**BIOCHEMISTRY AND MOLECULAR BIOLOGY 442**
**DNA MODULE – FALL 2018**

**Instructor:**
Heather Giebink, 101C Life Sciences Building, 814-867-0366, hug14@psu.edu

**Lecture:**
Friday 9:05AM - 9:55AM, Location 113 Carnegie

**Office Hours:** Always available by appointment through Starfish or Email. You are also free to stop into any lab section; however you may need to wait for me to become available. Drop-In Office Hours will be posted on Canvas and Starfish each Sunday evening.

**Lab Location:** 154 N. Frear

**Lab Sections:**
Section 1: Wednesday 8:30am-12:00pm**
Section 2: Thursday 8:30am-12:00pm**
Section 3: Thursday 1:35-5:35pm
Section 4: Monday 2:30-6:30pm
****Note:** *In the schedule of courses the class has a start time of 8:00am. However, most of the labs can be completed in 3.5 hours so the Wednesday/Thursday AM section will have a start time of 8:30am unless otherwise indicated.*

**TAs:**
Section 1: McCauley Meyer (mxm1357@psu.edu)
Section 2: Victoria Bonnell (vab18@psu.edu)
Section 3: Paige Teehan (pdt7@psu.edu)
Section 4: Nicholas Schultheis (nys5272@psu.edu)

**Prerequisites:** You are expected to have successfully completed Chem 202 or 210, AND EITHER BMB 251, BIOL 230W, or MICRB 201. It is to your advantage to have taken or be currently enrolled in BMB 211 or 401. Being able to handle dilutions and mathematic equations is helpful.

**Required Materials**
**Lab Manual:** You will need to purchase the lab manual from the Student Bookstore at 330 E. College Avenue. A .pdf of the first week's lab will be posted on Canvas to give you time to purchase the manual, but please purchase as soon as possible. .pdfs of the following labs will NOT be posted. The Student Bookstore only keeps a limited number of copies on hand, and if they run out you need to let them know that you need one so they can order more.

**Notebook:** You will need to purchase (or use leftover pages from a previously purchased) duplicate page lab notebook for this course. In this notebook you will need to write out your protocols for each lab in this notebook, and will hand in a copy of the notebook pages to your TA before leaving lab each week.

**Lab Dress Code and Safety Considerations:** You are required to wear closed toe shoes to lab. Safety goggles and a *limited number* of lab jackets are available for your use in the lab. Do NOT bring any food or drink into the lab. You are also expected to clean and return all appropriate supplies to the lab cubbies and dispose of all waste in an appropriate fashion. General disposal guidelines are provided for you in your lab manual, but please ask if you have any questions.

**LAB SCHEDULE**

| Week of | Experiment | Assignments Due |
|---|---|---|
| August 20th | Introduction to Lab and Pipetting Practicum | |
| August 27th | Experiment 1: Isolation of Genomic DNA | |
| September 3rd | No Labs Due to Labor Day | Lab Report 1 |
| September 10th | Experiment 2.1: PCR | |
| September 17th | Experiment 2.2: Analysis of PCR by Agarose Gel Electrophoresis | |
| September 24th | Experiment 3.1: Making a Recombinant Plasmid: Plasmids, Restriction Enzymes, and Ligation | Lab Report 2 |
| October 1st | Experiment 3.2: Making a Recombinant Plasmid: Competent Cells and Transformation | Draft Lab Report 3 |
| October 8th | Experiment 3.3: Making a Recombinant Plasmid: Alkaline Lysis Mini-Prep and Analysis of Recombinant Plasmids | |
| October 15th | Protein Module Begins | Lab Report 3 |

**LECTURE SCHEDULE**

| Date | Lecture Topics | Assignments Due |
|---|---|---|
| August 24th | • DNA Isolation techniques<br>• Use of CFUs to determine number of bacteria in a sample<br>• Using Absorbance to Assess DNA Concentration and Purity | Pre-lecture Quiz |
| August 31st | • No Lecture Due to Labor Day | |
| September 7th | • Polymerase Chain Reaction | Pre-lecture Quiz, Worksheet 1 |
| September 14th | • Agarose Gel Electrophoresis | Pre-lecture Quiz, Worksheet 2 |
| September 21st | • DNA Plasmids<br>• Cutting and Pasting: Restriction Enzymes and DNA Ligase | Pre-lecture Quiz, Worksheet 3 |
| September 28th | • Competent Bacteria and DNA Transformation | Pre-lecture Quiz, Worksheet 4 |
| October 5th | • DNA Plasmid Isolation (Mini Preps) and Clone Analysis | Pre-lecture Quiz, Worksheet 5 |
| October 12th | • Protein Module Begins | Worksheet 6 |

## OTHER IMPORTANT DATES

1. **Review Session: Sunday, October 21st: Location and Time TBD**

2. **DNA Exam : Tuesday, October 23rd: 7:45 – 9:45 pm, 101 Thomas**
   - Exam will cover all of the material covered in the DNA module of this lab course.

**Laboratory Reports**…… 150 points total**

| | |
|---|---|
| *Genomic DNA Isolation and Analysis* | ***30 pts*** |
| *PCR and Agarose Gel Electrophoresis* | ***45pts*** |
| *Recombinant DNA Plasmids* | ***75 pts*** |

**Draft Lab Report 3 …… 10 bonus points**

**Pipetting Skills Practicum and Calculation Challenge …….. 15 points**

**Exam…… 100 points**

**Worksheets***…… 10-15 points each**

**Pre-Lecture Canvas Quizzes****……. 3-5 points each**

**Duplicate Page Lab Notebook/TA Evaluation*****…..…….…… 15 points**

***Grade from DNA module will be 50% of final grade for the course. See section below for grade penalties due to absences, late submissions or collaborative work.**

**** The laboratory reports will be graded by the TAs based on the General Guidelines and Lab Report Guidelines provided at the end of each experiment. If you have a question about your grade, you must first see your TA. If you still disagree with the grade after meeting with your TA, you may fill out a lab report grade appeal form, and submit the completed form to Dr. Giebink for her to review.**

***** Worksheets will be handed out in lecture and uploaded to Canvas. They must be completed and handed in at the beginning of the next lecture. Answer keys will be posted following grading.**

******Pre-Lecture Quizzes will be posted on Canvas. In order to complete these quizzes you will need to read the material in the lab manual, listen to the online lecture(s), complete an online module, and then answer questions on the Canvas quiz. You only have 10 minutes to take the quiz so it is essential to take notes during the lecture.**

******* See your lab manual for guidelines as to what needs to be included in your duplicate page notebook. You will be expected to get your notebook pages signed and then hand in the duplicate copies at the end of each lab period.**

# Grade Penalties

There are grade penalties for each of the following situations:

1) **Late submission of lab reports – 5% per day (max of 5 days allowed) After the 5 days have passed you will receive a 0.**
    a. Type written lab reports must be uploaded to Canvas at the **BEGINNING** of your scheduled lab period (unless otherwise noted). The grade of those received after this time will be reduced by 5% of their total value per day (or part of day) of late submission up to a maximum of 25% (each weekend day also counts). Permission must be sought (and will only be granted on a case to case basis due to medical or otherwise unavoidable situations) from the instructor in order to turn in a lab report more than 5 days after the due date. Lab reports not turned in after the 5 days will result in a 0.

2) **Late submission of worksheets – 100% deduction if turned in after lecture begins.**
    a. Worksheets are meant to be completed both in lecture and during your incubation periods in lab. You may work with your classmates to complete the worksheets. The problems in the worksheet are designed to assist you in data analysis, writing your lab reports, and practice for the exam.

3) **Late submission of Pre-Lecture Quizzes - 100% deduction for Canvas Quizzes**
    a. Pre-Lecture Quizzes will be completed on Canvas, and the Canvas settings will be used to control access and submission dates. Late submission of Canvas quizzes will result in a 0.

4) **Unexcused absence from lab – 10 point deduction from cumulative lab report score**
    a. An unexcused absence from a lab will result in a 10 pt. deduction from your cumulative lab report grade. **If you have more than 3 unexcused absences it will result in a grade of an F for the DNA module.** See attendance section for more explanation of excused and unexcused absence.

5) **Collaborative work / minor plagiarism on lab reports**
    a. Each student must write their report **independently** (i.e. own graphs, text, style, layout, etc.). Lab reports which are deemed to be collaborative efforts based on qualities such as having the same style, graphs, fonts, text, placement, or sentence order will have up to 25% of the total possible points deducted.

6) **Major case of plagiarism on lab reports – score of 0 on lab report**
    a. Severe cases of plagiarism (in which 25% or more of the document is copied directly from another source) will result in a score of 0 for that assignment and will be reported as an academic integrity violation (see paragraph below for link with PSU policy).

## EXPECTATIONS

**ATTENDANCE:** Attendance is required for both lecture and lab. **Students should bring their lab manuals to all lecture sessions.** *Excused* absences may be made up during another laboratory session that same week (see beginning of syllabus for times), or during the pre-determined make up lab time. However, you must contact Dr. Giebink 867-0366 or hug14@psu.edu **PRIOR** to the **START** of your lab section if your absence is to be excused. If prior approval was not obtained and lab was missed, documentation explaining the circumstances of your absence is required. See the grade penalty section for consequence of unexcused absences.

If you miss a lecture for an unexcused or excused absence you are still responsible for all information presented. While .ppt slides will be posted on Canvas, theses slide do NOT contain all information that you are expected to know. I expect you to take notes on top of theses slides, and as well, there may be times in which I use the board or overhead to draw flow charts or to present practice problems. Any assignments due in lab or lecture must be turned in on time (in person or by email to the instructor) regardless of whether you have an excused absence or not. For any assignments turned in by email (in the case of an absence), it is the student's responsibility to make sure the document is formatted correctly, and readable.

**LECTURE:** I hope that the 50 min lecture session on Fridays will be both an informative and an interactive environment. My goal is to present background information that allows you to develop a basis for understanding the techniques that we will be using in the lab as well as to practice your analytical skills with data prediction and/or analysis of example data. **In order to get the most out of these 50 minute sessions, I expect students to come to class having already read the introduction for that week's lab as well as having completed the Pre-Lecture Quizzes and listened to the online lectures.** The online lectures will be used to present background information on the reagents or laboratory techniques to allow more time in lecture to be devoted to practicing analytical skills. The material in the online lectures will be tested on the exam.

**LAB:** During this session you will be required to complete the experimental protocols for the week. Some weeks this will take the entire time, while others it may not. However, **each week, you are expected to come to lab having already read the procedure from beginning to end in order to have an overview in your mind of the steps that you will be doing that day**. Many of the protocols are very detailed and mistakes are easy to make if you do not come prepared, focused, and with the overall experimental goals in mind.

The beginning of your 4 hour laboratory session will often be used to further explain or re-iterate important theoretical concepts, so **PLEASE COME ON TIME**. Late arrival to lab is not only disruptive, but it almost invariably results in mistakes being made due to missing key instructions. Repeat tardiness will be reflected in your grade through a deduction in the TA evaluation points.

**LAB CLEAN UP PROCEDURES: A portion of TA points will be awarded based on your adherence to safety protocols and proper clean up at the end of the lab. The following outlines the general expectations for clean up at the end of the lab period.**

1) Follow the general disposal guidelines below when cleaning up at the end of each lab period. If you are unsure of how to properly dispose of something, ask your TA.

2) Make sure to clean and return all appropriate supplies to your cubby (check the numbers to make sure you are putting supplies away in proper place!)

3) Wipe down your bench space with disinfectant and a wet paper towel before leaving.

4) If you have tubes or flasks that are being set on a cart to be autoclaved, please make sure to take ALL tape off of the tubes (including tape that was put on the flask by the prep room to indicate contents).

## SIMPLE TIPS FOR SUCCESS

1. Come to lecture and lab **ON TIME**. Not only can a late arrival to lab be reflected in your grade for the course, but inevitably it is the students that come late to lab that end of making mistakes that cause them to have to repeat steps or lose points on their lab reports.

2. Ask questions. Please ask questions during lecture or pre-lab introductions if there is something that is not clear to you. During a lab procedure, if you are unsure of how to proceed, ask your TA or instructor for clarification.

3. Take notes! There will be times either in lecture or in the pre-lab introduction that I will present material that is necessary to complete your lab reports.

4. Engage with the material – you will be given a number of worksheets and/or sample problems to help you better understand the material as well as increase your ability and confidence with data analysis.

5. Come and see me. If something is not making sense, unclear, or if you want to discuss your interpretations of your data, come and see me! Your TA is also available outside of class to assist you (within reason, please respect their designated office hours. They are taking classes, teaching, and performing their own research.)

6. The Eberly College of Science is committed to the academic success of students enrolled in the College's courses and undergraduate programs. When in need of help, students can utilize various College and University- wide resources for learning assistance. Visit: http://www.science.psu.edu/advising/success//

**ACADEMIC INTEGRITY**

All Penn State Policies regarding ethics and honorable behavior apply to this course. Academic dishonesty is not limited to simply cheating on an exam or assignment. The following is quoted directly from the "PSU Faculty Senate Policies for Students" regarding academic integrity and academic dishonesty: "Academic dishonesty includes, but is not limited to, cheating, plagiarizing, fabricating of information or citations, facilitating acts of academic dishonesty by others, having unauthorized possession of examinations, submitting work of another person or work previously used without informing the instructor, or tampering with the academic work of other students." Each student in this course is expected to work entirely on her/his own while taking any exam, to complete assignments on her/his own effort without the assistance of others unless directed otherwise by the instructor, and to abide by University and Eberly College of Science policies about academic integrity and academic dishonesty. Academic dishonesty can result in assignment of "F" by the course instructors or "XF" by Judicial Affairs as the final grade for the student. Refer to the following URL for further details: http://science.psu.edu/current-students/Integrity/Policy.html

**STUDENTS WITH DISABILITIES**

Penn State welcomes students with disabilities into the University's educational programs. If you have a disability-related need for reasonable academic adjustments in this course, contact the Office for Disability Services (ODS) at 814-863-1807 (V/TTY). For further information regarding ODS, please visit the Office for Disability Services Web site at http://equity.psu.edu/ods/. In order to receive consideration for course accommodations, you must contact ODS and provide documentation (see the documentation guidelines at http://equity.psu.edu/ods/guidelines/documentation-guidelines). If the documentation supports the need for academic adjustments, ODS will provide a letter identifying appropriate academic adjustments. Please share this letter and discuss the adjustments with your instructor as early in the course as possible. You must contact ODS and request academic adjustment letters at the beginning of each semester.

**CODE OF MUTUAL RESPECT AND COOPERATION**

The Eberly College of Science Code of Mutual Respect and Cooperation (http://science.psu.edu/climate/code-of-mutual-respect-and-cooperation/Code-of-Mutual-Respect%20final.pdf) embodies the values that we hope our faculty, staff, and students possess and will endorse to make The Eberly College of Science a place where every individual feels respected and valued, as well as challenged and rewarded.

**EDUCATIONAL EQUITY**

Penn State takes great pride to foster a diverse and inclusive environment for students, faculty, and staff. Acts of intolerance, discrimination, or harassment due to age, ancestry, color, disability, gender, gender identity, national origin, race, religious belief, sexual orientation, or veteran status are not tolerated and can be reported through Educational Equity via the Report Bias webpage.

**COUSELING AND PSYCHOLOGICAL SERVICES**
Many students at Penn State face personal challenges or have psychological needs that may interfere with interfere with their academic progress, social development, or emotional wellbeing. The university offers a variety of confidential services to help you through difficult times, including individual and group counseling, crisis intervention, consultations, online chats, and mental health screenings. These services are provided by staff who welcome all students and embrace a philosophy respectful of clients' cultural and religious backgrounds, and sensitive to differences in race, ability, gender identity and sexual orientation.

Counseling and Psychological Services at University Park (CAPS): 814-863-0395
Counseling and Psychological Services at Commonwealth Campuses
Penn State Crisis Line (24 hours/7 days/week): 877-229-6400
Crisis Text Line (24 hours/7 days/week): Text LIONS to 741741

## COURSE GOALS AND OBJECTIVES

**Hint: Use the learning objectives presented under the "analytical skills" section and the "theory" section as a study guide to make sure that you are prepared for the exam.**

What do I hope that you will take away from this class?
1) An increased ability to think analytically and critically
2) An appreciation and understanding of the theory behind a number of different basic molecular biology techniques
3) A set of technical skills that are basic tenets of laboratory work in the field of molecular biology.

To put it simply, in this class we will focus on both your "thinking" and your "doing skills". I have listed the learning objectives for the course below, and as you will see I have broken them up into three areas: 1) Technical Skills, 2) Analytical Skills, and 3) Experimental Theory (i.e. Learning Objectives for each lecture).

**Technical Skills:**
1) Display how to properly use micropipettes, and become comfortable using the pipettes with accuracy and precision even when pipetting small volumes such as 1 µl.
2) Isolate and assess the concentration and purity of genomic and plasmid DNA.
3) Display proper technique to plate and grow bacteria on solid media, as well as how to take bacteria from a single colony and grow those bacteria in liquid media.
4) Make, load, run, and visualize DNA samples on an agarose gel.
5) Calculate the number of live bacteria in a bacterial culture using serial dilutions.
6) Program and operate the Genesys 5 spectrophotometers in order to measure absorbance of samples at different wavelengths.
7) Demonstrate an ability to concentrate and focus in order to correctly set up multiple reactions with each one having multiple components (i.e. PCR samples, restriction digests, ligation reactions).

**Analytical/Critical Thinking/Problem Solving Skills:** While learning experimental/technical skills in molecular biology is a desired outcome of this class, an equally (if not more) important goal is to practice using and further develop your analytical skills. Regardless of the future path that you choose, the ability to think analytically in a variety of different situations will be a huge asset to both your personal and professional development. Below is a list of examples of how you will be expected to use analytical skills in this class. These skills will be assessed in lab reports as well as on your exam for the DNA portion of this class.

1) Describe experimental data verbally and in written form (i.e. gel pictures, or tables of numerical values)
2) Interpret results from agarose gels in order to assess purity, estimated concentration, and estimated size of DNA samples
3) Identify and evaluate appropriate positive or negative controls for an experiment.
4) Predict expected results of experiments based on understanding the theory behind those experiments.
5) Diagram experimental protocols in flow charts.
6) Formulate conclusions based on data sets.
7) Organize data into descriptive tables and graphs.
8) Solve mathematical problems that allow you to set up experiments (i.e. use C1V1 = C2V2) and analyze your data (i.e., calculate and properly use dilution factors; calculate DNA concentration by using a conversion factor based on Beer's Law; calculate yield and efficiency of a PCR reaction; estimate the size of a DNA fragment run on an agarose gel based on a linear regression of known standards; calculate efficiency of bacterial transformation reaction; calculate amounts of unknowns based on using a standard curve).
9) Calculate percent error and standard deviation and be able to discuss sources of error.

**Experimental Theory:** Below is a list of learning objectives for each week, along with one learning objective that you will see is pervasive across the course and applies to the lectures given every week.

<u>Every Week:</u>

1) State the purpose of the solutions and manipulations used in all experimental protocols.

<u>Lab 1: Genomic DNA Isolation</u>

1) Define "genome" and understand the difference between genomic, chromosomal, and plasmid/extrachromosomal DNA.
2) Recall the basic structure of the *E. coli* membrane.
3) Diagram and describe the 4 main steps to isolating genomic DNA from *E. coli*.
4) State the purposes of the solutions and/or physical manipulations in the protocol.
5) Predict how changing the concentration of a reagent or missing a step will impact the quantity or quality of the isolated genomic DNA.
6) Describe how chloroform/isoamyl alcohol phase separation isolates nucleic acids from other molecules in the cell.
7) Explain how the addition of ethanol to a solution of DNA results in the precipitation of DNA.

8) Calculate the concentration of double-stranded DNA using Beer's law.
9) Assess the purity of a DNA sample using A260:A280 ratios.
10) Explain why a low A260:A280 ratio can be indicative of protein contamination and why a high A260:A280 ratio can be indicative of RNA contamination.
11) Evaluate given results of a hypothetical genomic DNA preparation and be able to hypothesize where the experiment failed and how it should be corrected or repeated.
12) Calculate the number of live bacteria in a bacterial culture using serial dilutions.

Lab 2 – Part I: Polymerase Chain Reaction

1) Compare and contrast the basic requirements of an "in vivo" DNA replication reaction with the in vitro replication that occurs during PCR.
2) Explain the mechanism and required components for DNA polymerase activity (i.e. the creation of a phosphodiester bond).
3) Summarize the three steps of PCR, including the temperature, reaction, and length of each step as well as why/how those conditions are chosen.
4) Design PCR primers to amplify a desired DNA molecule.
5) Create a PCR protocol to amplify a desired DNA molecule.
6) Calculate PCR efficiency and use that value to assess your PCR. Propose a modification to a PCR protocol to increase efficiency.

Lab 2 – Part II: Agarose Gel Electrophoresis

1) Define agarose and demonstrate or illustrate how to pour an agarose gel.
2) Explain how DNA is separated by an agarose gel and how the percentage of agarose affects separation.
3) Recall how Ethidium bromide (EtBr) allows the visualization DNA.
4) Discuss the common problems associated with pouring/setting up an agarose gel and how they can affect your results.
5) Identify and describe the different forms (topoisomers) of a plasmid.
6) Diagram how different sizes (base-pair length) and topoisomers of DNA will run on an agarose gel in comparison to each other and in comparison to a standard DNA ladder.
7) Estimate the size (base-pair length) and mass (g) of experimental DNA molecules in comparison to a set of standards.

Lab 3 – Part I: Restriction Digest and DNA Ligation

1) List and describe the important features of a cloning plasmid.
2) Identify important features of a cloning plasmid on a plasmid map.
3) Explain the reaction that is catalyzed by restriction enzymes.
4) Predict the results of a restriction digest of a given plasmid and draw the bands as they would appear on an agarose gel (i.e. as undigested, complete, incomplete, or partial digest).
5) Explain the difference between "blunt" and "sticky" ends and recall what types of ends will ligate with each other.
6) Summarize the reaction mechanism of DNA ligase.

7) Develop a protocol to construct a recombinant DNA molecule using restriction enzymes and DNA ligase.
8) Compare and contrast non-directional and directional cloning and defend which one is preferred based on the given situation.

Lab 3 – Part II: Making Competent Cells and Transformation Reactions

1) Compare and contrast the benefits and disadvantages of gel purification in a cloning procedure.
2) Define transformation and competence.
3) Choose and interpret appropriate positive or negative controls for transformation reactions.
4) Evaluate given results of a hypothetical transformation reactions and be able to hypothesize where the experiment failed and how it should be corrected or repeated.
5) Describe how ampicillin and kanamycin function as antibiotics.
6) Describe how antibiotics select for bacteria containing specific plasmids.
7) Describe three ways bacteria fight against antibiotics.
8) Classify the ampicillin resistance, kanamycin resistance genes, or a novel antibiotic into the appropriate resistance mechanism category.
9) Describe and interpret ligation reactions run on agarose gels.

Lab 3 – Part III: Plasmid Purification and Analysis

1) Recall and describe the manipulations that allow you to purify plasmid DNA away from genomic DNA. (i.e. know all solutions in the mini-prep kit, what they do and how genomic DNA, proteins, and RNA is removed from the plasmid DNA)
2) Describe what topological form high quality, purified plasmid DNA should be in.
3) Explain how researchers can decrease the quality or plasmid DNA during the purification process.
4) Describe in words and diagram the differences between how high quality and damaged plasmid DNA would run on a gel.
5) Analyze restriction digest data of an unknown recombinant plasmid and identify which pieces come from which starting plasmid.
6) Utilize hypothetical recombinant plasmid maps to predict what a restriction digest of that plasmid would look like.
7) Use results from antibiotic selection as well as plasmid digestion to identify an unknown plasmid from a given set of plasmid maps.