

## PLP6636 FRONTIERS IN PLANT BIOTECHNOLOGY

### 3 credit hours

#### 1. Instructor:

Svetlana Y. Folimonova

Room 2565 Fifield Hall

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#### Course Time/ Location:

Lectures: Fifield Hall (room 2306), Tuesdays, periods 2 (8:30-9:20 AM) & 3 (9:35-10:25 AM)

Discussions: Fifield Hall (room 2306), Thursdays, period 3 (9:35-10:25 AM)

#### Course Materials Access: TBE

#### 2. Office Hours:

Office hours are Wednesdays and Fridays between 9 AM and 12 PM by appointment at the instructor's office (2565) Fifield Hall.

#### 3. Course Overview:

This course explores some of the exciting concepts in modern molecular biology that have been recently implicated in the development of novel cutting-edge genetic tools for use in the field of plant biotechnology. Special emphasis is given to small non-coding RNAs and their role in plant immunity along with RNA interference (RNAi)-based approaches in plant bioengineering, novel systems for targeted gene editing (such as CRISPR/Cas9 and others) and their practical applications. Other cutting-edge biotechnology advancements, including strategies for precise regulation of gene expression in plants, multigene engineering, and the use of plant pathogens as materials for nanotechnology, will be discussed. In this course, each two-hour lecture is followed by an in-class paper discussion, which gives the students an opportunity to improve the understanding of the fundamental concepts that have been discussed in the preceding lecture by reviewing and discussing a research paper that is selected accordingly to what has been taught during the earlier lecture.

Course Objectives: through this course, students will:

1. Become familiar with the advanced genetic tools that recently have become available in the field of biotechnology as a result of breakthrough discoveries in fundamental science and with their applications for plant improvement and production of new products in plants.
2. Learn some of the recently developed experimental procedures and methods that are used in the biotechnology research, their theory, applications, and limitations and learn how to properly select appropriate methodology while designing experiments.
3. Improve professional skills, including skills in developing a scientific idea as well as in critical reading of scientific literature and presentation skills.

Prerequisites: graduate standing and general knowledge of Genetics, Organic Chemistry and Biochemistry

#### 4. Course schedule of topics and assignments:

DATE	TOPIC	SUGGESTED READING (tentative, subject to change)
Aug 23, Tue <i>Lecture 1</i>	Course overview; Overview of Biotechnology	Newell-McGloughlin and Re (2007)
Aug 25, Thu <i>Discussion</i>	Overview of the Discussion part of the course	
Aug 30, Tue <i>Lecture 2</i>	New paradigms in biotechnology: progress enabled by the advancements in fundamental science	Liu et al. (2013); Saurabh et al. (2014)
Sep 1, Thu <i>Discussion</i>	Paper discussion 1	Discussion paper to be announced
Sep 6, Tue <i>Lecture 3</i>	Translating fundamental knowledge into applications I. Non-coding RNA. RNAi: Discovery and molecular mechanism I.	Montgomery (2004); Lindbo (2012); Carthew and Sontheimer (2009)
Sep 8, Thu <i>Discussion</i>	Paper discussion 2	Discussion paper to be announced
Sep13, Tue <i>Lecture 4</i>	RNAi: molecular mechanism II. RNAi in different systems	Axtell (2013); Bologna and Voinnet (2014); Castel and Martienssen (2013)
Sep 15, Thu	Paper discussion 3	Discussion paper to be

<i>Discussion</i>		announced
Sep 20, Tue <i>Lecture 5</i>	Small RNAs in inter-kingdom communications	Weiberg et al. (2015)
Sep 22, Thu <i>Discussion</i>	Paper discussion 4	Discussion paper to be announced
Sep 27, Tue <i>Lecture 6</i>	Applications of RNAi to plant biotechnology	Saurabh et al. (2014); Katoch and Thakur (2013)
Sep 29, Thu <i>Discussion</i>	Paper discussion 5	Discussion paper to be announced
Oct 4, Tue <i>Lecture 7</i>	The power and promise of RNAi technology in controlling citrus Huanglongbing disease. <i>The topic will be presented by Dr. Nabil Killiny, Assistant Professor at the UF, CREC</i>	Bove (2006); Harmon (2013); El-Shesheny et al. (2013); Hajeri et al. (2014)
Oct 6, Thu <i>Discussion</i>	Paper discussion 6	Discussion paper to be announced
Oct 11, Tue <i>Exam</i>	<b>MID-TERM EXAM</b>	
Oct 13, Thu <i>Discussion</i>	Paper discussion 7	Discussion paper to be announced
Oct 18, Tue <i>Lecture 8</i>	Translating fundamental knowledge into applications II. New approaches to gene manipulation. Strategies for targeted genome editing. ZFNs, TALENs, CRISPR/Cas systems	Gaj et al. (2013)
Oct 20, Thu <i>Discussion</i>	Paper discussion 8	Discussion paper to be announced
Oct 25, Tue <i>Lecture 9</i>	CRISPR/Cas system: interference mechanisms and applications	Sampson and Weiss (2013); Upadhyay et al. (2013); Barrangou (2013); Belhaj et al. (2013); Ran et al. (2013)
Oct 27, Thu <i>Discussion</i>	Paper discussion 9	Discussion paper to be announced
Nov 1, Tue <i>Lecture 10</i>	CRISPR/Cas system: interference mechanisms and applications (cont.)	Sampson and Weiss (2013); Upadhyay et al. (2013); Barrangou (2013); Belhaj et al. (2013); Ran et al. (2013)

Nov 3, Thu <i>Discussion</i>	Paper discussion 10	Discussion paper to be announced
Nov 8, Tue <i>Lecture 11</i>	The roles of CRISPR-Cas systems beyond adaptive immunity	Barrangou (2015); Westra et al. (2014)
Nov 10, Thu <i>Discussion</i>	Paper discussion 11	Discussion paper to be announced
Nov 15, Tue <i>Lecture 12</i>	Targeted genome editing in citrus: applications and perspectives. <i>The topic will be presented by Dr. Nian Wang, Associate Professor at UF, CREC</i>	
Nov 17, Thu	Paper discussion 12	Discussion paper to be announced
Nov 22, Tue <i>Lecture 13</i>	Nova/Frontline Harvest of Fear video (the video represents a debate over GM food crops; contains interviews with scientists, farmers, biotech and food industry)	
<b>Nov 24, Thu</b>	<b>HOLIDAY</b>	
Nov 29, Tue <i>Lecture 14</i>	Intellectual property rights and their relevance to agricultural biotech. <i>Lecture will be presented by John Byatt, Assistant Director of the UF Office of Technology Licensing.</i>	Dalton (2014)
Dec 1, Thu <i>Discussion</i>	Paper discussion 13	Discussion paper to be announced
Dec 6, Tue <i>Exam</i>	<b>EXAM</b>	

**REFERENCES FOR THE LECTURE SUPPLEMENTARY READING MATERIALS:**

Axtell MJ (2013) Classification and comparison of small RNAs from plants. *Annu. Rev. Plant Biol.* 64:137-159.

Barrangou R (2013) CRISPR-Cas systems and RNA-guided interference. *WIREs RNA* 4:267-278.

Barrangou R (2015) The roles of CRISPR-Cas systems in adaptive immunity and beyond. *Curr. Opin. Immunol.* 32:36-41.

- Belhaj K, Chaparro-Garcia A, Kamoun S, Nekrasov V. (2013) Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. *Plant Methods* 9:39.
- Bologna NG and Voinnet O. (2014) The diversity, biogenesis, and activities of endogenous silencing small RNAs in *Arabidopsis*. *Annu. Rev. Plant Biol.* 65:473-503.
- Bove JM. (2006) Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. *J. Plant Pathol.* 88:7-37.
- Carthew Richard W., and Sontheimer, Erik J. (2009) Origins and Mechanisms of miRNAs and siRNAs. *Cell* 136, 642–655
- Castel SE and Martienssen RA (2013) RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. *Nature Reviews* 14:100-112.
- Dalton M. (2014) DuPont's Corn to Take Root. *The Wall Street Journal* Feb. 12, 2014.
- Dawson WO, Folimonova SY. (2013) Virus-Based Transient Expression Vectors for Woody Crops: A New Frontier for Vector Design and Use. *Annu. Rev. Phytopathol.* 51:321-337.
- El-Shesheny I, Hajeri S, El-Hawary I, Gowda S, and Kiliny N (2013) Silencing Abnormal Wing Disc Gene of the Asian Citrus Psyllid, *Diaphorina citri* Disrupts Adult Wing Development and Increases Nymph Mortality. *PLOS One* 8: e25677.
- Gaj T, Gersbach CA, Barbas III CF. (2013) ZFN, TALEN, and CRISPR/CAS-based methods for genome engineering. *Trends in Biotechnology* Vol. 31-No.7:397-405.
- Gleba YY, Giritch A. (2011) Plant viral vectors for protein expression. *In Recent Advances in Plant Virology*, ed. C Caranta, MA Aranda, M. Tepfer, JJ Lopez-Moya. pp. 387-412. Norfolk, UK: Caister Acad. Press. 412pp.
- Hajeri, S., Killiny, N., El-Mohtar, C., Dawson, W.O., Gowda, S., 2014. Citrus tristeza virus-based RNAi in citrus plants induces gene silencing in *Diaphorina citri*, a phloem-sap sucking insect vector of citrus greening disease (Huanglongbing). *J. Biotechnol.* 176, 42-49.
- Harmon A. (2013) A Race to Save the Orange by Altering Its DNA. *The New York Times*, July 27, 2013.
- Hefferon KL. (2012) Plant virus expression vectors set the stage as production platforms for biopharmaceutical proteins. *J. Virol.* 433:1-6.
- Hefferon K. (2014) Plant virus vector development: new perspectives. *BioMed Research International* Vol. 2014, Article ID 785382.
- Katoch R. and Thakur N. (2013) Advances in RNA interference technology and its impact on nutritional improvement, disease and insect control in plants. *Appl. Biochem. Biotechnol.* 169:1579-1605.
- Lindbo J. A. (2012) A historical overview of RNAi in plants. *Methods Mol. Biol.* 984:1-16.

Liu W, Yuan JS, Stewart Jr CN. (2013) Advanced genetic tools for plant biotechnology. *Nature Rev. Genet.* 14:781-793.

Montgomery M. K (2004) RNA interference: historical overview and significance. *Methods Mol. Biol.* 265:3-21.

Newell-McGloughlin M. and E. Re. (2007) The evolution of Biotechnology. From Natufians to Nanotechnology. Springer. The Netherlands.

Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. (2013) Genome engineering using the CRISPR-Cas9 system. *Nature protocols* 8:2281-2308.

Sampson TR, Weiss DS. (2013) Exploiting CRISPR/Cas systems for biotechnology. *Bioassays* 36:34-38.

Saurabh S, Vidyarthi AS, and Prasad D (2014) RNA interference:concept to reality in crop improvement. *Planta* 239:543-564.

Upadhyay SK, Kumar J, Alok A, Tuli R. (2013) RNA-Guided Genome Editing for Target Gene Mutations in Wheat. *Genes, Genomes, Genetics* Vol. 3:2223-2228.

Weiberg A, Bellinger M, and Jin H. (2015) Conversations between kingdoms: small RNAs. *Curr. Opin. Biotechnol.* 32:207-215.

Westra ER, buckling A, and Fineran PC (2014) CRISPR-Cas systems: beyond adaptive immunity. *Nature Reviews Microbiology* 12:317-326.

## **PAPER SUGGESTIONS FOR DISCUSSIONS:**

### **Small RNAs as crucial regulators, mechanism and applications**

1. Patrice Dunoyer, Gregory Schott, Christophe Himber, Denise Meyer, Atsushi Takeda, James C. Carrington, Olivier Voinnet (2010). **Small RNA duplexes function as mobile silencing signals between plant cells.** *Science* 328: 912-916.
2. Attila Molnar, Charles W. Melnyk, Andrew Basset, Thomas J. Hardcastle, Ruth Dunn, David C. Baulcombe (2010). **Small Silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells.** *Science* 328:872-875.
3. Dinah Qutob, B. Patrick Chapman & Mark Gijzen (2013). **Transgenerational gene silencing causes gain of virulence in a plant pathogen.** *Nature Communications* 4:1349, DOI: 10.1038/ncomms2354.

4. Feng Li, Daniela Pignatta, Claire Bendix, Jacob O. Brunkard, Megan M. Cohn, Jeffery Tung, Haoyu Sun, Pavan Kumar, and Barbara Baker (2012). **MicroRNA regulation of plant innate immune receptors.** *PNAS* 109: 1790–1795.
5. Arne Weiberg, Ming Wang, Feng-Mao Lin, Hongwei Zhao, Zhihong Zhang, Isgouhi Kaloshian, Hsien-Da Huang, Hailing Jin (2013). **Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways.** *Science* 342: 118-123.

Extra: Robert H. Downen, Mattia Pelizzola, Robert J. Schmitz, Ryan Lister, Jill M. Downen, Joseph R. Nery, Jack E. Dixon, and Joseph R. Ecker (2012). **Widespread dynamic DNA methylation in response to biotic stress.** *PNAS* 109: E2183-E2191.

**New approaches to gene manipulation. Strategies for targeted genome editing. CRISPR/Cas systems**

6. Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D.A., and Horvath, P. (2007). **CRISPR provides acquired resistance against viruses in prokaryotes.** *Science* 315, 1709–1712.
7. Deltcheva, E., Chylinski, K., Sharma, C.M., Gonzales, K., Chao, Y., Pirzada, Z.A., Eckert, M.R., Vogel, J., and Charpentier, E. (2011). **CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III.** *Nature* 471, 602–607.
8. Saprunauskas, R., Gasiunas, G., Fremaux, C., Barrangou, R., Horvath, P., and Siksnys, V. (2011). **The *Streptococcus thermophilus* CRISPR/Cas system provides immunity in *Escherichia coli*.** *Nucleic Acids Res.* 39, 9275–9282.
9. Samuel H. Sternberg, Sy Redding, Martin Jinek, Eric C. Greene & Jennifer A. Doudna (2014). **DNA interrogation by the CRISPR RNA-guided endonuclease Cas9.** *Nature* 507:62-67.
10. Wenzhi Jiang, Huanbin Zhou, Honghao Bi, Michael Fromm, Bing Yang and Donald P. Weeks (2013). **Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice.** *Nucleic Acids Research* 2013,1–12.

Extra: Vladimir Nekrasov, Brian Staskawicz, Detlef Weigel, Jonathan D G Jones & Sophien Kamoun (2013). **Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease.** *Nature biotechnology* 31(8): 691-693.

Extra: Timothy R. Sampson, Sunil D. Saroj, Anna C. Llewellyn, Yih-Ling Tzeng & David S. Weiss (2013). **A CRISPR/Cas system mediates bacterial innate immune evasion and virulence.** *Nature* 497(9): 254-258.

### Viruses as tools in biotechnology

11. Sateesh Kagale, Shihomi Uzuhashi, Merek Wigness, Tricia Bender, Wen Yang, M. Hossein Borhan & Kevin Rozwadowski (2012). **TMV-Gate vectors: Gateway compatible tobacco mosaic virus based expression vectors for functional analysis of proteins.** *Scientific Reports* 2:874.
12. Subhas Hajeri, Nabil Killiny, Chooa El-Mohtar, William O. Dawson, Siddarame Gowda (2014). **Citrus tristeza virus-based RNAi in citrus plants induces gene silencing in *Diaphorina citri*, a phloem-sap sucking insect vector of citrus greening disease (Huanglongbing).** *Journal of Biotechnology* 176:42-49.

### Limitations of biotechnology approaches. Risk assessment

13. Lin Zhang, Dongxia Ho , Xi Chen, Donghai Li, Lingyun Zhu et al. (2012). **Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA.** *Cell Research* 22:107-126.

Extra: Kenneth W. Witwer, Melissa A. McAlexander, Suzanne E. Queen, and Robert J. Adams (2013). **Real-time quantitative PCR and droplet digital PCR for plant miRNAs in mammalian blood provide little evidence for general uptake of dietary miRNAs.** *RNA Biology* 10: 1080–1086.

### **5. Assessment:**

**Exams** – Mid-term exam (30% of student's grade); Final exam (45% of grade)

**Participation in class discussions** – 25% of grade

### **6. Attendance and Make-up Policy:**

Students are expected to attend all course lectures and in-class discussions as well as complete



required assignments on time. Students should arrive to the class on time. No cell phone use is allowed in the class. Along with the fact that these are firm requirements for participating in the course, the ability to fulfill these expectations reflects your professional characteristics. If you are unable to attend a class due to illness or an emergency, you must notify the instructor as soon as possible, preferably prior to the scheduled class. If you miss an exam for a valid and documented reason, a make-up exam will be scheduled with permission from the instructor. These 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>

## **7. Accommodations 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. The respective students should first register with the Disability Resource Center at 0001 Reid Hall, 352-392-8565, [www.dso.ufl.edu/drc/](http://www.dso.ufl.edu/drc/) and provide appropriate documentation.

## **8. Required and Recommended Textbooks:**

Although there is no required textbook, students are expected to read all the reading materials provided by the instructor, which will include review and research articles. Those will be provided in electronic format. Among those materials will be papers selected specifically for in class discussions that students are expected to study in details in order to actively participate in discussions.

## **9. Grades and Grade Points:**

In accordance with current University of Florida policy, grade points will be assigned as follows:

<u>Letter Grade</u>	<u>Grade Points</u>	<u>Percentage</u>
A	4.0	90 or above
A-	3.67	87-89
B+	3.33	84-86
B	3.0	80-83
B-	2.67	77-79
C+	2.33	74-76
C	2.0	70-73
C-	1.67	67-69
D+	1.33	64-66
D	1.0	60-63
D-	0.67	57-59

E	0.0	56 or below
WF	0.0	NA
I	0.0	NA
NG	0.0	NA
S-U	0.0	NA

Detailed and up-to-date information on UF grades and grading policies can be found at <https://catalog.ufl.edu/ugrad/current/regulations/info/grades.aspx>

### **10. On-line course evaluation:**

According to the UF Policy on Course Syllabi, “students are expected to provide feedback on the quality of instruction in this course by completing online evaluations at <https://evaluations.ufl.edu>. Evaluations are typically open during the last two or three weeks of the semester, but students will be given specific times when they are open. Summary results of these assessments are available to students at <https://evaluations.ufl.edu/results/>.”

### **11. Materials and supplies fees:**

None

### **12. Academic Honesty**

As a student enrolled at the UF, you committed yourself to the highest standards of honesty and integrity required by the honor code. You are expected to be consistent with this commitment. The following is the UF 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.” As it is stated by the UF student honor code, “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. 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: 2013-2014 Undergraduate Catalog).

It is expected that you will complete all work independently unless the assignment is designed as a group project as explicitly indicated by the instructor.

This policy will be firmly upheld at all times during this course.

For more information regarding academic honesty and student responsibilities, 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.

### **Campus Helping Resources**

The university's counseling resources are available for students experiencing personal problems that interfere with their general well-being and/or academic performance. The Counseling & Wellness Center provides confidential counseling services at no cost for students that are currently enrolled with the university.

- University Counseling & Wellness Center, 3190 Radio Road, 352-392-1575, [www.counseling.ufl.edu/cwc/](http://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, 352-392-1601, [www.crc.ufl.edu/](http://www.crc.ufl.edu/)