

BTEC 5414: Advance Plant Biotechnology
Fall 2020

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Office Hours are by Appointment

Time: Monday 5:00 pm – 7:20 pm and Wednesday 5:00 pm - 8:20 pm

Place: Experimental Sciences Building, Room 105

Purpose and Course Description: This course introduces students to advances in plant molecular biology and its applications in crop biotechnology. The laboratory part of this course focuses on vector construction, tissue culture, plant transformation and transgenic plant analysis.

Expected Learning Outcome: Students that successfully complete this course will: Have an advanced understanding of transgenic technology, plant genetics and their applications in biotechnology. Be knowledgeable of current laboratory techniques in this field.

Methods of Assessment of Learning Outcomes (Specific Course Requirements and Policies):

Learning outcome will be assessed through written examination and Lab reports. Participation in discussions and questioning during the class will also be used to assess learning outcomes outlined above. Course Assignments/Description of How Grades are Determined:

Participation/Attendance/pop quiz: 25%

Midterm: 25%

Final: 25%

Lab reports: 25%

Late Assignment Policy:

All assignments are considered late if they are not submitted on the assigned due date. For each day (not including weekends) that an assignment is late, a 10% deduction in the overall grade for that assignment will be enforced.

Grade Appeals:

Students initiating grade appeals should follow the official Grade Appeal Procedures outlined in the Graduate School catalog.

Attendance:

Lectures and labs will include information that is not in the assigned reading or handouts. It is therefore necessary and expected that you will attend and participate in every scheduled class and lab. There are no makeup classes or labs. If there is a reason for missing a class or a lab you must contact your instructor as soon as possible to make necessary arrangements to discuss the outcome of the absence. You may need to provide a note from your physician excusing your absence if you are absent from a class or a lab more than a day due to an illness.

Student Accessibility:

OP 34.22: Any student who, because of a disability, may require special arrangements in order to meet the course requirements should contact the instructor as soon as possible to make any necessary arrangements. Students should present appropriate verification from Student Disability Services during the instructor's office hours. Please note instructors are not allowed to provide classroom

accommodations to a student until appropriate verification from Student Disability Services has been provided. For additional information, you may contact the Student Disability Services office in 335 West Hall or 806-742-2405.

Academic Integrity:

It is the aim of the faculty of Texas Tech University to foster a spirit of complete honesty and a high standard of integrity. The attempt of students to present as their own any work not honestly performed is regarded by the faculty and administration as a most serious offense and renders the offenders liable to serious consequences, possibly suspension. "Scholastic dishonesty" includes, but not limited to, cheating, plagiarism, collusion, falsifying academic records, misrepresenting facts, and any act designed to give unfair academic advantage to the student or the attempt to commit such and act.

Cheating: Dishonesty in examinations, quizzes, or homework assignments, illegal possession of examinations, the use of unauthorized notes during an examination or quiz, obtaining information during an examination from the examination paper or otherwise from another student, assisting others to cheat, alteration of grade records, illegal entry to or unauthorized presence in an office are instances of cheating.

Plagiarism: Offering the work of another as one's own, without proper acknowledgement, is plagiarism; therefore any student who fails to give credit for quotations or an essentially identical expression of material taken from books, encyclopedias, magazines, internet web sites, and other reference works, or from the themes, reports or other writings of a fellow student is guilty of plagiarism.

Civility in the Classroom: Students are expected to assist in maintaining a classroom environment that is conducive to learning. In order to ensure that all students have an opportunity to gain from time spent in class, unless otherwise approved by the instructor students are prohibited from using cellular phones or beepers or engage in any other form of distraction. Inappropriate behavior in the class room will result in a request to leave the class.

Religious Holy Day:

By OP 34.19, a student who intends to observe a religious holy day should make that intention known in writing to the instructor prior to the absence. A student who is absent from classes for the observance of a religious holy day shall be allowed to take an examination or complete an assignment scheduled for that day within a reasonable time after the absence

TTU Resources for Discrimination, Harassment, and Sexual Violence:

Texas Tech University is committed to providing and strengthening an educational, working, and living environment where students, faculty, staff, and visitors are free from gender and/or sex discrimination of any kind. Sexual assault, discrimination, harassment, and other Title IX violations are not tolerated by the University. Report any incidents to the Office for Student Rights & Resolution, (806)-742-SAFE (7233) or file a report online at titleix.ttu.edu/students. Faculty and staff members at TTU are committed to connecting you to resources on campus. Some of these available resources are: TTU Student Counseling Center, 806-742-3674, <https://www.depts.ttu.edu/scc/> (Provides confidential support on campus.) TTU Student Counseling Center 24-hour Helpline, 806-742-5555, (Assists students who are experiencing a mental health or interpersonal violence crisis. If you call the helpline, you will speak with a mental health counselor.) Voice of Hope Lubbock Rape Crisis Center, 806-763-7273, voiceofhopelubbock.org (24-hour hotline that provides support for survivors of sexual violence.) The Risk, Intervention, Safety and Education (RISE) Office, 806-742-2110, rise.ttu.edu (Provides a range of resources and support options focused on prevention education and student wellness.) Texas Tech Police Department, 806-742-3931, <http://www.depts.ttu.edu/tttd/> (To report criminal activity that occurs on or near Texas Tech campus.)

Modules:

I. Plant Genomes

The Plant gene

The plant cell.

Structure and function of gene and regulatory elements.

The Plant Genome

structure Variations in plant genomes, polyploidy and complex genomes.

Plant Repetitive/Satellite DNA and Telomere repeat sequence.

Single Copy vs multiple copy sequences.

Regulation of gene expression

Transcription and translation.

Post-transcriptional and post-translational control.

II. Plant Tissue Culture

Introduction to plant tissue culture

Cell theory and cellular totipotency.

Tissue Culture

Infra-structure requirements and sterile techniques.

Media constituents, environmental effects.

Callus and suspension cultures.

Tissue Culture Methods

Organogenesis.

Somatic embryogenesis.

III. Molecular Biology Methods

DNA and RNA Manipulation, Amplification and Detection

Nucleic acid modifying Enzymes.

Blotting techniques.

Polymerase chain reaction (PCR).

Real time PCR and droplet PCR.

DNA Cloning Cloning and sub-cloning.

Cloning vectors.

Bacterial and yeast transformation methods.

Methods for recombinant screening.

Methods Bacterial selection and counter selections.

Site specific mutagenesis.

Sanger and automated Sanger sequencing.

Advanced DNA Cloning Advanced cloning and mutagenesis.

Gateway cloning.

Gibson cloning.

Golden Gate cloning.

DNA Cloning (Plant) Vectors for plant transformation.

Vector Design and Construction.

Promoters and selectable markers.

Epitope tagging.

Functional Analysis of plant genes

Forward Genetics Approach.

Chemical Mutagenesis and radiation Mutagenesis.

T-DNA and transposon mutagenesis.

Reverse Genetics Approach.

Over-expressing genes.

Gene silencing- RNAi, siRNA, amiRNA and Gene editing.

Detection of Protein-Protein Interactions

Yeast two-hybrid system.

Bimolecular fluorescence complementation (BiFC).

Co-immunoprecipitation and pull-down assays.

Visualizing Proteins Immuno-detection.

Fluorescence and Luciferase reporters.

Bioluminescence resonance energy transfer (BRET).

Fluorescence resonance energy transfer (FRET).

IV. Plant Transformation Methods

Introduction to Plant Transformation

History of genetic modification of plants.

Impact of biotechnology on plant agriculture.

Transgenic Plant Controversy.

Biological Methods

Agrobacterium tumefaciens-mediated transformation

Crown gall disease.

The biology of Agrobacterium.

Structure-Function of Ti Plasmid.

Explants Co-cultivation.

Vacuum Infiltration.

Floral Dip Inoculation.

Agro-drench.

Agro-infection.

V. Analysis of Transgenic plants

Transgene Copy Number and Organization

Regulations and Biosafety

Segregation analysis.

Southern blot analysis.

Quantitative and Droplet Digital PCR.

Identifying T-DNA flanking sequencing.

Analysis of Gene Expression

Northern Blot analysis.

RT-QPCR.

RNAseq analysis.

Protein Expression

Western blot analysis.

Phenotypic Analysis

Phenotyping for biotic, abiotic stress and hormonal response.

Field Testing of Transgenic Plants

Laboratory Exercises.

I. Tissue Culture

1. MS media preparation.
2. Tobacco and Arabidopsis seed serialization.
3. Plating seeds

II. Gateway cloning

1. Donor plasmid prep
2. LR reaction and E. coli transformation
3. Plasmid DNA extraction.
4. Screening for recombinants using PCR

III. Handling Agrobacterium

1. Competent cell preparation.
2. Agrobacterium transformation.
3. Screening using colony PCR.

IV. Plant transformation systems

4. Organogenesis- Tobacco. Lab. Exercise: (xanthi) transformation.
5. Floral dipping- Arabidopsis. Lab. Exercise (Colombia).

V. Analysis of transgenic plants

1. Segregation analysis Lab. Exercise-Kanamycin resistance.
2. Genotyping by PCR Lab. Exercise-Kanamycin gene.
3. Expression analysis using qRT-PCR. PCR- Lab. Exercise -Relative quantification.
4. Estimating copy number using qPCR- Lab. Exercise- Absolute quantification.

VI. Functional analysis of plant genes

1. T-DNA KO mutant analysis. Lab Exercise. Arabidopsis T-DNA KO genotyping.
2. Phenotyping Transgenic and T-DNA KO Arabidopsis plants for abiotic stress response.