**BY271L: Biology of Microorganisms Laboratory**

**Summer 2018; CH273, MW 10:30-12:50**

**Stuff you need to buy:**

Laboratory safety glasses

Lab coat (must stay in lab till end of semester)

Permanently bound graph paper notebook (e.g., a composition book) -- only need one per student team, will stay in laboratory

Dry erase marker

Extra-fine tip black sharpie

**Overview:** While all science is ultimately rooted in experimentation, microbiology stands out in that it is defined by a standard set of experimental methods that are used at all levels, from basic research to clinical practice. Some of these methods are based on cutting-edge molecular technologies such as PCR and DNA sequencing, whereas others (e.g. colony isolation on agar plates) are little changed from the lab notebooks of Koch and Pasteur over a century ago. In this laboratory course you will master aseptic technique and the handling of cultured microorganisms, as well as learn the basics of modern molecular microbiology and bioinformatics. You will do this in the context of a semester-long project that will start with the unusual activity of isolating soil bacteria and using them as paints to make a living work of art. Much of what you'll do in this course is open-ended and exploratory -- learn the techniques and see where they take you.

By the end of the semester you will be expected to be proficient with the theory and practice of the following methods:

1. Aseptic technique and laboratory safety protocols
2. Isolation of clonal microbial populations using agar plates
3. Enumeration of bacteria by viable count and optical density measurement
4. Polymerase chain reaction (PCR)
5. Gel electrophoresis
6. DNA sequence alignment and the Basic Local Alignment Search Tool (BLAST)
7. Statistical methods for analyzing experimental data: *t* tests and linear regression
8. Technical writing and peer review

**Grading:**

* 3 manuscripts
	+ Lead author manuscript 30%
	+ Computer/Reviewer manuscript 15% each
	+ Final manuscript 10%
* Lab Worksheet (team) 10%
* Presentation (team) 10%
* Notebook checks (team) 10%

Your final grade for BY271 will be calculated as (lecture grade x 0.67) + (lab grade x 0.33).

**Team Work:**

As in lecture, you will be expected to work in teams. Each team will consist of 2 or 3 people. All the rules and suggestions from lecture for team work apply here as well. Additionally, you should strive to divide the day's labor in a sensible way between team members in order to get the procedures done correctly and on time. It's best to plan this out BEFORE you get to lab so you can finish on time!

**Falling Behind:**

Perhaps unlike other lab classes you've taken, in this course you HAVE to get each procedure to work. Since nobody has a clue what you've isolated, you can't write reports that make any sense unless you get the procedures to work.

If something doesn't work, *don't worry*. Mistakes happen and science is often frustrating -- and you're not an expert lab hand yet, that's why you're taking the class. Make sure your TA knows you need to repeat a procedure and he/she will have the reagents and media ready for you in the next class period. MAKE SURE YOU'VE READ YOUR PROTOCOLS thoroughly, and understand what you're supposed to be doing and WHY -- the more comfortable you are with the procedures, the less chance there is that you'll mess something up.

**Attendance:**

This is a hands-on course, so you absolutely HAVE to be in lab to do the work. Not only that, but your group depends on you, and if you don't show up you're taking advantage of them. **Each unexcused absence will result in a -10 penalty from your final lab grade.**

**SAFETY FIRST!**

You will be given a handout detailing lab safety procedures and protocols on the first day of class. MEMORIZE THESE and practice them religiously both here and for the rest of your life when you're working around biohazardous materials! There are hazards in the laboratory, including open flames, mildly pathogenic microbes, and chemicals that can irritate or burn your skin. Developing good lab safety habits is the only way to keep yourself and others safe and healthy in the long term.

**Dress Code:**

Part of lab safety is dressing properly for lab. Every day you will put on your lab coat and safety glasses as soon as you get to lab. Backpacks, purses, and other similar items will be hung on hooks at the edge of the class -- they must not be placed on the floor. Whenever you will be handling cultures or molecular biology reagents, you will wear disposable lab gloves. You MUST wear closed-toe shoes and will not be permitted into lab otherwise. Additionally, you should avoid wearing anything that could get in the way of your work or could get caught in a Bunsen burner flame, including dangling necklaces, bracelets, shirts with wide sleeves, and long hair that isn't pulled back.

**MICROBES DO NOT LEAVE THIS LAB!**

The environmental microbes we will be working with are considered **BIOSAFETY LEVEL 2**, meaning they have a moderate risk of causing infection but a very low risk of being communicable or causing serious harm to otherwise healthy adults. It is important, however, that we minimize the risk of transporting these cultures out of the lab, either on our belongings or (worse) within our bodies. Several policies will be in place to minimize this risk:

1. You will not use personal pens, pencils, or notebooks in the lab. Anything you write with or on stays in the lab permanently.
2. You will not use your personal electronics, including your phones, in the lab. The one exception will be to take photographs of plates or microscope images using your phone -- but this must be done by an individual who has removed his/her gloves, washed his/her hands, and is not personally handling any living material.
3. In class you will use our laboratory laptops to work with data and to collect notes. The laptops are numbered so you can make sure to use the same one every day. You may email any of this information to yourself so you can continue to work on it at home.
4. Before you contact any cultures, you will put on your personal protective equipment: lab coat, gloves, and safety glasses. All of these items will remain in the lab throughout the semester.
5. After removing your gloves for the day, wash your hands before leaving the lab. NEVER touch a door knob or any of your personal belongings while wearing gloves!
6. Before and after working with cultures, wipe your bench down with disinfectant.

**ASSIGNMENT EXPLANATIONS:**

1. **Manuscripts:** All the "lab reports" you will write in this course will be structured as professional scientific manuscripts, consisting of five sections: Abstract, Introduction, Methods, Results, and Discussion. You will write 3 lab manuscripts including a final manuscript that will include new experiments that you design and execute yourself. A rubric will be provided for how these manuscripts will be graded, but broadly speaking, your score will be based on 1) clarity of your argumentation, 2) correctness of your quantitative reasoning, 3) graphical presentation of the data, and 4) correct separation of the material into the 5 required sections.

Each of these 3 manuscripts will be written using a "scaffold" approach. For each paper, one group member will be the **Computer**, one will be the **First Author**, and one will be the **Reviewer**. The Computer will be responsible for number-crunching and generation of the raw data that will form the meat of the paper. The Computer will also put together the figures (including photographs) and tables for the paper. The First Author will do the large majority of the writing, taking the output from the Computer and using it to build a logical and complete manuscript. The Reviewer will read the First Author's first draft and write a detailed critique of it that addresses unclear writing, faulty reasoning, missing elements, and any other substantive problems with the original draft. The First Author will revise the manuscript based on the Reviewer's suggestions and will be solely responsible for the final paper turned in to the TA.

**Each of these segments -- the first draft/review, the data spreadsheets, and the final manuscript -- will be turned in for grading.** Because the First Author has the hardest job, he/she gets more points than anyone else. The individual group members will ONLY be graded on the quality of their individual task. For instance, if your First Author writes a really bad first draft, but you as Reviewer respond with a constructive review, you'll get full points for your component. If you're First Author and your Computer makes a giant math error, but you correctly draw conclusions based on the analyses you're given, the Computer will lose points, but not you. Note, however, that as First Author, you are responsible for producing a good paper, so if one or the other of your group members fails to fulfill their role in a timely manner, you still have to get the paper together and turn it in.

Please note that **the final manuscript is the most challenging**. One of the first things you will need to decide on as a team is who should be the First Author on this manuscript. This individual should be comfortable with taking on a challenging scientific role, and also on having a sizable chunk of his/her lab grade (40%) up in the air until the very end of the semester.

1. **Original Research and Final Manuscript:** Early in the semester, you and your classmates will isolate a number of microorganisms from the soil. Over the course of the semester, you will collect a large amount of data about these organisms. Additionally, you will create "paintings" using your isolated organisms, and you will observe how these paintings change over time. From these collected observations and your knowledge of the identities of your organisms and the environment from which they were isolated, you will construct a hypothesis about their ecology that can be tested with experiments you design, using the skills you learn over the course of the semester. The final weeks of lab will be devoted to performing these experiments and analyzing the data, and your final lab will outline this work. In addition to the scaffolded scores for this manuscript, your team will be eligible for up to 10 more points based on the creativity, depth, and thoroughness of the experiments. **The team should be thinking about this manuscript from the moment they isolate their bacterial unknowns!** The depth of your thinking about these organisms will be reflected in this final project, and will also probably make it enjoyable and exciting for you.
2. **Lab Worksheet:** There are 3 possible projects, prior to the final project, that you can write manuscripts on, but you only have to pick 2. For the third project, your team will turn in a shorter worksheet-style report. This will include the required figures and data analysis as well as a brief description (one or two paragraphs) of the significance of the findings. Specific guidelines will be provided for each project.
3. **Presentation:** Students will present their findings from their original research to the class. Presentations will be hand-crafted posters that will be created in class. Please note that this research is *real scientific research*, and students who are interested in presenting their work in BY271 at the UAB student research expo or any other conference are encouraged to do so -- we will help!
4. **Lab Notebook:** Each student team will keep a lab notebook. Daily dated entries will be made, and methods, observations, and data will be recorded here. Teams will prepare short plans prior to each lab session detailing how the day's responsibilities will be distributed between team members; these will be written (or typed) outside of lab and then taped or glued into the lab notebook. Detailed methods don't need to be included; a note such as "procedure was followed according to page 75 in the lab manual" is sufficient. However, any deviations from the manual should be noted as they occur. In some cases your lab manual has questions that it requests you to answer in your notebook; try to keep up with these, as they will help you when it's time to write your manuscripts. Notebooks will be spot-checked periodically throughout the semester and a single grade will be assigned for the entire team.

**Note on manuscript due dates:** These are *suggestions* only. We strongly encourage you to get your papers in on time so that you can get feedback from your TA, but we also want you to take the time required to produce quality writing. Therefore, ***no penalties will be assigned for late manuscripts*** other than the hardship of not getting timely grades back.

**We pledge to have all written work graded and returned within a week of it being turned in.** We are also available to consult on early drafts (within reason).

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| **Date** | **"Current" Lab** | **Assignments Due** |
| Jun 4 | Biosafety/Aseptic Technique/Pipetting |  |
| Jun 6 | Field Sampling |  |
| Jun 11 | Isolation of Unknowns/Statistics |  |
| Jun 13 | Microscopy/Gram Stain |  |
| Jun 18 | Growth Curves/Environmental Tolerances |  |
| Jun 20 | Data Analysis; PCR |  |
| Jun 25 | Electrophoresis/Metabolisms/Mandalas |  |
| Jun 27 | Agar Art | **Growth Curve Report Due** |
| Jul 2 | DNA Sequence Analysis; Bergey's; Hypotheses |  |
| Jul 4 | **HOLIDAY** |  |
| Jul 9 | Stress Resistance | **Niche Report Due** |
| Jul 11 | Stress Resistance; Data Analysis | **Hypothesis Due** |
| Jul 16 | Hypothesis Experimentation |  |
| Jul 18 | **Stress Report Due** |
| Jul 23 |  |
| Jul 25 |  |
| Jul 30 | Presentation preparation and Peer Review |  |
| Aug 1 | Presentations |  |
| Aug 9 | **LECTURE FINAL EXAM** | **Final Paper Due** |

**BY 271 Disclaimer**

Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. I understand that working with unknown environmental bacterial isolates carries health risks. I have been instructed in, and am confident that I understand, the safety requirements and procedures for working with Biosafety Level 2 organisms. If at any point I am unsure or unconfident in my ability to perform a procedure, I will immediately ask for assistance from my TA. I will also notify my TA of any exposures or spills as soon as possible.
2. I will inform my TA immediately if I have or develop any medical conditions that I believe will suppress my immune system or otherwise cause an increased vulnerability to bacterial infection. I am not required, however, to provide any details other than that I believe I have an elevated risk of infection.
3. I understand that the novel data I collect in this course is the property of UAB and will be publicly available for use by other students and researchers in the future. I understand that my contributions will be acknowledged (unless I expressly request to remain anonymous) and that I will have the opportunity to contribute as a co-author on any publication made using data that I personally collected.

Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_