Biol 390: Molecular Techniques, spring, 2024

Instructor:

Dr. Ming-Mei Chang, ISC 352 (office)/ 346 (lab), Phone: 245-5416, Email: chang@geneseo.edu

Office Hours

In-person: Monday (12 - 1:30 pm) OR Email for appointments

Virtual: Friday (11:30- 1 pm)

<https://teams.microsoft.com/l/meetup-join/19%3ameeting_YmMwMGVhZTgtMmIzOC00NTA2LTkzYjMtMDdiNTU5MjhhZDY4%40thread.v2/0?context=%7b%22Tid%22%3a%2202ce934f-066a-4d00-b828-cedba7cf4f79%22%2c%22Oid%22%3a%227efe60b1-d70f-497d-87d4-69b35dcc9a14%22%7d>

Meeting ID: 285 711 468 921 Passcode: aXQFYD

Learning Outcomes:

You might have learned various molecular techniques from previous courses. This lab enhances your knowledge and practical skills on molecular techniques used in research. Three main learning outcomes are:

To learn and practice techniques commonly used in cell and molecular biology.

In addition to working with chemicals, i.e., making solutions and dilutions, you will learn principles and practice techniques of genomic DNA isolation and quantification, degenerate PCR, agarose gel electrophoresis, TOPO cloning, restriction mapping, web-based sequence analysis, total RNA isolation and quantification, reverse transcription and real-time PCR, bacterial recombineering with and without Cas9, colony PCR, and sequence analysis to verify proper nucleotide edition, and associated techniques of microbial culture. Each weekly laboratory consists of a pre-laboratory lecture and discussion, followed by a three-hour wet lab. Your understanding of the subject and efforts is as important as the experimental results obtained. After completing this course, you should be familiar with each technique covered and capable of performing and applying them to related studies.

To gain skills in collecting, analyzing, interpreting, and communicating experimental results with others.

You have to keep electronic versions of WELL-WRITTEN weekly lab notes that include all the information and data of each lab, which will be used to write up three reports in the format of scientific research papers and help you study for the tests.

To be able to work as a team.

Most biological studies, particularly cell and molecular biology research, involve teamwork. In this lab, you work in pairs. Joint efforts between you and your partner are required to complete each lab. Although we meet for regularly scheduled laboratory periods, sometimes the lab must be started the night before, completed the next day, or wait for three hours of incubation time for bacterial recovery and growth. You, your partner, or both must return to complete it. To reduce stress, you should avoid having another class, meeting, or work scheduled immediately after the lab. Be a good team player.

Grading:

Individual Lab Note	15%
One Quiz and Three Tests	25%
Group Homework	25%
Three Group Lab Reports	30%
Participation	5%

All assignments, except calculations (handwriting) and lab reports (online submission), must be submitted in hard copies at the beginning of the lab on the due date.

Your grade[1] is based on the scale listed below.

A (100 - 93%)	A^-(92 - 90%)	B^+ (89 - 87%)	B (86 - 83%)	B^- (82 - 80%)
C^+ (79 -77%)	C (76 -73%)	C^- (72 -70%)	D (69 - 60%)	

Individual Weekly Lab Note (LN)[2]

Each student should write a weekly lab note with detailed information about the week's lab immediately after completion. Five weekly lab notes will be collected at the BEGINNING of the following week's labs (See the due date on the next page). Keeping well-written weekly lab notes throughout the semester is crucial because they provide information for writing lab reports later. For each weekly lab note, you should START with a NEW Word document in the PROPER ORDER:

1. DATE, TITLE, NAME, and LAB PARTNER on the FIRST page
2. PURPOSE/OBJECTIVES, including OVERALL for the module and SPECIFIC objectives for the week
3. RESULTS
4. INTERPRETATION and DISCUSSION
5. CONCLUSION and FUTURE DIRECTION/ NEXT STEPS

*See **LAB NOTE** below for details.*

One Quiz and Three Tests

A quiz on the calculation of making solutions and dilutions in the 2nd week's lab. Each lab module includes several exercises extending over multiple weeks, each building on the foundation established in the previous week(s). Before coming to the lab, you must review what you learned from the previous week(s) and read the materials for the week. At the end of each lab module, there is a test on what you have learned throughout the module. Since the course has no textbook, you must understand the background information, prelab lecture, homework, and techniques involved, which will be in the test.

Group Assignment and Homework (GH)[2]

In-class group assignments and homework help you understand the lab and its applications better. The in-class assignment is due at the end of the lab period. Others are due at the BEGINNING of the following lab.

Three Group Laboratory Reports (LR)[3]

After completing each module, the report is due at the **BEGINNING** of the following lab. It should be written in the format of PRIMARY scientific research papers (peer-reviewed papers reporting original data by authors and published by a non-commercial publisher), including TITLE, ABSTRACT, INTRODUCTION, RESULTS, DISCUSSION, and REFERENCES unless indicated otherwise.

*See **LAB REPORT** below for details.*

[1]*Any grade disputes must be initiated **WITHIN ONE WEEK** after the assignment/test is handed back.*
[2]*No late weekly lab notes or homework is allowed without legitimate reasons. "Too busy" is NOT legitimate.*
[3]*There is a 10% penalty per day for late submissions.*

Course Materials and Supplies:

A *Sharpie fine-point permanent marker, a lab coat, a USB drive,* and a *3-ring binder* are required for the lab. Lab protocols are posted in Brightspace. Before the lab, you must PRINT, READ, and PLACE the lab protocol in the 3-ring binder. Doing so helps you understand the pre-lab lecture. Never view protocols on your electronic devices while conducting the experiments, as you can easily make mistakes. Making a physical mark as you complete each step in a multi-step procedure is also an excellent practice. PowerPoint slides of the pre-lab lecture will be posted after each lab.

Lab Policy and Public Health:

We meet weekly for 14 lab periods, among which 12 involve wet labs, and two only focus on data analysis and discussion. Although attendance is mandatory, your health and well-being are critical to learning. You might miss labs for legitimate reasons, but you can not miss more than two labs during the semester, as falling behind too much can affect your learning and grade, and you may need to consider withdrawing. Although I can work with you on keeping up, you may miss some course content and the hands-on experience, a critical component of the course. Thus, extended absences impact your ability to perform fully in the lab. For extended absences, you need to contact the Dean of Students, Dr. Sancilio (sancilio@geneseo.edu), who can assist you in determining the best

course of action. If you are experiencing cold or flu-related symptoms but feel well enough to attend, wear a well-fitting mask, come to the lab, and maintain physical distance as much as possible.

Accommodations:

SUNY Geneseo reasonably accommodates persons with documented physical, emotional, or learning disabilities. Students should consult with the Office of Disability Services (Erwin 22, Email: access@geneseo.edu, Phone: 585-245-5112) and individual faculty regarding any needed accommodations early in the semester.

Academic Policies of the Biology Department-ACADEMIC DISHONESTY:

Be aware of the College policies concerning academic dishonesty. According to College policies, the school may handle any alleged cheating and plagiarism as a disciplinary problem (see the College Bulletin). Be especially aware that **academic dishonesty** includes *putting your name on a group project without contribution* and *turning in lab reports copied from reports of previous semesters' classes. If your name is on a project, you must ensure the work is authentic and adequately referenced; you are also responsible if your group member plagiarizes material*. The college faculty will take all the necessary steps to deter academic dishonesty. All cases will be reported to the Dean of the School for possible disposition as a College disciplinary matter.

Tentative Schedule:

WK	DATE	LAB EXERCISE	DUE
1	1/23	Pipetting; Calculation on making solutions & dilutions; Introduction to Module I	In-class GH
		Module 1 Cloning NBS-containing Sequences by Degenerate PCR	
2	1/30	1-1 Genomic DNA Isolation; Degenerate PCR; Quiz 1	
3	2/6	1-2 Agarose Gel Electrophoresis; Degenerate PCR Primer Design	LN 1-1, GH 1-1
4	2/13	1-3 DNA Ligation and Bacterial Transformation (*Seal and store plates the next day)*	LN 1-2, GH 1-2
5	2/20	1-4 Plasmid DNA Isolation; Sample Preparation for Sequencing; Restriction Digest (*Start bacterial culture the night before*)	GH 1-3
6	2/27	1-5 Agarose Gel Electrophoresis; Web-based Sequence Analysis; Data Analysis and Discussion	LN 1-4, GH 1-4
		Module 4 Studying Gene Expressions by RT-qPCR	
7	3-6	4-1 Total RNA Isolation and quantification; Test 1: Module 1	GH 1-5, **LR Module 1**
8	**3/13**	**Spring break**	
9	3/20	4-2 Reverse Transcription; Real-time PCR	LN 4-1, GH 4-1
10	**3/27**	**Diversity Summit (No Class)**	
11	4/2	4-3 Data Analysis and Discussion; Test 2: Module 4	GH 4-2
		Module 3 Bacterial Recombineering with and without CRISPR-Cas9	
12	4/9	3-1 Bacterial recombineering (*Three-hour incubation)*	**LR: Module 4**
13	4/16	3-2 Colony PCR; Agarose Gel Electrophoresis; PCR Product Purification	GH 3-1
14	4/23	3-3 Data Analysis and Discussion	LN 3-2, GH 3-2
15	4/30	3-4 Cas9-assisted recombineering (*Three-hour incubation)*	**LR Module 3**
16	5/7	3-5 Colony PCR; Agarose Gel Electrophoresis; PCR Product Purification	GH 3-4
	5/10	Test 3: Module 3 (8 - 11:20 am, the first day of the Final)	GH 3-5

LAB NOTE

Like working on research projects, you should organize your weekly experimental results in an electronic copy format containing detailed information about each lab, which will be helpful for the lab write-up and tests later. Keeping up your lab notes should be ongoing throughout the semester. You need to i) make your lab note in an organized way and clearly to show evident care taken to make it easy to understand; ii) have the content of each lab note in A PROPER ORDER specified as follows:

1. DATE, TITLE, NAME, and LAB PARTNER on the top of the FIRST page
2. PURPOSE/OBJECTIVES, with OVERALL for the module and SPECIFIC objectives for the week
3. RESULTS:
   a. Include the RAW DATA obtained in the lab and convert them into organized
      - TABLES with TITLES on the TOP and FOOTNOTES at the BOTTOM, or
      - FIGURES with proper labels and FIGURE LEGENDS containing TITTLES followed by a brief description at the BOTTOM or on the SIDE.
   b. Ensure each result is presented as one or two statements in text and cite TABLES and FIGURES
   c. Briefly describe what you did or the step number in the protocol when recording an observation.
   d. Show all your CALCULATIONS.
4. INTERPRETATION and DISCUSSION
   You should interpret/discuss your results but NOT repeat the results. For example, what do the results mean? Did you get the expected results? Why or why NOT? This part is as important as the RESULTS because it shows your understanding of the lab.
5. CONCLUSION and FUTURE DIRECTION/ NEXT STEPS

LAB REPORT

The new trend in writing: Use active, short, and concise sentences.

***Title*:**

The title should reflect the paper's content and be appropriate for the readers (*describe the subject* or *summarize the results*). It shows the factors tested, the measured effects or responses, the specific topic or organism under study, and the researcher's name (s). Be as concise as possible.

***Abstract/Summary*:**

Write this part last after you have all the others completed.

The abstract is the "preview" of what will come in the paper and should be less technical. It should be a shorter version (1-2 paragraphs) of the paper. Readers can quickly scan the paper and decide whether to read more. It can be apart from the paper by briefly introducing background information and telling what you did, what happened as a result, and what you concluded. It summarizes the introduction leading to the objective/purpose, methods, results, and conclusions.

- ❑ 2-3 **introductory sentence(s)** leading to the purpose of the study
- ❑ 2-3 sentences on what you did, **materials/methods,** including the organism (A brief description of the experiment)
- ❑ Summarize the **major findings/results** (1-2 sentences each)
- ❑ **Major conclusion(s)** for the paper

Introduction:

The introduction should clearly state the questions to answer or tasks to accomplish for your study. Summarize the relevant literature to help readers understand why the study is interesting/ important, and include enough background information to make your report understandable. What do you want to accomplish? What question(s) do you ask? End with one or two sentences to address what you accomplished OR explain the question(s) you asked. The cited papers in this section can be used for discussion later. It is usually 2-4 paragraphs.

Results:

The results should contain tables and figures. Before citing and showing them in the text, there should be a brief description of the contents. Tables and figures/graphs should be numbered and labeled. For graphs, the **dependent** variables should be on the vertical (Y) axes and the **independent** variables on the horizontal (X) axes. Linear, semi-log, or log graphs should be used where appropriate. The figure should have a stand-alone legend that readers can understand without reading the text. The table should have a title on top and footnotes at the bottom if needed. NO raw data (directly from the lab, like those shown in PowerPoint) or significant discussion**,** speculation, or explanation in the Results

Discussion:

HIGHLIGHT and DISCUSS *significant* results, but DO NOT repeat them.

Require literature citations to support your claims in the discussion.

Discuss if your results

- ❑ Relate to the original question/purpose of the experiment
- ❑ Support/achieve your hypothesis/objective
- ❑ Are consistent with previous studies *(citations)*
- ❑ Fit into the big picture

For unexpected results, try to explain why OR use other interpretations or further research to answer the questions raised by your results. END with 1 or 2 sentences to summarize your **conclusion** and emphasize why it is relevant. The discussion should include *conclusions from your results* and *whether your data are consistent with previous studies, relevant models, or hypotheses*.

Literature Cited:

Any facts or ideas not from you must be attributed to the source where you found them. Indicate such references by citing the corresponding paper(s) at the appropriate place in the text and listing a complete citation under "*Literature Cited*" at the end. All references cited MUST be cited in the text. Use the **AMA** format (PubMed) and ensure consistency throughout the section.

Final Check: Scientific content

Are the directions followed and the reasoning accurate? Are all possible inferences made?

Are writing style, grammar, and spelling appropriate?