AGSC 4630/5630 – INTRODUCTION TO GENE EDITING WITH CRISPR/CAS9

Spring 2022

Instructor Information

Instructor:	Sonali Roy, Ph.D.
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Office Hours:	Tuesday 1:00-4:00 PM or by appointment

Course Identification

Course Number:	AGSC4630/AGSC5630
Course Name:	Introduction to Gene Editing with CRISPR/Cas9
Credit Hours:	3 Credit Hours
Lecture Meeting:	N/A
Class Location:	Plant Biotechnology Teaching Lab
Prerequisites:	Students must have completed *at least* one of the following courses AGSC 3109 Principles and methods of Biotech I, AGSC 3710 Biotech and Society, BIOL 4110 Molecular Genetics, AGSC 5160 Animal Genetics and Breeding, AGSC 5190 Plant Breeding
Lab:	There are 4 lab sessions, two of which are bioinformatics based and require laptops/computers and access to the internet. Two lab trainings require students to be present on site and at the bench.

Course Description/Overview

Gene editing technologies are facilitating precise engineering of plant genomes and they provide a rapid alternative to traditional plant breeding methods. This course will introduce students to these cutting-edge technologies with a focus on CRISPR/Cas9 based genome editing and prepare them for a career in plant biotechnology in a changing climate. Learn the theory behind the Noble-prize winning discovery and receive hands-on training in how to design and implement a CRISPR/Cas9 mediated gene edit. This course covers the biology, ethics and applications of CRISPR/Cas9 in agriculture and industry and is designed for upper-level advanced undergraduate and graduate students enrolled at TSU for a degree in Agricultural sciences or Biological sciences.

Course Resources

Resource Website(s)

- CRISPR-P http://crispr.hzau.edu.cn/CRISPR2/
- NCBI BLAST https://blast.ncbi.nlm.nih.gov/Blast.cgi

Softwares

- Benchling https://www.benchling.com/ or Geneious (paid)

Textbook

- Genome Engineering via CRISPR-Cas9 System 1st Edition by Vijai Singh (Editor), Pawan K. Dhar PhD (Editor)

- CRISPR 101 3rd Edition Addgene (Open source: https://info.addgene.org/download-addgenes-ebook-crispr-101-3rd-edition)

Learning Objectives

The learning objectives of the courses are:

(1) To introduce students to the concepts of Genome Engineering and Gene Editing.

(2) Demonstrate an understanding of the biology behind CRISPR/Cas9 mediated targeted genome editing.

(3) To identify and investigate practical uses of this technology in plant biology and agriculture, microbiology and food sciences industry.

(4) Apply their knowledge and independently design a guide RNA targeted to a gene and identify a cloning strategy.

(5) Understand the ethical challenges and societal risks which come with powerful technologies such as CRISPR/Cas9.

Expectations or Outputs

By the end of this course, you will be able to

- (1) Tabulate the similarities and differences between at least three methods of gene editing in use today during written assignments and/or tests with >70% accuracy.
- (2) Draw a multi-panel schematic diagram to summarize steps of CRISPR/Cas9 mediated DNA targeting, cleavage and repair in bacteria in written tests.
- Tabulate advantages and disadvantages of CRISPR as a technology.
- (3) During classroom discussions, you should be able to present at least three examples of practical uses of gene editing in the life sciences industry by visiting websites of start-ups and companies that use gene editing.
- (4) Identify PAM sites, select guide RNAs, determine gRNAs with least off target editing and design primers to PCR amplify and clone the selected gRNA during laboratory sessions using tools available online for at least three of five target genes/organisms.

- Transform cloned gRNA constructs in to yeast and record visually observable differences between edited/unedited strains and analyze sequencing data to identify gene edits, during laboratory exercises.
- (5) Debate for or against the use of gene editing in a given case-study in an essay less than 1000 words outlining at least three arguments that explains your stance.

Assessments Methods and Grading Scale

Assessment methods

Grades for the course will be based on the following assessments (will be discussed on first week of the class). Assessment differences between undergraduate and graduate classes will be in terms of percent score allotted to the midterm exam and a project/essay which will be required of graduate students only. The scoring method is outlined below.

Assessment Methods	Weight	Due date
Mid-term exam	*20% undergraduates, **10%** graduates*	***TBD***
Lab work and participation	*4*10 = 40%*	*2 weeks*
Homework, Assignments	*10%*	*each week before class*
Final exam	*20%*	***TBD***
Class participation, attendance (poll), contribution on the discussion board and classwork	*10%*	*In class*
*Project (Graduate Students Only)**	***10%***	
Total	***100%***	

Grading Scale

As per the TSU grading policy, your final grade will be reported based on the following grading scale.

90-100: "A" 80-89: "B" 70-79: "C" ≤ 69: "F"

Course Policies and Expectations

- This is an inclusive classroom. I as an instructor am committed to creating and maintaining a safe learning space where you will be treated with respect and dignity. All individuals are provided equitable opportunities to participate, contribute, and succeed. I welcome all students including but not limited to members of minority and marginalized groups such as African Americans, Latinx, Native Americans,

international peoples, women, people with disabilities, and members of the LGBTQIA+ community.

- I only expect that you have a deep interest and enthusiasm for the subject and diligently invest time in your own learning by completing assignments on time. Peer-learning and peer-mentoring which is one of the most effective ways of understanding complex concepts, is highly encouraged.

- If you have special learning requirements, please come speak to me before the class and we can find something that will work for us.

- Email and zoom meetings are the best way to contact me.

University Policies

Academic Integrity

Academic regulations and procedures are governed by University policy. Academic dishonesty cases will be handled in accordance the University's policies. More on academic integrity: http://www.tnstate.edu/library/infoliteracy/academic_integrity.aspx

Disability accommodation

TSU is committed to creating inclusive learning environments and providing all students with opportunities to learn and excel in their course of study. Any student with a disability or condition which might interfere with his/her class performance or attendance may arrange for reasonable accommodations by visiting the Office of Disability Services (ODS). ODS is located in Kean Hall, room 131 and can be reached at 963-7400 or http://www.tnstate.edu/disabilityservices/ You will be required to speak with ODS staff and provide documentation of the need for an accommodation. If you qualify for an accommodation you will be provided with a document stating what type of classroom accommodations are to be made by the instructor. It is your responsibility to give a copy of this document to the instructor **as soon as you receive it**. Accommodations will only be provided **AFTER** the instructor receives the accommodation instructions from ODS; accommodations are not retroactive. You must follow this process for each semester that you require accommodations.

Sexual misconduct, domestic/dating violence, stalking

TSU recognizes the importance of providing an environment free of all forms of discrimination and sexual harassment, including sexual assault, domestic violence, dating violence, and stalking. If you (or someone you know) has experienced or is experiencing any of these incidents, there are resources to assist you in the areas of accessing health and counseling services, providing academic and housing accommodations, and making referrals for assistance with legal protective orders and more.

Please be aware that most TSU employees, including faculty and instructors, are "responsible employees", meaning that they are required to report incidents of sexual

violence, domestic/dating violence or stalking. **This means that if you tell me about a situation involving sexual harassment, sexual assault, dating violence, domestic violence, or stalking, I must report the information to the Title IX Coordinator.** Although I have to report the situation, you will still have options about how your situation will be handled, including whether or not you wish to pursue a formal complaint. Our goal is to make sure you are aware of the range of options available to you and have access to the resources you need.

You are encouraged to contact TSU's Title IX Coordinator to report any incidents of sexual harassment, sexual violence, domestic/dating violence or stalking. The Title IX coordinator is located in the Office of Equity and Inclusion, McWherter Administration Building, Ste. 260 and can be reached at 963-7494 or 963-7438. For more information about Title IX and TSU's SART or policies and procedures regarding sexual, domestic/dating violence and stalking please visit: www.tnstate.edu/equity.

If you wish to speak to someone confidentially, who is not required to report, you can contact the TSU Counseling Center, located in the basement of Wilson Hall, at 963-5611 or TSU Student Health Services, located in the Floyd Payne Campus Center room 304, at 963-5084. You may also contact the following off campus resources: Sexual Assault Center of Nashville at 1-800-879-1999 or www.sacenter.org or the Tennessee Coalition to End Domestic & Sexual Violence at 615-386-9406 or www.tncoalition.org.

Harassment & discrimination

Tennessee State University is firmly committed to compliance with all federal, state and local laws that prohibit harassment and discrimination based on race, color, national origin, gender, age, disability, religion, retaliation, veteran status and other protected categories. TSU will not subject any student to discrimination or harassment and no student shall be excluded from participation in nor denied the benefits of any educational program based on their protected class. If a student believes they have been discriminated against or harassed because of a protected class, they are encouraged to contact the Office of Equity and Inclusion at McWherter Administration Building, Ste. 260, 615-963-7494 or 963-7438, www.tnstate.edu/equity

Course Outline

Course title: Introduction to Gene Editing with CRISPR/Cas9

Credit Hours: 3 Hours

Course description: This course introduces the student to gene editing technologies used in agriculture leading to the discovery and development of CRISPR/Cas9 tools to edit plant genomes. It covers the biology, ethics and applications of CRISPR/Cas9 in agriculture and industry. This course is directed towards students with an interest in synthetic biology and plant genome engineering.

Learning objectives: By the end of this course, the student should be able to

(1) Tabulate the similarities and differences between at least three methods of gene editing in use today during written assignments and/or tests with >70% accuracy.

(2) Draw a multi-panel schematic diagram to summarize steps of CRISPR/Cas9 mediated DNA targeting, cleavage and repair in bacteria in written tests.

•Tabulate advantages and disadvantages of CRISPR as a technology.

(3) During classroom discussions, you should be able to present at least three examples of practical uses of gene editing in the life sciences industry by visiting websites of start-ups and companies that use gene editing.

(4) Identify PAM sites, select guide RNAs, determine gRNAs with least off-target editing and design primers to PCR amplify and clone the selected gRNA during laboratory sessions using tools available online for at least three of five target genes/organisms.

•Transform cloned gRNA constructs in to yeast and record visually observable differences between edited/unedited strains and analyze sequencing data to identify gene edits, during laboratory exercises.

(5) Debate for or against the use of gene editing in a given case-study in an essay less than 1000 words outlining at least three arguments that explains your stance.

Prerequisite

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1. A basic understanding of Molecular biology is required. At least one of the following courses is pre-requisite for attending this class AGSC 3109 Principles and methods to Biotech, AGSC 3710 Biotech and Society, BIOL 4110 Molecular Genetics, AGSC 5160 Animal Genetics and Breeding, AGSC 5190 Plant Breeding.

2. Access to the internet.

References:

Following text books are recommended but not required. Reading material will be provided by the instructor including research articles and review papers. Students are encouraged to find additional

- Genome Engineering via CRISPR-Cas9 System 1st Edition by Vijai Singh (Editor), Pawan K. Dhar PhD (Editor)
- CRISPR 101 3rd Edition Addgene (Open source)

Class schedules of fall, 2021

Week	Topics
1	Introduction to Genome Engineering (Zinc Finger Nucleases, TALENs, CRISPR/Cas9)
2	Discovery of CRISPR/Cas9 in bacteria and cellular repair mechanisms
3	Somatic and germline editing and nucleases besides SpCas9
4	Basic and Advanced CRISPR methods and delivery
5	CRISPR/Cas9 complications, off-target mutations, mosaics
6	Guide RNA characteristics, design and multiplex gRNAs
7	**Lab:** Designing a guide RNA for targeted genome editing
8	**Lab:** gRNA cloning and purification
9	**Lab:** Yeast transformation
10	**Lab:** Genotyping and identifying CRISPR edited lines
11	CRISPR/cas9 Applications in plant biology research and agriculture
12	CRISPR/cas9 Applications in food and human sciences and energy industry.
13	**Student Engagement:** CRISPR/Cas9 in popular science, guest lecture – practicing scientist.
14	CRISPR/Cas9 Bioethics and environmental regulations
15	Final exam

Policies:

Attendance:

Attendance is required for all classes.

Electronic devices:

Laptops are required for two/four of the hands-on training classes in this course.

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