

MCB 473/573: RECOMBINANT DNA METHODS AND APPLICATIONS Spring 2020

Information contained in the course syllabus, other than the grade and absence policy, may be subject to change with advance notice, as deemed appropriate by the instructor.

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OFFICE HOURS: You are encouraged to discuss any problems you may have with the lab or lecture material. You can talk with the Instructor or a TA during lab. However, if you would like office hours, Stephanie Capaldi is available **by appointment only.** If you wish to meet with the graduate TA you can arrange a day and time that is suitable for both of you. **Appointments can be made by e-mail or in class.**

LEARNING OUTCOMES

- In this course you will gain practical experience in the most up to date techniques used in biology research labs today. This experience will allow you to apply these methods to investigate biological processes and problems in graduate research programs, industry and in the health-related professions.
- Through the lectures and course assignments we will explore the fundamental biological concepts behind the experiments in the lab so that you understand the purpose and mechanism of the methods you are using. This will also strengthen and advance your understanding of key biological principles that you have heard about in other courses.
- You will gain experience in data analysis and interpretation. This experience will allow you to identify flaws in experimental design and critique your own data.
- You will be introduced to scientific writing and communication, where you will learn how to write and present your data in the form of a research paper. By the end of the course you will be familiar with the style and content of each section of a research paper. You will also learn how to use the published literature to appropriately support and question your findings.

COURSE OBJECTIVES

The techniques covered in this course include: Agarose Gel Electrophoresis, Restriction Enzyme Digestions, Ligation of DNA fragments, Polymerase Chain Reaction (PCR), DNA Sequencing, Bioinformatics e.g. BLAST, Bacterial and yeast transformations, Plasmid and PCR purification, Flow Cytometry, Protein gels (Western blots), RNA isolation and purification, Reverse Transcription and DNA Microarrays.

The course will be divided into two parts. First, you will learn techniques commonly used to clone DNA in bacteria. Here you will amplify and then insert a mystery gene into a plasmid. You will then sequence your construct and use bioinformatics programs to identify the mystery gene. In the second half of the course, you will study the effects of nutrient deprivation on the Environmental Stress Response in yeast. In this module, you will measure this response in certain strains using GFP. Interesting strains will then be selected to determine the effects of nutrient deprivation on all of the genes in the organism using DNA microarrays.

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For the first part of the course you will work with a lab partner. However, in the second part you will work on your own project.

COURSE WEBSITE: All of the course material will be posted in advance on D2L, <u>http://d2l.arizona.edu</u>. This includes your graded assignments and announcements. If you cannot access this site at any time please contact Stephanie Capaldi immediately. Please check the course site regularly and **always** <u>before every lab session</u>.

LAB & LECTURE ROOMS: Lecture is in the Henry Koffler Building, Room 209 (4:00 – 4:50 PM) and lab is in the Henry Koffler Building, Room 541.

ACADEMIC AND LABORATORY ETHICS: One of the best ways to understand the experiments in the lab is to discuss them with your lab mates and with the course staff. Although it is completely acceptable (and encouraged) to discuss your data with your lab mates and the course staff you must write up this data in your lab notebook entries and in the appropriate course assignments in your own words. All written assignments (Lab reports, Interpretation Questions and Notebook entries) are to be each student's own work. All tests and quizzes must be taken on your own i.e. no collaboration is permitted.

CODE OF ACADEMIC INTEGRITY: Students are encouraged to share intellectual views and discuss freely the principles and applications of course materials. However, graded work/exercises must be the product of independent effort unless otherwise instructed. Students are expected to adhere to the UA Code of Academic Integrity as described in the UA General Catalog. See: <u>http://deanofstudents.arizona.edu/academic-integrity/students/academic-integrity</u>.

PLAGIARISM AND MCB473/573: All of your assignments-lab reports, interpretation questions, and your daily notebook entries must be entirely your own work. You cannot paraphrase each other's work and to avoid this we advise you not to share drafts with each other. In the research papers you will be expected to compare your findings to the published research articles. You may also use information from textbooks. If you discuss material from a paper or textbook, you must paraphrase the material and then cite the source in your reports. You cannot copy material

from other students (past or present), the scientific literature, textbooks or the lab manuals.

Plagiarism will not be tolerated. If a student is caught copying material from another student or from the scientific literature or course manuals appropriate action will be taken, which may include loss of credit. If you are unsure of what constitutes plagiarism please speak to a member of the MCB473/573 staff, we would be happy to advise you.

The University Libraries have some excellent tips for avoiding plagiarism, available at <u>http://www.library.arizona.edu/help/tutorials/plagiarism/index.html</u>.

Selling class notes and/or other course materials to other students or to a third party for resale is not permitted without the instructor's express written consent. Violations to this and other course rules are subject to the Code of Academic Integrity and may result in course sanctions. Additionally, students who use D2L or UA e-mail to sell or buy these materials are subject to Code of Conduct Violations for misuse of student e-mail addresses.

LABORATORY SAFETY: In the MCB473/573 lab you will encounter some hazardous chemicals. Before you attend the first lab session you must read and understand the lab safety sheet at the end of this document and complete the D2L online chemical safety test.

TEXTBOOK: All the theory and biological concepts behind the experiments in the lab will be covered in lecture and therefore **there is no textbook for the course**. However, if you do want to read more about the techniques and concepts covered AN INTRODUCTION TO GENETIC ENGINEERING, 3rd Edition, Desmond S.T. Nicholl, Cambridge University Press, 2008 and MOLECULAR BIOLOGY OF THE CELL, 6th Edition, Bruce Alberts are recommended.

MATERIALS YOU MUST PROVIDE FOR LAB: You will need to purchase either a lined or grid bound notebook, a lab coat and a calculator for lab. All the data that you collect in the lab must be kept in the notebook, keeping a good notebook is essential for writing your two research papers. You will be given instructions in the first lecture on writing in the lab notebook. Before every lab you must prepare the 'Aims' and 'Protocol' sections for the lab session. During lab you must report all your observations, annotate gels and tables in the 'Data and Observations' section. During lab or at home you must discuss the significance, outcome and any problems associated with the lab session in the 'Discussion' section. LAB NOTEBOOKS WILL BE CHECKED OFTEN IN LAB BY THE MCB473/573 STAFF AND WILL CONTRIBUTE TO YOUR LABORATORY PERFORMANCE GRADE.

ABSENCE AND CLASS PARTICIPATION POLICY

The UA's policy concerning Class Attendance, Participation, and Administrative Drops is available at: <u>http://catalog.arizona.edu/policy/class-attendance-participation-and-</u>

administrative-drop

The UA policy regarding absences for any sincerely held religious belief, observance or practice will be accommodated where reasonable, <u>http://policy.arizona.edu/human-resources/religious-accommodation-policy</u>.

Absences pre-approved by the UA Dean of Students (or Dean Designee) will be honored. See: <u>https://deanofstudents.arizona.edu/absences</u>

Participating in the course and attending lectures and other course events are vital to the learning process. As such, you are required to attend both the lab sessions and lectures. Please note that attendance at every lab session and <u>arrival on time</u> WILL CONTRIBUTE TO YOUR LABORATORY PERFORMANCE GRADE. To limit disruption to a lab session please inform both Stephanie Capaldi and your lab partner of your absence in advance if at all possible. **Regardless of the reason for your absence it is your responsibility to obtain materials to complete any assignments or to write-up the notebook entries that you missed.**

DEAN APPROVED ABSENCES

An official University of Arizona Dean's note must be provided to Stephanie Capaldi in advance for any Dean approved absences. Appropriate accommodations will be made for absences that may affect the assessments described below.

ASSESMENTS: Your final grade in this course will be determined as follows

LABORATORY PERFORMANCE	15 %	
SCIENTIFIC COMMUNICATION	40 %	
ACADEMIC PERFORMANCE	45 %	

DUE DATES OF ALL ASSIGNMENTS AND EXAMS ARE LISTED IN THE COURSE SCHEDULE ON D2L. Please note that there are no bonus points or extra credit for any of the assignments or assessments in this course.

LAB PERFORMANCE (TOTAL 15%)

Your lab performance will be assessed in two ways as follows;

<u>Notebook Entries (10% of overall grade)</u>-Complete lab session entries including 'Aims', 'Protocol', 'Data and Observations' and 'Discussion' sections **for every lab session**. Your entries will be checked and assessed at random without prior notice by a TA during the lab sessions. No entries on 'loose' sheets of paper or in any other form (including electronic) other than in your notebook are acceptable.

<u>In-Lab Performance (5% of overall grade</u>)-This assessment will be divided equally between five criteria;

1) Lab technique - your ability to follow the protocols carefully and accurately (you will NOT be assessed on the results (quality or whether or not experiments work-we understand that you are learning. In addition, experiments often fail for even the most experienced researchers due to no fault on their part!)

2) Lab safety – your ability to follow the correct handling and disposal procedures of hazardous chemicals and waste as outlined in this syllabus, lab manuals or following instruction from the MCB473/573 staff. Being dressed appropriately for lab (see the document at the end of this syllabus). Following standard lab practice rules e.g. no eating or drinking in the lab.

3) Participation - answering questions in lab and in particular lecture. Taking on an equal share of the lab work in your group. Not dominating or doing most of the lab work in your group.

4) Organization – your ability to complete your experiments in a methodical, steady but timely manner. At the end of a lab session, you must clean up your bench, discard reagent waste appropriately, rinse apparatus and place in the appropriate bins for the dishwasher, and wipe down your work area. Maintaining a clean and tidy bench will also contribute to your lab performance grade.

5) Lab Attendance and Punctuality-your attendance and punctuality will contribute to your lab performance grade.

SCIENTIFIC COMMUNICATION (TOTAL 40%)

The scientific communication portion of this course will be assessed as follows;

<u>Lab Report Module II (10% of overall grade)</u>- you will submit four separate assignments for this report, weighted as follows;

- 1) Abstract (2% of overall grade)
- 2) Introduction (2% of overall grade)
- 3) Methods (1% of overall grade)
- 4) Results and Discussion (5% of overall grade)

Lab Report Module III (25% of overall grade)

You will submit four separate assignments for this report, weighted as follows;

- 1) Abstract (5% of overall grade)
- 2) Introduction (5% of overall grade)
- 3) Methods (2.5% of overall grade)
- 4) Results and Discussion (12.5% of overall grade)

Please note, that unfortunately due to time and few members of staff, we will be unable to read any portion of your reports by e-mail prior to submission. However, we are happy to read your work and advise you during lab or in office hours, but again please note that there are a lot of you and few of us so prepare your drafts and schedule a time to meet with a staff member well in advance of the due date of the assignment.

Oral Presentation (5% of overall grade)

You will give a five minute power point presentation in module III.

ACADEMIC PERFORMANCE (TOTAL 45%)

The academic performance portion of your grade is divided as follows;

<u>Test 1 (15% of overall grade</u>)- This test will cover all the lab and lecture material in module II.

<u>Test 2 (15% of overall grade</u>)- This test will cover all the lab and lecture material in module III.

There is no Final Exam for this course. All tests are closed book. The tests are 50 minutes long and take place during the lecture period. **IN GENERAL NO MAKE-UP EXAMS ARE OFFERED, SO PLEASE SCHEDULE ANY APPOINTMENTS OR INTERVIEWS OUTSIDE THE TEST DATE TIMES.**

<u>Quiz 1 (3% of overall grade)</u>- This online quiz will cover basic lab calculations. You can prepare for this quiz by studying the 'Calculations' and 'Using Stock Solutions in a Molecular Biology Lab' manuals posted under the 'General Information' section in the contents page on D2L. The quiz contains practice quizzes that you can take before you feel ready to take the final quiz. You then have one attempt at the final quiz.

Interpretation Questions (12% of overall grade)

Six sets of Interpretation Questions (problem sets) will be assigned during the course (for due dates please see the course schedule). Each set of questions is worth 2% of your final grade. You should complete these questions on your own for two reasons. First, they will help teach you how to analyze and interpret data. Second, they are very similar, in format, to the questions you will be given in the tests. You should look at these questions well in advance of their due date so that you can discuss any problems with the TAs or instructor during lab. The MCB473/573 staff are happy to 'guide' you and put you on the 'right track' with these questions- however, they will not give you the answers or check your solutions.

GRADUATE STUDENTS ONLY (MCB 573)

You are required to complete one additional assignment. As a result, the final report on Module III will count for 20% of your final grade. The remaining 5% will be assigned to the additional assignment.

Please note: This is the expected number and types of assessments for the course. However, due to unexpected circumstances or unanticipated changes in the lab experiments these assignments may change, however, the OVERALL grading scheme (i.e. lab performance (15%), scientific communication (40%) and academic performance (45%)) will not be changed.

SUBMITTING ASSIGNMENTS: All assignments must be submitted in electronic format on D2L.

All completed 'Interpretation Question' sets must be submitted by the posted deadline. All sections of the two lab reports (Lab Report Module II and Lab Report Module III) must be submitted by the posted deadline. Late submissions will be penalized as follows: up to 1hr after the deadline will be penalized by 6 percentage points, >1hr and up to 24hrs after the deadline will be penalized by 12 percentage points, >24hrs and up to 48hrs after the deadline will be penalized by 25 percentage points and >48hrs and up to 72hrs after the deadline will be penalized by 50 percentage points. Late submissions more than 72hrs after the deadline will not be graded and will receive zero points.

The On-line quiz must be submitted by the posted deadline; if a technical problem is encountered, Stephanie Capaldi must be e-mailed immediately (you will not be allowed to take the quiz if you do not inform Stephanie Capaldi about the problem before the quiz deadline).

Note- Students sometimes experience problems submitting assignments through D2L. Make sure that you receive confirmation of your submission (keep this for your <u>It is your responsibility</u> to ensure that your work is correctly records). submitted, therefore, give yourself adequate time before the deadline to submit and receive confirmation of submission (submitting work too close to the deadline is most likely to lead to submission problems). Also, make sure you submit the final or correct draft!, only assignments submitted in the drop box on time can be graded without penalty (if you submit the wrong draft and don't submit the correct version on time penalties above will apply if you wish the correct version to be graded. If the correct version is not submitted within 72hrs after the deadline we can only assess the version submitted in the drop box). IF YOU DON'T RECEIVE CONFIRMATION OF YOUR SUBMISSION IN ADEQUATE TIME BEFORE THE DEADLINE OR IF YOU ARE UNSURE, FOR ANY REASON, THAT YOUR SUBMISSION HAS WORKED, E-MAIL YOUR ASSIGNMENT IMMEDIATELY TO STEPHANIE CAPALDI. IF IT IS RECEIVED BEFORE THE DEADLINE IT WILL BE GRADED WITHOUT PENALTY. THEREFORE, ASSIGNMENTS RECEIVED AFTER THE DEADLINE DUE TO ANY D2L PROBLEMS OR ANY OTHER COMPUTER PROBLEMS EXPERIENCED DURING SUBMISSION WILL BE PENALIZED OR NOT GRADED (SEE ABOVE).

GRADING SCALE AND POLICIES: <u>DURING</u> THE COURSE THE STANDARD **NUMERICAL SCALE (I.E. 90-100% = A ETC)** <u>WILL NOT</u> BE APPLIED. Your scores for each assessment will appear as a percentage in your gradebook. After the major assessments (the two lab reports and tests) have been graded the <u>approximate</u> letter grades corresponding to the percentages will be posted so that you can better judge your score. Thus, these letter grades will not follow the numerical scale, but instead will reflect your performance compared to the rest of the class.

REQUESTS FOR INCOMPLETE (I) OR WITHDRAWAL (W) must be made in accordance with University policies, which are available at http://catalog.arizona.edu/policy/grades-and-grading-system#incomplete and http://catalog.arizona.edu/policy/grades-and-grading-system#incomplete and http://catalog.arizona.edu/policy/grades-and-grading-system#incomplete and http://catalog.arizona.edu/policy/grades-and-grading-system#incomplete and http://catalog.arizona.edu/policy/grades-and-grading-system#Withdrawal respectively.

DISPUTE OF GRADE POLICY: Exam regrade requests must be made within the time frame posted with the exam results on D2L. No regrades will occur after the posted

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deadline. For all other assignments, any queries/disputes must be emailed to Stephanie Capaldi within <u>1 week</u> after your feedback is posted.

CHANGES IN THE COURSE CALENDER: This is a lab course and therefore we cannot predict the outcome of the experiments and as a result there may be changes in the schedule of certain experiments. Such changes may impact assignment deadlines and test dates. As a result, the course calendar on the course site may change. The MCB473/573 staff will make every attempt to give you as much advance notice as possible of any changes in the schedule. **Please check the website frequently for updated information.**

EXPERIMENTS OUTSIDE OF CLASS HOURS: Unfortunately, some experiments cannot be stopped between lab sessions. Sometimes you may be required to come to lab on a day and time outside your scheduled lab session. We appreciate that you have other classes so there will only be a few of these special days during the semester, and they will be as brief as possible.

BREAKS DURING THE LAB SESSIONS: During the lab sessions there will often be some 'downtime' during which you are waiting for completion of a procedure. Use this time wisely. You can update your notebooks, discuss your experiments with your partner or the TAs, discuss any difficulties you are having with the lecture material or problem sets. You may also use this time to take a break or get some food, however, please check with a member of staff first as important instructions or a discussion may be taking place during the break.

LAPTOPS & CELL PHONES: Please silence your cell phones and refrain from interacting with your text message service during lecture. Do not interact with your text messaging service or make or receive calls in the lab <u>unless it is an emergency</u> (go outside of the lab to deal with such situations). Laptops are not required during the lab sessions except on a few occasions when you will be given prior notice. Please do not use electronic devices at your lab bench, you may spill a chemical onto your device that may damage it or may be toxic or hazardous. If you do need to access your phone, please follow the rules in the lab safety and practice section of this document. For lab you are required to print out a copy of the manual to follow the protocols, this should remain in your bench drawer.

THREATENING BEHAVIOR POLICY: The UA Threatening Behavior by Students Policy prohibits threats of physical harm to any member of the University community, including to oneself. See http://policy.arizona.edu/education-and-student-affairs/threatening-behavior-students.

UA NONDISCRIMINATION AND ANTI-HARASSMENT POLICY: The University is committed to creating and maintaining an environment free of discrimination; see http://policy.arizona.edu/human-resources/nondiscrimination-and-anti-harassment-policy. Our classroom is a place where everyone is encouraged to express well-formed opinions and their reasons for those opinions. We also want to create a tolerant and open environment where such opinions can be expressed without resorting to bullying or discrimination of others.

ACCESSIBILITY AND ACCOMMODATIONS: At the University of Arizona, we strive to make learning experiences as accessible as possible. If you anticipate or experience barriers based on disability or pregnancy, please contact the Disability Resource Center (520-621-3268, <u>https://drc.arizona.edu/</u>) to establish reasonable accommodations.

Our goal in this classroom is that learning experiences be as accessible as possible. If you anticipate or experience physical or academic barriers based on disability, please let the course instructor know immediately so that we can discuss options. You are also welcome to contact the Disability Resource Center to establish reasonable accommodations.

If you have reasonable accommodations, please plan to meet with the course instructor during office hours or by making an appointment to discuss accommodations and how the course requirements and activities may impact your ability to fully participate.

Please be aware that the accessible table and chairs in this room should remain available for students who find that standard classroom seating is not usable.

- All exam and certain classroom accommodations will only be granted if we
 receive appropriate documentation from the DRC. Therefore, the DRC must be
 notified of all accommodations as soon as possible so that they can verify and
 then supply us with all the necessary information. <u>The DRC must be notified of
 special accommodations at least 2 weeks before the first test.</u>
- No accommodations will be made retroactively (as stated by the Dean of Students Office and DRC).
- We realize that unforeseen issues may arise during the semester that may impact your performance in this course. Again, please see the course instructor as soon as possible so that we can try to find a good solution that works for all concerned.

MCB473/573 Lab Safety and Practice (adapted from the 'Introduction to Experimental Biology and Communication course (7.02) from Massachusetts Institute of Technology

General Safety Rules

1) No eating or drinking in the lab. No food wrappers or cartons can be thrown into the trash bins in the lab, this includes water bottles.

2) Place all bags/coats under the benches in the communal areas. No bags can be left in the aisles between benches.

3) Wear a labcoat.

4) Wear appropriate clothing.

-No open toe shoes, wear sensible shoes

-Tie long hair back

-We advise you to wear long pants in the lab. Long pants are part of PPE requirement for hazardous chemical use in the Lab Chem Safety Manual.

5) Use gloves anytime you are working at the bench, this protects you and prevents you from contaminating your experiments. You are required to wear a lab coat for this class. Remove all protective clothing and gloves before leaving the lab.

6) Glove rules

- Remove them to handle non-lab equipment. e.g phones, elevator, doors, computers

- Don't touch your face etc. with your gloves when working at the bench. Do not wear gloves outside of the lab.

7) Be aware of your bench space. You may have little room to work in and may have to share a bench with 3 others. Take care when using tips and pipettes so that you don't stab your bench mate! Keep your bench space clean and free of clutter. This may have a huge impact on the outcome of your experiments!

8) No smoking. There is a universal ban on smoking in all University buildings.

9) Wash your hands with soap at the designated sink in the lab before you leave the lab after each laboratory session. Of course, you must also wash your hands as appropriate during the lab sessions e.g. accidents (see below).

Dealing with accidents/injury

1) All injuries or accidents no matter how minor must be reported immediately to the instructor. OSHA requires that "close call" that could have led to significant harm or injury also be reported.

2) Eyewash. If you get a chemical or recombinant material in your eye immediately wash your eye using the eyewash (remove contact lenses first) and then inform the instructor.

3) If you spill hazardous chemicals on yourself immediately wash the affected area, which may require using the shower in the lab.

4) If you spill a chemical on the floor or bench etc. inform a TA immediately, they will clean up the spill. The location of the spill kits, eye wash, shower and first aid kit will be pointed out to you on the first day of class.

Specific Safety Hazards associated with MCB473/573

1) Caustic/toxic chemicals. Pay special attention to safety warnings regarding caustic and/toxic chemicals used on certain days. Use all appropriate precautions. When using

particular hazardous chemicals you will be instructed to use them in a special designated area of the lab such as the fumehood. The chemical inventory and MSDS for this course can be accessed online.

- 2) EtOH jars. If you set your EtOH jar on fire do the following;
- a. Do not panic
- b. Cover the jar (e.g. with a glass lid)
- c. Do not pick the jar up, it will be scorching
- d. Inform a T.A immediately if the fire gets out of control

3) UV radiation. You will be using UV light to visualize DNA. UV light can cause extensive and irreversible damage to eyes and skin. If you have to use an open UV source always wear the safety shields next to the UV transilluminators and a lab coat to protect your arms.

Disposal of Pipetteman tips

All used pipette tips must be collected separately from tubes in the labeled 'tip waste' receptacles on your benches. When this beaker is full its contents must be disposed of in the large cardboard bin labeled 'tip waste'.

Procedures for handling/disposal of hazardous or toxic chemicals/waste

Handling toxic or hazardous chemicals- Before you come to lab each day you must carefully read over the protocols for the day in your manual. If hazardous chemicals are part of a protocol they will be discussed before you begin your experiment. Here, you may be required to wear special clothing e.g. safety glasses together with your lab coat, and if applicable follow specific disposal instructions of hazardous waste. Disposal of toxic or hazardous chemicals- Certain chemicals cannot be disposed of down the sink. Chemicals that cannot be disposed of down the sink are collected in LABELLED waste bottles in the hood. Certain chemicals are incompatible and cannot be collected in the same bottle. ALWAYS READ THE LABEL ON THE WASTE BOTTLE BEFORE ADDING YOUR CHEMICAL. IF IN DOUBT ASK A TA. IF YOU ACCIDENTALY ADD THE WRONG CHEMICAL TO A WASTE BOTTLE IMMEDIATELY INFORM A TA, PARTICULAR COMBINATIONS OF INCOMPATIBLE CHEMICALS CAN BE EXPLOSIVE. The instructor or TA will discuss the procedure for disposing of hazardous chemicals before you begin an experiment.

Procedure for handling/disposal of bacteria and yeast cultures-Wear gloves. Lysol or bleach must be added to cultures or media, left for a few minutes and then poured down the sink. After a procedure involving bacteria or yeast do not leave media waste on the bench, by the next day it will be full of microrganisms that may ruin subsequent experiments. Gloves and plates that have contacted yeast or bacteria, and tubes containing certain recombinant DNA materials must be placed in the biohazard waste bin.