BIOL/BIOC 4502/5502: Bacterial Diversity and Physiology Laboratory
Fall 2022  LIFE A217

Professor: Dr. Calvin A. Henard  TA: Spencer Lee, BMB Ph.D. student

Contact Information:  Email: Calvin.Henard@unt.edu
Office: Science Research Building (SRB), Room 132
Office Phone: (940) 565-3280
Office Hours: by scheduled appointment

Course Pre-requisites, Co-requisites, and/or Other Restrictions: Previous or current enrollment in BIOL/BIOC 4501.

Course Description and Objectives: Microbial diversity laboratory is largely focused on microbe isolation techniques, including the use of selective and differential growth medium, and molecular-based microbial profiling. Over the semester, we will quantify, isolate, and identify the symbiotic yeast and bacteria found in kombucha (a commercially available probiotic drink). During this process students will learn:

• Techniques used to isolate microbes from mixed cultures using differential/selective growth medium
• Targeted metagenomic workflows
• DNA isolation and PCR
• BLAST analysis
• Phylogenetic analysis

Benchling Electronic Lab Notebook: Go to benchling.com and create a free account using your school email. Request to join the group BIOL 4502 Fall 2022. You will use benchling over the course of the semester to document your lab activities. You will insert images/cfu calculations, and/or videos into benchling organized by date to document your lab results. It should contain a detailed protocol that contains a complete list of the materials used, the process, and weekly results. For the kombucha experiment, document the development of the symbiotic culture of bacteria and yeast (SCOBY) with pictures and/or videos. You should also include weekly written observations (color changes, SCOBY thickening, cloudiness/clarity, and smell) and document any potential issues that arise. If you observe mold (green or black growth on the top of the kombucha/SCOBY) Dump it and restart the process. By the end of the semester you should be able to describe the microbial processes occurring during the kombucha development. After the kombucha experiment is complete, you will need to graph your resultant data and insert them (cfu/mL of 4 growth medium, isolated microbe micrographs, isolated microbe sequence identification and/or phylogenetic analysis) into your benchling.

Electronic lab notebooks will be checked weekly on Friday. No entry will result in point deductions.
### Topic Schedule

<table>
<thead>
<tr>
<th>Date</th>
<th>Weekly Topic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept 1</td>
<td>Lab Safety/ Lab objectives/Benchling overview</td>
</tr>
<tr>
<td>8</td>
<td>Selective/Differential Medium/Streaking/Dilutions (GFP-expressing E.coli)</td>
</tr>
<tr>
<td>15</td>
<td>Kombucha inoculation and experiment start (Booch Protocol)</td>
</tr>
<tr>
<td>22</td>
<td>Kombucha microbe quantification and isolation</td>
</tr>
<tr>
<td>29</td>
<td>Kombucha microbe quantification and isolation (A#1)</td>
</tr>
<tr>
<td>Oct. 6</td>
<td>Metagenomics pipeline overview/ Kombucha DNA isolation</td>
</tr>
<tr>
<td>13</td>
<td>PCR with 16s/ITS primers</td>
</tr>
<tr>
<td>20</td>
<td>DNA electrophoresis</td>
</tr>
<tr>
<td>27</td>
<td>cPCR with Brett 18s primers/CLEAN-UP</td>
</tr>
<tr>
<td>Nov. 3</td>
<td>16s Metagenomics data analysis (REMOTE A#2)</td>
</tr>
<tr>
<td>10</td>
<td>ITS Metagenomics data analysis (REMOTE; A#3)</td>
</tr>
<tr>
<td>17</td>
<td>BLAST and Phylogenetic analysis of Brett 18s amplicon (REMOTE; A#4)</td>
</tr>
<tr>
<td>24</td>
<td>Thanksgiving *NO LAB</td>
</tr>
<tr>
<td>Dec. 1</td>
<td><strong>Finalized Electronic notebook due</strong></td>
</tr>
</tbody>
</table>

*Please note this schedule is tentative and subject to change by the instructor.*

### Attendance:
This is a practical lab course. As such, you must be present to perform the experiments and participate in lab activities. 25pts will be deducted from your participation/attendance for each absence up to 4 absences. **More than four absences will result in an automatic F for the overall course grade.**
The lab will be computational in nature throughout November. The November lab dates will be held remotely as indicated.

### Grading:
Your final course grade will include the lab notebook, 4 assignments, and participation:

- Electronic Lab Notebook: 200pts
- Assignments (4): 200pts
- Lab participation/attendance: 100pts

Grading will follow a standard scale:

- (448 – 500 points) A
- (398 – 447 points) B
- (348 – 397 points) C
- (298 – 347 points) D
- (< 297 points) F

### UNT Policies

#### Academic Integrity Policy

Academic Integrity Standards and Consequences. According to UNT Policy 06.003, Student Academic Integrity, academic dishonesty occurs when students engage in behaviors including, but not limited to cheating, fabrication, facilitating academic dishonesty, forgery, plagiarism, and sabotage. A finding of academic dishonesty may result in a range of academic penalties or sanctions ranging from admonition to expulsion from the University. **If I identify plagiarism on an Exam, you will get an automatic 50 unless the violation is egregious, then you will receive a 0.**
ADA Policy
The University of North Texas makes reasonable academic accommodation for students with disabilities. Students seeking reasonable accommodation must first register with the Office of Disability Access (ODA) to verify their eligibility. If a disability is verified, the ODA will provide you with a reasonable accommodation letter to be delivered to faculty to begin a private discussion regarding your specific needs in a course. You may request reasonable accommodations at any time; however, ODA notices of reasonable accommodation should be provided as early as possible in the semester to avoid any delay in implementation. Note that students must obtain a new letter of reasonable accommodation for every semester and must meet with each faculty member prior to implementation in each class. Students are strongly encouraged to deliver letters of reasonable accommodation during faculty office hours or by appointment. Faculty members have the authority to ask students to discuss such letters during their designated office hours to protect the privacy of the student. For additional information, refer to the Office of Disability Access website (http://www.unt.edu/oda). You may also contact ODA by phone at (940) 565-4323.

Emergency Notification & Procedures
UNT uses a system called Eagle Alert to quickly notify students with critical information in the event of an emergency (i.e., severe weather, campus closing, and health and public safety emergencies like chemical spills, fires, or violence). In the event of a university closure, please refer to Canvas for contingency plans for covering course materials.

Acceptable Student Behavior
Student behavior that interferes with an instructor’s ability to conduct a class or other students' opportunity to learn is unacceptable and disruptive and will not be tolerated in any instructional forum at UNT. Students engaging in unacceptable behavior will be directed to leave the classroom and the instructor may refer the student to the Dean of Students to consider whether the student's conduct violated the Code of Student Conduct. The University's expectations for student conduct apply to all instructional forums, including University and electronic classroom, labs, discussion groups, field trips, etc. Visit UNT's Code of Student Conduct (https://deanofstudents.unt.edu/conduct) to learn more.