GENERAL INFORMATION

Objectives

The purpose of this laboratory course is to introduce you to a sampling of the morphologically and physiologically diverse members of the Prokaryotes. The emphasis is on the enrichment, purification, and identification of organisms taken from natural habitats, but we will also explore genetic phenomena using model bacteria. The Bacteria and Archaea domains are so vast and diverse that you can study only a miniscule portion of the organisms and their isolation techniques in a one-term course. We will not study, fungi, algae, protozoans, slime molds, nor a number of other microbial groups. Each deserves its own course.

- **Enrich, purify, identify, and explore genetic systems of the diverse Prokaryote group**
  - wild strains (from natural habitats)
  - lab strains (used as model systems)
- **Learn standard lab and microbiology techniques**
  - sterile technique, bacterial husbandry, microscopes, etc
- **Learn to follow a protocol**
  - follow directions (written and verbal)
  - learn proper techniques related to clean-up, hazards, and safety
- **Exposure to real-life lab work scenarios and experience**
  - perform actual experiments
  - juggle multiple projects and experiments at once
  - gain independence in a laboratory setting

Format

Microbiology (Bi330) is a required pre- or co-requisite for *Microbiology Lab*. If a student withdraws from Bi330, they must withdraw from Bi331. Students failing to do so will be dropped from Bi331 upon our request to the Registrar.

A lecture for the laboratory is scheduled for 9:00-9:50 am on Mondays in Pacific Hall 123. In the lecture, background information and technical suggestions will be given. The goal with the Monday lectures is to maximize your time working in the lab, and so introductions and overviews won't routinely be given in the lab. Attendance will be taken via iClicker; this is to help incentivise you to attend these lectures so that you will be prepared when you arrive at your lab. If you miss the lectures, you will likely be unprepared to perform the exercises for those weeks.

Laboratories meet twice per week for up to 2 hours and 20 minutes each session in Klamath 33. Most sessions will last the full time, so do not expect to get out early. You must attend your regularly scheduled lab section and you are not permitted to swap lab sessions with any students. During many weeks, you will also have additional lab work outside of your normal section time. You will have access via your UO ID card to the laboratory from 8:00am-9:00pm Monday through Friday, and 8:00am-6:00pm on Saturday and Sunday. A buddy-system will be mandatory for working in the lab after hours (5:00pm-9:00pm Monday-Friday) and on the weekends (see description of the buddy-system on page 5). You may work in the lab at any time except when there is another class in session or during the weekly staff-only lab prep session (see class schedule on page iv).

You will need a notebook in addition to this manual for this course. The notebook can be of any type that suits you. The idea is that you will take detailed notes about your results and observations throughout the term.

We will begin most sessions with a brief introduction about the work to be done that day, and then commence with the lab exercises. We will do three types of exercises: general exercises (GEs), enrichments (ENs), and projects (Ps). General exercises will be done by all students at the same time and are designed to introduce you to some of the commonly used techniques in the many branches of microbiology. The enrichments are for the concentration or isolation of specific groups of microorganisms from mixed populations; all students will perform these, but once begun, the enrichments won't require coordinated efforts by the entire class. The projects are larger scale experiments that will take 1 to 6 weeks each to complete; some are group (2-4 students) projects that, once begun, will require group members to coordinate daily or weekly tasks.

Assignments and grading

Grades will be assigned on points earned out of a possible ***450*** based upon the following criteria:

5-minute Quizzes (70 points [most are 5 points each]). There will be a short quiz, to be taken on canvas prior to your lab, on 13 of the labs in which new exercises will be performed. The first quiz will be 15 points to be taken prior to your first lecture; the remaining 12 quizzes will be 5 points each and taken before each lab (due 30 minutes before your designated lab section). The questions will cover the procedures and background found for those exercises in the lab manual. The purpose of these quizzes is to motivate you to read the material before arriving in lab. All quizzes are open note (you may use the lab manual to find answers); however, you may not use any other sources. Since there is a time limit on these quizzes, it is highly suggested to read to lab manual first before starting your timed quiz. The low score of one of the twelve 5-point quizzes will be dropped (total points = 15+[11*5] = 70 points).

Written Exams (100 points [50 points each]). There will be 2 closed-note exams: one given during lab lecture in week 5 and one given during your lab section in week 9. These are intended to test your knowledge of the techniques we use and of the physiological, ecological, and biochemical characteristics of the organisms that we study. No make-up exams will be given unless prior arrangements are made or a valid medical or travel excuse is provided.

Lab Practical Final Exam (75 points). During the 10^{th} week you will take a lab practical exam. Grading will be based upon your ability to perform some of the standard microbiological techniques and to analyze results from isolations and tests that were previously done in the lab. Since this exam is based on lab experience during the term, make sure you participate activly in ALL lab exercises. Many help hours will be cancelled during week 10 due to grading for the practical exam.

Worksheets (85 points [10 points each; one at 5 points]). Short worksheets for general experiments will be required for 9 of the general exercises. Due dates will be listed in the schedule (see page iii). Sloppy work will be penalized. Calculations must be clear, graphs must be constructed via computer programs or done on graph paper, mistakes must be erased, and multiple pages must be stapled.

Project 1 (P1): Identification of 2 Unknowns (50 points [15 points each; 20 points for key]). You will be given a mixture of 2 species from bacterial groups that we will have studied. Your mission will be to purify and identify them to the species level based upon an identification key of your design. You will submit a copy of your key during week 4 when you receive your unknowns.

Summary (50 points). You will write a summary for one of the other two lab projects (P2:The nitrogen cycle in a biological filter or P3: Genetic analysis of prodigiosin biosynthesis). A detailed reubric can be found on canvas. A few brief notes: the text of the summary must be typed, double-spaced, 12 point font, 1-inch margins, be no more than two pages long, and must include:

- A statement of the goal of the experiment in the context of relevant background information (cite the papers available on canvas!).
- The methods, **without procedural details** (such as dilutions, reagents, standards, etc.), employed to address the question.
- A summary of the results you obtained. You should include a table, relevant figure, graph, and/or statistical analysis, but provide these on one separate page.
- Analysis of the results and conclusions that you can draw from the them

Attendance/Discretionary (15 points). This will be based upon attendance (iClicker during lectures), participation, group cooperation, workstation cleanup, punctuality, etc. If you forget your clicker remote or it malfunctions during lecture, do not ask your instructor for attendance points for that day. It is your responsibility to remember your clicker remote and keep it in good working order (keep batteries on hand). You will be permitted to miss one lecture without it impacting your grade. All lectures will be in-person-only; there will be no video or zoom options. If you have to miss a lecture for any reason, make sure to look over the lecture slides (posted to canvas), borrow notes from your peers, and come to office hours to get up to speed.

Special note about lab attendance. You are expected to attend all labs. We will be moving at a very brisk pace from week 1-8 and will be covering a lot of ground in each lab session. You will be directly working with living bacterial species during every single lab session during the term. Furthermore, our prep team has quick turn arounds to set up for lab experiments performed in the Monday/Tuesday lab sessions versus the Wednesday/Thursday lab sessions. All of this taken together means that there are NO MAKE-UP LABS available, although we can provide you with data for some worksheet exercises. If you have to miss lab for any reason (COVID, other illness, family emergency, etc.), YOU MUST NOTIFY YOUR INSTRUCTOR VIA E-MAIL AT LEAST 2 HOURS PRIOR TO THE LAB SECTION(S) YOU WILL MISS in order to be given data to do the associated worksheet. Since quizzes are all done virtually through Canvas, you will still be responsible for these items regardless of absence (see Quizzes above). If you do not notify your instructor of your absence as described above or are more than 10 minutes late for your lab, you will be counted as absent. Although not recommended, you may miss one lab without it impacting your grade. Regardless of reason, points will be deducted for additional absences (beyond the one permitted) or being late to lab. If you are absent for more than 3 *unexcused* sessions you will receive a failing mark for the course.

Student Experience Survey (5 points). The end of course student experience survey is available in DuckWeb from Wednesday 5-31-23 at 8am through Friday 6-9-23 at 5pm. At the end of the

course, please fill out the student experience survey. This is an important professional courtesy that helps your instructors improve their teaching approaches and course material; the majority of your instructors truly care about receiving your constructive feedback. Take a screenshot at the end of your submission confirming you completed the survey for Bi331, with your name visible, and upload it to canvas to earn the 5 points. You may upload a JPEG, JPG or PDF file. Do not include any of your actual feedback/answers in your screenshot so as to ensure your submission remains anonymous. You will have until Tuesday 6-13-23 at midnight (11:59 PM) of Finals Week to upload your screenshot for points, but make sure you complete the student experience survey on DuckWeb by the Friday 6-9-23 due date.

Lab care and safety

Safety
A separate safety sheet will be available on canvas. Keep this handy at all times and strictly follow the rules and guidelines.

Common spaces
Please keep the common spaces clean and organized; these include some benches, all incubators, and lab shelves. Don't leave old cultures behind—it is your responsibility to dispose of them properly when your general exercises and enrichments have been completed. Wipe up spills with 70% ethanol (EtOH), clean common tools and equipment, put away your source materials, and remove labels from tubes, caps, and flasks.

Your bench
Though you will work at the same bench throughout the term, you will be sharing the space and tools with several students who are in other sections. It is important that you keep your workspace neat and clean, and that you keep track of your tools. If something is missing, please ask your instructor or TA to find a replacement. "Borrowing" from an unoccupied bench means that the students who work at that space in another section will be missing some of their tools or supplies. Furthermore, to help promote safe practices, you will be required to do the following upon entering the room and before leaving:

At the beginning of your session:
- Wash your hands at the sink
- Wipe down your bench and tools with 70% EtOH
- Pick up your designated lab coat

At the end of your session:
- Turn off gas
- Turn off under-shelf light
- Turn off water
- Put away microscope and lock microscope cabinet
- Clean up/straighten your lab bench
- Return the lab coat/ to the proper location
- Wipe down your bench and tools with 70% EtOH. Be particularly thourough if there is oil, stain, or a bacterial spill on the bench.
- Wash your hands at the sink

Buddy-system

You will be required to adhere to a buddy-system when you work in lab after hours (5:00pm-9:00pm Monday-Friday) and on weekends. You and your buddy will be required to sign a log-in sheet to confirm who has been in lab and to note any problems that arise (e.g. gas/bunsen burner being left on, broken glass, injuries, etc.). Buddy-systems in scientific research labs are common and meant to help protect an individual's safety (not being alone in buildings and to protect against lab-related issues like bacteria, fire, chemicals, gasses, etc.). You will need to coordinate coming into lab with at least one of your fellow Bi331 students, either from your direct lab section or from one of the other lab sections). You are not to allow non-enrolled friends (or anyone else) into the building. Any access point in the Science Complex allows people to roam freely though too many spaces and buildings, and there have been some issues in the past with people holding the doors for others. Not adhering to these rules could lead to the loss of building/Klamath 33 key card access at any time during the quarter. To set up your key card access, please work with Annie Rogers in the Biology office (*arogers2@uoregon.edu*); she will require your University identification number (9 digits long) and the alphanumeric prox number (4-5 digits in the upper righthand corner; it often starts with an A, B, or C), both of which are found on the backside of your student ID card. If any issues arise relating to your key card access over the term, please contact her directly.

Slack

To help you coordinate with your fellow students regarding experiments, coming to lab after hours and on weekends, and/or meeting to study, a class Slack account will be made. Please make sure to download the app on either your phone and/or your computer; it functions similarly to other texting apps but without you having to share your cell phone number. You will need your uoregon email to join our class Slack channel. Keep in mind that if you do not use Slack actively (such as sending a message), it may kick you off. Please remember that this channel is an extension of the University, and all codes of conduct will apply. If evidence of inappropriate behavior or cheating is detected, you risk being ejected from the Slack channel, along with any other appropriate disciplinary measures.

The Slack channel is designed to be used solely by you and your classmates and not to communicate with the teaching staff. All questions should be sent to your instructor, GEs, and BULAs via email and all official class communication will be done through canvas announcements.

Cleaning glassware

Reuseable glassware (test tubes, flasks, beakers) must be rinsed in the sink and then placed in the large wash tub. Remove tape and any other labeling. Graduated pipettes (1 ml, 5 ml, 10 ml) must be placed in the pipette cylinder containing 10% bleach next to the sink. All spectrophotometer and durham tubes should be bleached and tossed in bihazard boxes.

Sterile supplies

Sterile liquids (water, saline, mineral oil, etc.) are single use items; that is, if you take some portion from a tube or vial, do not return the tube or vial to the rack or shelf from which you took it. Sterility cannot be guanarateed for the next user. Unused portions are to be discarded. Agar plates removed from their bags, but that have not been opened, may be returned to the plate tub at the center island bench.

Stain bottles

You will use a lot of stains throughout the term. When a stain bottle runs out, place it in the specified container on the wash cart and retrieve a full bottle from the stains kept in cartons on the shelf on the east wall.

Microscopes

You will be assigned a microscope that you will use throughout the term. Three other students (one each from the other three lab sections) will use the same microscope. You will have access to no other microscopes, nor will any other students have access to yours. They will be kept in locked cabinets, and you will be provided a key for that cabinet. You must return the key at the end of the term; if you fail to do so, your UO account will be charged (the current replacement cost is over $20!). If you come to the lab during open hours without your key, you will not have access to your microscope. We instituted this policy in response to the theft of a microscope in the past.

Each supply drawer should have a small dropper bottle of immersion oil for microscopy. These bottles invariably leak, so always keep the oil bottle in the small container in which it is found in your tool tray.

Labeling

You will use dozens of tubes, flasks, and Petri dishes throughout the term, and keeping track of your cultures and enrichments will be crucial. Use fine point Sharpies and write DIRECTLY ON THE GLASS for the labeling of all serial dilution tubes, spectrophotometer tubes, flasks, bottles, and differential test tubes. Do not label plastic or metal caps of tubes or flasks (we can use tape for this if necessary), and never label the frosted glass area on a flask or tube. Always include your initials and lab section (I, II, III, or IV) and whenever appropriate, the source of material.

Label Petri dishes on the bottom (the part with the agar). Don't use tape for the labels, as the tape will prevent you from observing the agar from the bottom side. Keep labels as small as possible (don't try to include the whole history of the culture—do that in your notebook). Remember, you'll want to look at the colonies, and this is hard to do against a heavily labeled background. Petri plates with agar are stored in the inverted position with the agar side up. This prevents condensation that forms in the lid from dripping onto the colonies on the agar (which can cause contamination or cells to spread into films rather than remain in isolated colonies).