the online source for majority of the course modules. All modules will be uploaded in the “module” section of Canvas. Assignments will also be uploaded in the “module” section of Canvas under the “Assignments” folder.



Announcements regarding general course information will be posted in Canvas throughout the semester. Students need to login with GatorLink username and password for access. If you do not have a GatorLink ID go to <http://gatorlink.ufl.edu> or to the Help Desk: 392-HELP for assistance. Canvas is a learning management system, that can be accessed from a web browser or one of the Canvas mobile apps. In order to use Canvas, you will need some basic skills to use a computer or mobile device. This link provides information regarding technology requirements and skills you may need to use Canvas: <https://community.canvaslms.com/t5/Canvas-Basics-Guide/What-tools-and-computer-skills-do-I-need-to-use-Canvas/ta-p/446129>.

**Basic technical skills required before engaging in this online course include:**

Being able to use the learning management system Canvas as explained in the previous section, using email with attachments, creating and submitting files in commonly used word processing program formats, downloading and installing software, following tutorials for software use, using presentation and graphics programs, using the video conferencing platform zoom including screen sharing and chat functions.

**Assignment objectives:**

**Assignment I (5% of grade, due date Jan. 14th):** Answer complex long essay questions in review of covered course topics. This exercise and the feedback from the instructor will inform the student of the level of detail required for the take home exam to obtain perfect scores.

**Assignment II (6 % of grade, due date Feb. 11th):** In silico recombinant DNA technology exercise, involves retrieval of a genomic DNA sequence from online database, development of a cloning strategy to subclone the promoter from the specific genomic sequence 5' of a reporter gene with terminator for subsequent analysis, analysis of transcription factor binding sites in the promoter with a software tool, design of primers with a software tool, browsing annotated plasmid DNA sequence files for evaluation of cloning strategies.

**Assignment III (15 % of grade, March 23<sup>rd</sup> and March 30<sup>th</sup>):** Presentation of recent original research article that will be randomly assigned by instructor describing molecular improvement of crops through transgenic approaches. Instructor will be available the week before the presentation to provide feedback on the presentation draft and answer any open questions (due date TBD).

**Assignment IV (15 % of grade, April 6<sup>th</sup> and April 13<sup>th</sup>):** Presentation of recent original research article that will be randomly assigned by instructor describing molecular improvement of crops through gene editing approaches. Instructor will be available the week before the presentation to provide feedback on the presentation draft and answer any open questions (due date TBD).

**Exam objectives:**

**Exam 1 (take home 14 %, of grade, due date Feb. 4<sup>th</sup>):** 15 to 20 questions with sub-questions, requiring long essay-type answers in covering the following course topics in detail: Gene expression and regulation in prokaryotes and eukaryotes (genome

organization, transcription, processing of transcripts, translation, protein folding and sorting, regulation of gene expression, degradation of RNA and proteins).

**Exam 2 (timed and scheduled exam, 20% of grade, March 18<sup>th</sup>):** Mix of long essay and short answer questions covering the following course topics: 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, mechanisms and applications of gene silencing for crop improvement, DNA repair pathways, new biotechnologies (cisgenics, intragenics, genome editing including targeted mutagenesis, gene targeting, base editing, prime editing, epigenetic editing).

**Exam 3 (timed and scheduled exam, 25% of grade, April 20<sup>th</sup>):** 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.

## **CLASS POLICIES**

**Instructor response plan:** The instructor strives to provide frequent feedback and short response times. Same day response to emails and discussion postings can be expected, feedback on assignments, and exam grades will typically be provided within three business days of the submission. Grading of the take home exam will take 5-8 business days from submission due to the time required for reviewing the long essay answers in this exam.

### **Attendance and Participation:**

Students are expected to log on in on time to scheduled synchronous paper discussion and review sessions have cell phones turned off and camera turned on. Students are expected to be prepared (have completed modules and read papers prior discussion) and participate in class discussions during synchronous online sessions, ask questions and push for clarity.

### **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.

Requirements for class attendance and make-up exams, assignments, and other work in this course are consistent with university policies that can be found at:

<https://catalog.ufl.edu/ugrad/current/regulations/info/attendance.aspx>

### **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. 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/>.

### **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>. Furthermore, you are obligated to report any condition that facilitates academic misconduct to appropriate personnel. If you have any questions or concerns, please consult with the instructor or TAs in this class.

### **Netiquette**

is a set of rules for behaving properly online. Something about cyberspace makes it easy for people to forget that they are interacting with other real people. The following bullet points cover some basic expectations to communicating online:

Be sensitive to the fact that there will be cultural and linguistic backgrounds, as well as different political and religious beliefs, plus just differences in general.

Use good taste when composing your responses in Discussion Forums. For example, swearing and profanity must be avoided. Also consider that slang can be misunderstood or misinterpreted. Don't use all capital letters when composing your responses as this is considered "shouting" on the Internet and is regarded as impolite or aggressive. It can also be stressful on the eye when trying to read your message. Be respectful of others' views and opinions. Avoid "flaming" (publicly attacking or insulting) as this can cause hurt feelings and decrease the chances of getting all different types of points of view.

Be careful when using acronyms. If you use an acronym it is best to spell out its meaning first, then put the acronym in parentheses afterward, for example: Frequently Asked Questions (FAQs). After that you can use the acronym freely throughout your message.

Use good grammar and spelling, and avoid using text messaging shortcuts. Test your microphone and camera before synchronous online meetings, to provide for an enjoyable interaction during the meeting.

### **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.

### **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,

<https://disability.ufl.edu/>

### **Campus Helping Resources**

Students experiencing crises or personal problems that interfere with their general wellbeing 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.

- **U Matter, We Care:**

If you or a friend is in distress, please contact [umatter@ufl.edu](mailto:umatter@ufl.edu) or 352 392-1575 so that a team member can reach out to the student.

- **Counseling and Wellness Center:** <http://www.counseling.ufl.edu/cwc>, and 392-1575; and
- **University Police Department:** 392-1111 (or 911 for emergencies).

### **Academic Resources**

- **E-learning technical support**, 352-392-4357 (select option 2) or e-mail to Learning-support@ufl.edu. <https://lss.at.ufl.edu/help.shtml>.
- **Career Connections Center**, Reitz Union, 392-1601. Career assistance and counseling. <https://career.ufl.edu/>
- **Library Support**, <http://cms.uflib.ufl.edu/ask>. Various ways to receive assistance with respect to using the libraries or finding resources.
- **Teaching Center**, Broward Hall, 392-2010 or 392-6420. General study skills and tutoring. <https://teachingcenter.ufl.edu/>
- **Writing Studio, 302 Tigert Hall**, 846-1138. Help brainstorming, formatting, and writing papers. <https://writing.ufl.edu/writing-studio/>

- **Student Complaints On-Campus:** Visit the Student Honor Code and Student Conduct Code webpage for more information. <https://sccr.dso.ufl.edu/policies/student-honor-%20code-student-conduct-code/>
- **On-Line Students Complaints:** View the Distance Learning Student Complaint Process. <https://distance.ufl.edu/getting-help/student-complaint-process/>.

**NOTE: The instructor reserves the right to change any information contained in this and other handouts in this course.**