Texas Tech University
Center for Biotechnology and Genomics **Fall 2014**

BTEC 5338
Methods in Biotechnology

Meeting:	**Lecture**	**Section 001**	**M**	**1:00-1:50**	**Room 120 ESB**
	Laboratory	**Section 501**	**MW**	**2:00 - 4:50**	**Room 105 ESB**

Instructors: Office Hours are by Appointment

Dr. Jatindra N. Tripathy	**jatindra.n.tripathy@ttu.edu**	**834-1837**
Dr. Ruwanthi Wettasinghe	**ruwanthi.wettasinghe@ttu.edu**	**834-0973**
Dr. Susan San Francisco	**susan.san-francisco@ttu.edu**	**834-4752**
Ms. Vijay Twaritha	**twaritha.vijay@ttu.edu**	**Teaching Assistant**

Topic: Genome, Gene and Protein

Literature:

Mobus, E. and E. Maser. (1998). Molecular cloning, overexpression, and characterization of steroid-inducible 3α-hydroxysteroid dehydrogenase/carbonyl reductase from *Comamonas testosteronii*. J. Biol. Chem. 273(47): 30888-30896.

Oppermann, U.C.T., Filling, C., Berndt, K.D., Persson, B., Benach, J., Laderstein, R., and H. Jornvall. (1997). Active site directed mutagenesis of 3β/17β- hydroxysteroid dehydrogenase establishes differential effects on short-chain dehydrogenase reductase reactions. Biochemistry 36: 34-40.

Maser, E., Mobus, E., and G. Xiong. (2000). Functional expression, purification, and characterization of 3α-hydroxysteroid dehydrogenase/carbonyl reductase from *Comamonas testosteronii*. Biochem. Biophys. Res. Comm. 272: 622-628.

Required Texts:

Ninfa, A.J., Ballou, D.P., and Benore, M**.** *Fundamental Laboratory Approaches for Biochemistry and Biotechnology*, Second Edition, 2010, John Wiley and Sons.

Brown, T.A.. *Gene Cloning & DNA Analysis, An Introduction*, Sixth Edition, 2010, Wiley-Blackwell

Students are expected to read assigned materials before attending class.

Course Description:

This course is broadly divided into two parts: Gene cloning and Recombinant Protein expression and characterization. Each part has two modules. Module I and II in gene cloning covers cloning and subcloning of a foreign gene. Module III, IV and V cover recombinant protein over expression, purification and characterization respectively.

Reading assignments, additional materials, required experimental protocols and course announcements will be provided by the instructor as needed.

Lecture/Laboratory Schedule:

Introduction:	From clone to protein: An overview to the cloning, overexpression, and characterizationof 3-alpha-HDR from *Comamonas testosteroni*
	Introduction to bioinformatics
	Lab safety training and lab notebook maintenance
	Reagents and Buffers
Cloning:	Cloning strategies – T/A cloning
	Polymerase Chain Reaction (PCR)
	Electrophoresis
	Transformation of *E. coli*
	Blue-white screening of recombinants
	Plasmid miniprep and DNA quantitation
	DNA Sequencing
Subcloning:	Subcloning strategies, vector selection, design and modification
	Restriction digestion
	Colony PCR
Protein Overexpression:	Selection of host, mechanism of recombinant protein expression in *E.coli.*
Protein Purification:	Cell lysis methods, Concept of Epitope tagging (IMAC)
Protein characterization:	Analysis of proteins by SDS-PAGE
	Analysis of proteins by Western technique
	Protein Identification methods
	Protein quantitation
	Enzyme activity and 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.**

Special Conditions:
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 (AccessTECH). No requirement exists that accommodations be made prior to completion of this approved university process.

Religious Holy Day:
A student who intends to observe a religious holy day should make that intention known 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.

Assessments and Grading:
There will be five 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."

1. Exams: Two-hour exams and a **comprehensive final** will constitute **10%, 15%** and **25%** to the course grade respectively. These are tentatively scheduled for October 6 and November 10 and the final on December 9 (1:30-4:00 p.m.).

2. Lab Notebooks: You must keep a notebook. Each experiment (or tutorial) should be represented 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.**

3. 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.

4. Lab Participation: Active participation such as 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

labware 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.

Learning Outcome:
The fully prepared student will: Be able to use various bioinformatic tools to locate genes of interest; will be able to design constructs that can be used to over-express proteins; will be able to isolate and purify these plasmids and carry out restriction-digest analysis of the purified plasmids; will be able to transform bacterial cells and screen for transformed cells using antibiotic resistance; will be able to isolate DNA and analyze sequencing data; will be able to release expressed proteins from bacterial cells and purify the proteins using a variety of chromatographic techniques; will be able use gel electrophoresis to monitor DNA and protein purity and to estimate their molecular masses; will be able to measure DNA and protein concentrations and measure enzyme activities so that specific activity can be calculated.

Academic Honesty: 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. Students who are assigned a grade of F in a course because of academic dishonesty, will have the reason for this grade assignment noted on their academic transcripts.