KLINGLER
College of Arts & Sciences
MARQUETTE UNIVERSITY

Experimental Molecular Biology Laboratory
BIOL 4102/5102. Fall 2017

Instructor **Dr. Edwin Antony**
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Office hours **By appointment**

Class Hours:

Monday – Lecture – 3:00 – 3:50 pm. Wehr 100
Tuesday Lab (section 401) – 1:00 – 4:50 pm. Wehr 103
Thursday Lab (section 402) – 1:00 – 4:50 pm. Wehr 103

Teaching Assistant
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Course Objectives: This course will provide hands-on experience with techniques and concepts commonly used in molecular biology, biochemistry, and biophysics research laboratories. Students will work as teams of two to uncover the function of a unique gene/protein. The course will follow an "independent research" approach where the students will first learn how to setup a hypothesis and design appropriate experiments to test the hypothesis. The lecture sessions will focus on the theory behind the experiments, why/how the experiments are performed, and discussions will heavily focus on data analysis and interpretation.

Learning objectives include:
1. Acquire a thorough understanding of standard cloning, molecular biology, protein expression and protein quantitation techniques typically used in molecular biology and biochemistry laboratories.
2. Learn to apply these approaches in a "real-life" research setting.
3. Learn 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.

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 bacterial pathogens. It truly will be an amazing journey of exploration and hope you bring a ton of enthusiasm and curiosity to the lab.

Our pathogens of interest for Fall 2017 are:
1. *Staphylococcus epidermidis* (STAEQ).
2. *Bacteroides fragilis* (BACFN)
3. *Bordetella pertussis* (BORPE)

4. *Campylobacter jejuni* (CAMJE)
5. *Chlamydia psittaci* (CHLPS)
6. *Clostridium tetani* (CLOTE)
7. *Ehrlichia chaffeensis* (EHRCR)
8. *Ehrlichia ruminantium* (EHRSS)
9. *Francisella tularensis* (FRATT)
10. *Nocardia asteroides* (NOCAS)
11. *Escherichia coli* (model)

Attendance: Students must attend ALL classes and labs. Due to the staggered nature of the experiments, it is impossible for the instructor to accommodate make-up classes. A calendar detailing the list of experiments are presented in the table below. Please avoid double-booking with other events/interviews. **Exceptions to this rule, under dire circumstances, will be rarely considered; however written consent from the instructor MUST be obtained at least two weeks in advance.** 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: 11/20/2017 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 A-, 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.

Grading: This course will be graded using six criteria.

1. Pre-lab quizzes (5 %)
2. Laboratory notebook (10 %)
3. Laboratory reports (30 %)
4. Poster presentation (20 %)
5. Art project (5 %)
6. Final paper (30 %)

Final letter grades (A-F) will be assigned based on the percentage of the total points. More information about how the class will be graded will be discussed/decided half-way through the semester.

Pre-lab quiz (5 points total)
A short pre-lab quiz will be administered at the beginning of each class based on the week's reading assignments. 5 such quizzes will be administered.

Laboratory Notebook (10 pts total)
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 do occur while writing in yur notebook and you might have to change reaction conditions during an experiment. Such changes 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 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 (30 pts total)
You are required to write two laboratory reports. The due dates for the two reports are detailed in your schedule. The instructor will present guidelines and provide examples during the first meeting.
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 partner.
- ***b.*** ***Objective/Introduction*****:** This is a brief summary of the purpose of the experiment.
- ***c.*** ***Methods*****:** DO NOT reproduce the experimental protocol that was handed out to you. Describe what you did using complete sentences. The format of the methods is similar to a scientific publication. Please refer to the example provided.
- ***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 answers should follow a discussion format (paragraphs and complete sentences), rather than an enumerated list.

Poster Presentation (20 points): You will generate a poster based on your research findings and formally present it to the department. Instructions will be given two-three weeks before the event.

Art (5 points): You will be generating a piece of 'cover art' as part of the course. You should generate an artistic rendition that integrates three aspects of your research: a) function of the protein, b) some aspect of the disease and c) a thematic rendering of your bacterium of investigation.

Final Manuscript/Paper (30 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.

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Schedule

Week	Project Details
Week 1 (8-28, 8-29 or 8-31)	**<u>Introduction to SSB protein</u>** - Lab Safety, pipetting basics, weighing and buffer preparation. - Transformation into bacterial cells - Grow 4L biomass - Transform Monday - Start O/N culture on Tue - Start 4L culture on Wed morning, induce on Wednesday, Express overnight at 25 deg C or 3hrs at 37 Deg C. Spin down and save cells on Thursday.
9-4 *Labor Day*	*No Lecture*
Week 2 (9-5 or 9-7)	**<u>SSB Protein Prep - 1</u>** - Check protein overexpression using SDS-PAGE - Lyze cells and fractionate proteins over Ni-NTA resin (affinity chromatography). Freeze away fractions containing protein. - -Cleave off HisTag with overnight incubation using protease. - Design primers for mutagenesis. - Work on sequence alignment and select residues for mutagenesis
Week 3 (9-11, 9-12 or 9-14)	**<u>SSB Protein Prep - 2</u>** - Test protease cleavage conditions. - Incubate protein with protease, collect sample every hour. - Analyze protein using SDS-PAGE. - Setup Quickchange reaction and finish transformation. - Structure module using PyMol (Bring laptop computer)
Week 4 (9-18, 9-19 or 9-21)	**<u>SSB Protein Prep – 3</u>** - Cleave His Tag off protein - Fractionate protein over Ni-NTA resin. - Pool fractions containing SSB protein and dialyze into ssDNA column buffer. - Seed colonies for miniprep on Monday. - Miniprep for clones on Tuesday and Thursday – Mail sequencing reactions.
Week 5 (9-25, 9-26 or 9-28)	**<u>SSB Protein Prep – 4. Protein Concentration Measurement and Mutagenesis</u>** - Purify protein over ssDNA column. - Measure protein concentration using Bradford Assay and Absorbance. - Dialyze protein against storage buffer. - Bring Laptop computer to class. - Use DNA Star to align sequences and check for positive clones. - *Dr. Antony will be away for Seminar. TA will handle class*.
Week 6 (10-2, 10-3 or 10-5)	**<u>SSB-DNA Binding EMSA (electro mobility band shift assay)</u>** - **REPORT 1 DUE** - EMSA based measurement of SSB-DNA binding. - If you have identified positive clones; start mutant protein purification. - Transform on Monday and follow procedure to purify SSB protein containing mutation.
Week 7 (10-9, 10-10 or 10-12)	**<u>SSB-DNA Binding Trp Quenching</u>** - Continue Mutant protein purification. - Trp Quenching experiment to measure protein:DNA interactions
Week 8 (10-16, 10-17 or 10-19) *Fall Break*	- **Fall Break 10-19 – 10-22**

Week 9 (10-23, 10-24 or 10-26)	**Measurement of DNA Binding Site Size** - Use Trp quenching and [NaCl] to obtain site-size for your SSB. - Continue purification of mutant SSB protein.
Week 10 (10-30, 13-31 or 11-2)	**Measurement of DNA wrapping – FRET** - DNA wrapping around SSB measured using Forster Resonance Energy Transfer. - Finish purification of mutant SSB protein. Measure concentration of mutant protein. - **REPORT 2 DUE**
Week 11 (11-6, 11-7 or 11-9)	**Mutant versus wildtype – DNA binding EMSA** - Compare DNA binding activity of wildtype versus mutant protein using EMSA. - Compare DNA binding activity of wild Type versus mutant using Trp Quenching.
Week 12 (11-13, 11-14 or 11-16)	**Mutant versus wildtype – DNA binding site-size and FRET** - Compare DNA binding activity of wildtype versus mutant protein. - Measure site-size of mutant versus wildtype protein. - Does the mutant protein wrap DNA to similar degree as wild type protein?
Week 13 (11-20, 11-21 or 11-23)	- **Thanksgiving break (Thu)** - Work on Final Paper and Poster.
Week 14 (11-27, 11-28 or 11-30)	- 27th – Dr. Antony away for seminar. TA will go over posters on Monday. - Practice poster presentations. - **PRINT POSTERS** (Thursday and Friday)
Week 15 (12-4, 12-5, 12-7)	- Poster presentation (no labs this week) - Date and time TBD - **FINAL PAPER DUE**
12-9	- FINALS WEEK