

Experimental Molecular Biology Laboratory BIOL 4102/5102. Fall 2016

Marquette University, Department of Biological Sciences

Instructor Dr. Edwin Antony
Office WLS 209
Telephone 414-288-1474
Email edwin.antony@mu.edu
Office hours M 3:00-4:00 PM or by appointment

Class Hours:

Monday – Lecture – 3:00 – 3:50 pm. Wehr 100

Tuesday (section 401)/Thursday (section 402) – Labs – 1:00 – 4:50 pm. Wehr 103

Teaching Assistant: Jerrin Cheriyan

Contact Information: Jerrin.cheriyam@mu.edu

Course Objectives: This course will provide hands-on experience with techniques, concepts and approaches commonly used in molecular biology & biochemistry research. Students will investigate the function of a gene/protein and the lab will use an “independent research” approach. The lecture session will focus on the theory behind the experiments and discuss why/how the experiments are performed and results interpreted. *This course is communication intensive; therefore, written lab reports and reading of scientific literature will be major components.* Learning objectives include: (1) understanding standard molecular biology, protein expression and protein quantitation techniques typically used in molecular biology and biochemistry labs; (2) applying these approaches in a “real-life” research setting; (3) learning to communicate science using written and verbal forms - aka “the scientific thought process”. The course is appropriate for all pre-health, chemistry and biology professionals, and those preparing for life science-oriented graduate programs and industrial careers.

Prerequisites: Prior general chemistry, general biology, and/or basic organic chemistry laboratory experience is expected.

Textbooks and Lab Material: There is no specific textbook required for this class. Handouts for each lab, including reading assignments will be uploaded on the course website: www.teachwithSSB.com

1. A laboratory notebook with carbon-copy pages is required and will be available at the campus bookstore and must be purchased before the first lab.
2. Lab coats must also be purchased before the first lab.

Grading: This course will be graded based on total points received for:

- a) Pre-lab quizzes
- b) Laboratory notebook
- c) Laboratory reports
- d) Poster presentation
- e) Art project
- f) Final paper

Final letter grades will be assigned based on the percentage of the total points. More information about how the class will be graded will be discussed half-way through the semester. Attendance at all of the assigned meetings is essential. **Make-up of any missed meeting will only be allowed for excused absences obtained through written documentation.** Unexcused absences in the lab will automatically result in a loss of points associated with that specific lab. All laboratory reports turned in after the deadline will have 40% of the possible points deducted for the first day that it is late, with 10% of the possible points deducted for each additional day that it is late

As per MU's code, "Students are allowed a total of six (6) absences in a 3-credit course (excused or unexcused). Points for unexcused absence will be deducted as described in the above paragraph. Any student exceeding the allowable number of absences may be Withdrawn for Excessive Absences (WA) prior to the semester calendar deadline of: 11/20/2016 for the fall term. If a student exceeds the number of allowable absences after this deadline, the course final grade will be lowered (½ a letter grade; A to AB, etc.) per absence above the allowable number."

Assessment: Assessment of the course will include a Course Evaluation (administered by MU) at the end of the course. Information from the Course Evaluation will be used to improve the course in subsequent years.

University Academic Integrity Policy: This course will adhere to the MU Academic Policies and Procedures, which is published in the 2015–2016 Undergraduate Bulletin and can be accessed at: <http://bulletin.marquette.edu/undergrad/academicregulations/#academicintegrity>.

ADA accommodations: Accommodations will be made for students with disabilities in conjunction with Marquette University policy. Please contact the Office of Disability Services at 414-288-1645. They are located at 707 Building, fifth floor. (email: ods@marquette.edu). More information is available on their website: Marquette.edu/disability-services.

Supplies: Eye protection, lab coat, full-length pants, and closed-toe shoes are required in the laboratory. Please dress appropriately for a lab environment where chemicals and corrosives will be present and you will be working with these solutions while making buffers, etc.

Pre-lab quiz (10 points each)

A short pre-lab quiz will be administered at the beginning of each class based on the week's reading assignments.

Laboratory Notebook (20 pts each)

You will be keeping a laboratory notebook containing the title, date, objectives, materials and methods, and results for each experiment. This is one of the most important exercises in the class. Include tables for all dilutions and show all calculations in this section. Prepare the **title and objectives sections BEFORE coming to the laboratory**. Be sure to note any problems or observations in the results section. Such observations will aid in your discussion in the laboratory report. An example of how to keep a proper notebook will be discussed before the first lab.

Try to keep your notebook as neat as possible. Mistakes are made and are common, but clearly cross-out "wrong" sections (if any). DO NOT ERASE!!! Always write in INK!! A research notebook must be organized such that ANYONE could pick it up and repeat your work without any questions

(and hopefully get the same results and observations). Laboratory notebooks are scientific records and can be submitted as evidence in court trials, so learn now how to keep a neat, detailed (but succinct) notebook! The carbon-copy pages of your notebook must be turned in at the end of lab each day BEFORE you leave the lab. There are no excuses to this rule.

Laboratory Reports (25 pts each)

You are required to write your own laboratory report for each experiment. The report is due at the beginning of your next lab or as instructed. Some experiments will be completed in a single day, while others will take multiple days to complete. A formatted template will be provided to help with writing your lab reports. Specific instructions will be given with each lab. However, each report must contain the following elements:

- a. **Title:** The title should include experiment number, experiment name, the date(s) performed, the due date, your name, and the name of your lab partners.
- b. **Objective/Introduction:** This is a brief summary of the objective or purpose of the experiment.
- c. **Methods:** DO NOT reproduce the experimental protocol that was handed out to you or followed during the course of the day. Describe what you did using complete sentences (in past tense). If part of the procedure is a repeat of a method used in a previous lab (e.g. running an SDS or agarose gel), it is sufficient to state that the procedure "was performed as described previously in experiment X." However, if you modified the procedure in any way, you must explain what the modification was.
- d. **Results:** All results must be described with complete sentences in paragraph format. Whenever possible, results should also be presented in a table or as a graph and inserted into the lab report as figure with a figure legend.
- e. **Discussion:** Succinctly summarize your results. Address any unexpected observations. The discussion section of the lab report should describe in detail what your results mean and the implications they might have on future experiments. With each lab protocol you will be given several questions or problems that you should address while discussing the results of your experiment. The answering of these questions should be done in discussion format (paragraphs and complete sentences), rather than an enumerated list.

Poster Presentation (300 points): You will generate a poster based on your research findings and formally present it to the department.

Art (100 points): You will be generating a piece of 'cover art' as part of the course. You will be working with Ms. Lynne Shumow at the Haggerty Museum of Art to generate an artistic rendition that integrates three aspects of your research: a) a function of the protein, b) some aspect of the disease and c) a thematic rendering of your bacterium of investigation.

Final Manuscript (1000 points): This will be a 'journal style' manuscript detailing your exploration of your SSB protein of choice. More details will be provided on the first day of class.

You will have the privilege of exploring the function of an unknown SSB protein. SSB stands for single stranded DNA binding protein. While we expect the protein to have some common characteristics with the SSB protein from *E. coli*, each SSB protein will be unique. There is an opportunity to find something amazing and possibly a target for drug development against one of these deadly pathogens. It truly will be an amazing journey of exploration and hope you bring a ton

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of enthusiasm and curiosity to the lab!!

Our pathogens of interest for Fall 2016 are:

1. *Bacillus anthracis* (anthrax).
2. *Listeria monocytogenes* (listeriosis)
3. *Chlamydia trachomatis* (chlamydia)
4. *Clostridium perfringens* (food poisoning)
5. *Helicobacter pylori* (gastritis)
6. *Streptococcus pneumoniae* (pneumonia)
7. *Enterococcus faecalis* (septicemia)
8. *Thermus aquaticus* (model organism for structural and functional investigation)
9. *Escherichia coli* (model)

Schedule

Week	Project Details
Week 1 (8-29, 8-30 or 9-1) PCR SSB Gene	Introduction to SSB protein. Lab Safety, Pipetting basics, Weighing and Buffer preparation. PCR amplify the SSB gene and run it on an Agarose Gel.
9-5 <i>Labor Day</i>	<i>No Lecture</i>
Week 2 (9-6 or 9-8) Cloning	Cut PCR product with restriction enzymes. Ligation and transformation into DH5 cells. Grow cultures for miniprep next week.
Week 3 (9-12, 9-13 or 9-15) Screening for Clones	Miniprep DNA. Screen for clones using restriction enzymes. Meeting with Artist at Haggerty Museum (during digest and analysis).
Week 4 (9-29, 9-20 or 9-22) Protein Overexpression	Transformation into bacterial cells Grow 4L biomass Check protein overexpression using SDS-PAGE Gel Freeze Cell Pellet.
Week 5 (9-26, 9-27 or 9-29) Protein Purification	Lyse cells. Purify protein using affinity chromatography Analyze proteins on SDS-PAGE gel
Week 6 (10-3, 10-4 or 10-6) Limited Proteolysis	Proteolytic cleavage of SSB proteins Protein-protein interactions Enzymes as catalytic machines
Week 7 (10-10, 10-11 or 10-13) Crystallization	Setup SSB apo and SSB-DNA crystal screens
Week 8 (10-17, 10-18 or 10-20) <i>Fall Break</i>	Fall Break
Week 9 (10-24, 10-25 or 10-27) Complementation	(Dr. Antony at NIH Study Section) Bumping assay to check if your SSB gene can support function in <i>E. coli</i> . Dr. Origanti will be teaching this class
Week 10 (10-31, 11-1 or 11-3) DNA Binding	Measure protein concentration Test DNA binding activity of SSB protein (EMSA)
Week 11 (11-7, 11-8 or 11-10) DNA binding	SSB binding kinetics using stopped flow instrument and intrinsic tryptophan fluorescence.
Week 12 (11-14, 11-15 or 11-17) FRET	DNA wrapping around SSB measured using Forster Resonance Energy Transfer
Week 13 (11-21, 11-22 or 11-24)	Thanksgiving break (Thu)
Week 14 (11-28, 11-29 or 12-1)	Week reserved for art with Lynne Shumow and possibly crystallization.
Week 15	Poster presentation

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(12-5, 12-6, 12-8)	

SUMMARY OF GRADED ELEMENTS

BIOL 4102/5102 Fall 2015 Possible Points		
Element	Description	Max. Points
<i>Take home assignments</i>	2 assignments. 50 points each.	100
<i>Pre-laboratory quizzes</i>	11 total, lowest scored one will be dropped. 10 points each.	100
<i>Daily laboratory notebook</i>	11 total labs, lowest one will be dropped. Due at the conclusion of the lab. 50 points each.	500
<i>Laboratory reports</i>	11 experimental reports (as per schedule above). Lowest scored one will be dropped. Due at beginning of next lab. 100 points each	1000
<i>Poster Presentation</i>	Poster presentation of research findings.	300
<i>Final Manuscript</i>	Manuscript written like a research article with appropriate references.	1000
Total		3000