

**Colorado State University Department of Biochemistry**  
**BC 404 Comprehensive Biochemistry Lab**  
Fall Semester 2017

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Office Hours: Monday 2:00 – 4:00PM
- Teaching Assistant: Tyler Biebighauser  
Yates 307  
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Office hours: Wednesday 4-5PM in Yates 307/310
- Schedule: 307 and 308 Yates, TR 2:00-4:50PM
- Textbook: Laboratory Notebook (in the bookstore under BC404; \$13.50)  
**A USB flashdrive**
- Course objectives: **Objective #1:** *Students will be exposed to a laboratory that closely mimics a research laboratory setting and mentality.*
- Objective #2:** *Students will be able to plan, execute, and problem solve common molecular biology and protein chemistry techniques including: PCR, agarose and polyacrylamide electrophoresis, bacterial transformation, vector and PCR restriction digestion and ligation, protein expression, protein purification, qRT-PCR, enzymatic characterization, site-directed mutagenesis.*
- Objective #3:** *Students will understand the underpinning theory and experimental design for the experimental techniques listed above leading to the transfer of such knowledge in future research that the student may perform.*
- Objective #4:** *Students will demonstrate proficiency in working with a partner to plan and direct a “mini” research project.*
- Canvas: I will use this site to post all the protocols and procedures you will be using to successfully execute the biochemical technique. I also plan to use this site to post your current grade and lecture notes.

Class Structure: What can you expect in the next 16 weeks?

1. 1<sup>st</sup> 8 weeks:
  - a. This portion of the class is designed to:
    - i. Place our gene of interest into a high yield expression vector, express and enzymatically characterize the protein.
    - ii. Help you practice the skills that will be necessary for you to succeed in the second portion of the class.
2. The last 8 weeks.
  - a. In this portion of the lab you will design a point mutant that will destroy, or enhance (if you're really good) some aspect of the protein's function. We will then assess your success using kinetics and possibly other biophysical techniques.
3. Lectures
  - a. Remember a portion of the objectives is to teach you the theory of the biochemical techniques you are performing. This will help you problem solve and ensure the success of your experiments.

Assessment: Your grade will be derived from a number of places some of which is wrapped up in your ability to perform the experiments.

**1. Exam- 100 points**

- a. The exam will be a comprehensive take home exam that will test your knowledge gained in both lectures and the lab.

**2. Problem Sets-180 points**

- a. Problem sets are designed to test your knowledge of theory for particular techniques as well as your skill in working with data and designing experiments.

**3. Project – 100 points**

- a. The Project points will be comprised of 3 portions:
  - i. Design – 40 points
  - ii. Execution/Participation – 35 points
    1. These points are a protection for a person whose partner “slacks off” during this portion of the lab. Poor participation and attendance during this period will result in strict penalties.
  - iii. Write-up – 25 points

**4. Notebooks- 50 points**

- a. 25 of these points will be given when you hand-in your completed notebook at the end of the semester. Points will be given for:
  - i. Whether it is complete or not including, written objectives, methods (“a diagram of the setup with sufficient detail for reproducibility”, results (data

- including graphs, gel images, etc.), and conclusions for each day in lab.
  - ii. Legibility and organization.
  - iii. Line of reasoning for your conclusions based on results.
  - iv. Dates consistent with the course layout. In other words, it was being completed as you worked, not at the end of the semester.
- b. 25 of these points will be given in at least two lab notebook checks. KEEP THEM UPDATED and you won't lose points. The following criteria will be assessed when the notebooks are collected.
- i. 2.5 points for written objectives of all the labs from the last check up to the current lab (that day).
  - ii. 2.5 points for written methods of all the labs from the last check up to the current lab (that day).
  - iii. 2.5 points for written results of all the labs from the last check up to the last lab fully executed.
  - iv. 2.5 points for written conclusion of all the labs from the last check up to the last lab fully executed.
  - v. 2.5 points for legibility and organization.
  - vi. There will not be any partial credit for these points. Either you get the 2.5 points or you don't. For example if you have all the purposes for the labs written except one then you lose 2.5 points.

**5. Lab participation – 20 points**

- a. Unexcused absences will result in automatic deduction of at least half of these points, no questions asked.
  - i. Multiply occurrences will result in additional points being deducted.
- b. Poor effort resulting in “lousy” data or slow progress will result in points being lost.
- c. Poor organization resulting in loss of samples will result in points being lost.

To be Successful: Here is a short-list of ways to be successful:

1. Come prepared knowing exactly what you are going to do and have your notebook prepared to take down data.
  - a. LISTEN at the beginning of class for changes and additional instructions.
2. Be careful in the way you proceed, do not rush through experiments.
  - a. Rushed science is usually bad science.
3. Carefully label your reagents and your products. Make sure you know where you have stored them.

- a. Never throw away something unless you are sure you don't need it. Each lab procedure will tell you what to keep and what to throw away upon the completion of the lab.
4. Talk to your fellow classmates about things you are confused about.

Grades: The following is a complete breakdown of point accumulation:

<u>Assignment</u>	<u>Points</u>
Exam (1)	100
Notebooks	50
Problem sets	180
Project	100
<u>Lab participation</u>	<u>20</u>
<b>Total</b>	<b>450</b>

<u>Grade</u>	<u>Percentage</u>
A+	97-100%
A	90 - < 97%
B+	87 - < 90%
B	80 - < 87%
C+	77 - < 80%
C	70 - < 77%
D	60 - < 70%
F	below 60%

What this means is that you are guaranteed at least those grades if you have those percentages. A curve may or may not apply to this class. I have, in the past, used a minus policy and reserve the right to do so depending on the distribution of the grades.

Attendance: Attendance to every class is mandatory for each experiment and the lectures. You must perform the experiments with your partner or you will start to lose points. There are obvious exceptions to this rule like a death in the family, extreme illness or a University excused absence. Missing class because you need to take a nap is not acceptable. If you need to miss a class come and see me and we can talk about whether it is a valid excuse and how we can arrange to make it right. In the case of an emergency or a tragedy deal with it first and then come see me.

Lab Safety: Some general policies regarding lab safety:

- 1) No open toed shoes (shorts are fine).
- 2) No “horse-play” in the lab.
- 3) No eating or drinking in the lab.
- 4) Place Backpacks on racks.

Academic Integrity: This course will adhere to the Academic Integrity Policy found in the Colorado State University [General Catalog](#).

End of the Semester: **I WILL NOT NEGOTIATE GRADES AT THE END OF THE SEMESTER.** It is my expectation that you will accept the grade assigned to you and take responsibility for YOUR work throughout the semester. Grade negotiation always leads to someone receiving special treatment and is a policy that I cannot abide as I desire to maintain an atmosphere of academic honesty and integrity. If you are concerned about your grade please come and talk to me **DURING** the semester when something **can be done** about it.

The Class Schedule: Below is a class schedule detailing each day and the experiment we will be running on that particular day.

BC404-FA17 Daily Schedule – Subject to change	
Date	Experiment/Schedule
8/22	Lab Check-in, Introduction Lecture 1- Introduction to Subcloning and LDH
8/24	Cloning: PCR amplify LDH/Pipet Check Lecture 2 – Subcloning PCR and Following
8/29	Cloning: PCR gel/Restriction enzyme digest Lecture 3 - Electrophoresis
8/31	Cloning: Digest Purification
9/5	Cloning: Ligation/Transformation Lecture 4 – Transformation and Plasmid Design
9/7	Cloning: DNA purification/ DNA sequencing sample
9/12	Cloning: Sequence analysis Lecture 5 – DNA Sequencing

9/14	<b>Protein Expression: BL-21 pLysS transformation</b> <b>Lecture 6 – <i>E. coli</i> Expression of a Foreign Protein</b>
9/19	<b>Protein Expression: Overnights</b> <b>Lecture 7 – qRT-PCR</b>
9/21	<b>Protein Expression: Expression Harvest</b> <b>qRT-PCR: RNA isolation/Quantitation</b> <b>qRT-PCR: cDNA synthesis</b>
9/26	<b>qRT-PCR: qRT-PCR experiment</b>
9/28	<b>Lecture 8 – qRT-PCR data analysis/Protein Purification</b> <b>Introduction to Chromatography Systems</b>
10/3	<b>Protein Purification: Ni-affinity column</b>
10/5	<b>Lecture 9 – Enzyme Kinetics</b> <b>Enzyme Kinetics</b>
10/10	<b>Pour SDS-PAGE gel</b> <b>Lecture 10 – Introduction to Student Project/Pymol</b>
10/12	<b>SDS-PAGE Gel</b> <b>Lecture 11 – Kinetics analysis/SDS-PAGE electrophoresis</b>
10/17	<b>Protein Quantitation</b> <b>Lecture 12 – Protein Quantitation</b> <i>Project Primers DUE</i>
10/19	<i>Project DUE</i>
10/24 – 12/5	<b>Your Project</b>
12/7	<b>Hand-in exam/notebook/project write-up</b> <b>Clean up and checkout</b>