Texas Tech University  
Center for Biotechnology and Genomics  

Fall 2019 Course Syllabus  

Course Number: BTEC 5338-001 (Lecture)  
BTEC 5338-501 (Lab)  
Course Name: Methods in Biotechnology  
Course Coordinator: J. N. Tripathy  
Title: Research Associate Professor  
Office: EXPSC Room 103  
Phone: (806) 834-1837  
Email: jatindra.n.tripathy@ttu.edu  

Course Schedule:  

<table>
<thead>
<tr>
<th>Lecture</th>
<th>Section 001</th>
<th>T</th>
<th>1:00-1:50</th>
<th>Room 120 ESB</th>
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<tbody>
<tr>
<td>Laboratory</td>
<td>Section 501</td>
<td>TR</td>
<td>2:00-4:50</td>
<td>Room 105 ESB</td>
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Instructors:  

Lecture  
Dr. Jatindra N. Tripathy  
jatindra.n.tripathy@ttu.edu  
(806)8341837  

Lab  
Dr. Xiaomei Shu  
Xiaomei.shu@ttu.edu  
(806)834 8760  
Miss Yinxue Li, TA  
Yinxue.li@ttu.edu  

Office hours are by appointment only.  

Course Description:  
This course is designed as an introductory lab course for biotechnology majors. The overall goal of this course is to equip student with a strong foundation in recombinant DNA technology including recombinant protein expression and characterization. The course is broadly divided into two parts: (1) Gene cloning and (2) Recombinant protein expression and characterization. Four experimental modules cover the entire course. Module I introduces the strategy and procedures to clone gene, Module II covers sub cloning, Module III covers over-expression of foreign gene in Escherichia coli, purification of recombinant protein from E.coli, and Module IV covers recombinant protein characterization.
**Required Texts:**


**Literature:**


(full text available at  http://www.jbc.org/content/273/47/30888.full.pdf+html)

The instructor as needed will provide reading assignments, additional materials, required experimental protocols and course announcements.

Students are expected to read assigned materials before attending class.

**Learning Outcome:**

The fully prepared student will be able to:

✓ Describe the various strategies used to clone a gene.
✓ Use various bioinformatic tools to carry out *in silico* analysis-
  • to design the gene construct.
  • to locate gene of interest in the genome.
  • to do restriction analysis in a DNA sequence to locate restriction site.
  • to find out the right ORF for cloning.
  • to analyze DNA sequencing data (chromatogram).
  • to translate gene sequence to amino acid sequence.

✓ Isolate ORF from the genome and clone it into a cloning vector.
✓ Sub-clone (transfer from one vector to another) the ORF to an expression vector.
✓ Isolate and purify plasmids and carry out restriction-digest analysis of the plasmids.
✓ Transform bacterial cells and screen transformed cells using antibiotic resistance.
✓ Screen the recombinant colony from a non-recombinant one.
✓ To carry out agarose gel electrophoresis to analyze the purity of DNA and estimate the size.
✓ To carry out SDS-PAGE analysis to analyze protein purity and to estimate molecular mass.
✓ Isolate recombinant proteins from bacterial cells and purify the proteins.
✓ Prepare protein purification table
✓ Describe the importance of purification table during protein purification.
✓ Measure DNA concentrations, protein concentrations and enzyme activities.
✓ Calculate specific activity, purification fold, and yield of the protein at various purification steps.
✓ Write and maintain a lab notebook.

Lecture/Laboratory Schedule:
Module 1: Gene Cloning (Week 1 to Week 5)
From gene to protein: An overview to the cloning, overexpression, and characterization of 3-alpha-HDR from *Comamonas testosteroni*.

Introduction to bioinformatics (in silico analysis of DNA sequence) Lab safety training and lab notebook maintenance, Preparation of different solutions (concentrated, percentage, molar).

Cloning strategies, T/A cloning, Polymerase Chain Reaction (PCR), Agarose Gel Electrophoresis, Transformation of *E. coli*, Blue-white screening of recombinants Plasmid miniprep and DNA quantitation DNA Sequencing.

Module II: Sub Cloning (Week 6 to Week 10)
Sub cloning strategies, expression vector selection, vector design and modification, Restriction digestion and analysis, colony PCR, recombinant screening.

Module III: Recombinant Protein Overexpression and Purification (Week 11 to 13)
Selection of expression host, mechanism of recombinant protein expression in *E.coli*. Cell lysis methods, epitope tagging.

Module IV: Recombinant Protein Characterization (Week 14, 15)
Immobilized Metal Affinity Chromatography, SDS-PAGE, Western blot Protein quantitation, enzyme activity assay and enzyme kinetics.

Attendance:
Lectures and labs will include information that is not in the assigned text 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.

Assessments and Grading:
There will be four criteria used to determine your final grade in this course. No other forms of assessment will be used and no “extra credit” will be considered. All assignments, exams, quizzes and notebook collections will be considered late if they are not submitted on the assigned date that they are due, and may receive a grade of “0.”
Exams: Two-hour exams and a comprehensive final will constitute 10%, 15% and 25% of the course grade respectively. These are tentatively scheduled for October 1 and November 5 and the final on December 7 (1:30-4:00 p.m.).

Lab Notebooks: You must keep a notebook. Each experiment (or tutorial) should be present in the notebook and all data and observations must be recorded during the lab- not copied over afterwards. Each should have a Title, a description of the Purpose of the experiment, a Description or Flow Diagram of the experimental procedures, a section for recording Results and a Summary and Discussion of the results. The Notebooks should be well-organized, and should reflect an understanding of the background principles, the purpose for the experimental techniques being used, and how the experimental procedures are linked to each other and the overall “flow” of the laboratory topic and project goals. The notebook will account for 30% of the final grade. Recording results on a piece of scrap paper while doing an experiment is completely unacceptable.

Quizzes: There will be unannounced quizzes during the semester, which will constitute 10% of the total grade. These will cover past material as well as expected reading prior to class.

Lab Participation: Active participation such as in-class and lab discussions, suggestions, and answering questions is strongly encouraged. Bench work in the lab is done in a group setting with two students in a group. Both members of the group should take equal responsibility to successfully complete lab experiments. Each person should be courteous and considerate of his/her lab partner and need to provide equal opportunity to follow lab protocols. Orderly conduct during the lab is expected at all times. Once the experiments are completed the chemicals, reagents, biological agents, and enzymes needed to be stored appropriately. All lab ware and bench surfaces should be cleaned before leaving the lab. Lab participation grade will be 10% of the total grade.

A final letter grade will be determined by performance on the above criteria, with consideration given to performance of the class as a whole. In prior years, the A/B cutoff for final grades has been around 90%, the B/C cutoff around 80%, the C/D cutoff around 70%, and the D/F cutoff around 65%. A grade of “I” (Incomplete) will be awarded by the instructor prior to the end of the semester only when failure to complete the work has been due to causes beyond the student’s control and when class performance has been satisfactory. Texas Tech regulations require that a form explaining the reason for the Incomplete and the method to be used to make up the missed work be submitted, after being signed by both the student and instructor, to the Registrar. Incomplete grades that are not replaced by an A, B or C grade within one year are automatically replaced by an F.

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/](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/ttpd/ (To report criminal activity that occurs on or near Texas Tech campus).