MICR/MBMB 480 FALL 2016

Mondays and Wednesdays 2:00-6:00 pm (Primary)

Tuesdays and Thursdays (As needed, limited time frames)

GRADING, SCHEDULE & LAB RULES

Instructors:

Dr. Derek Fisher	Office: Life Science III room 1007
Dr. Bethany Rader	Office: Life Science II room 191
Dr. Douglas Fix	Office: Life Science II room 139

Grading: Each of the three instructors will be in charge of one third of the course. There will be 100 points for each third of the course, awarded as follows:

Exam	25
Lab Reports and Assignments	40
Quizzes (3)	15
Laboratory Skills	20
Total Possible Points	100

Part of your grade will be the instructors' assessment of your lab skills. This is not based on attendance (**since you are expected to attend every lab**), but on how well you follow the directions, how you work with others in a group, and how well you take responsibility for the materials and equipment you use in class. Please note that you will not be penalized in this area if one of your experiments fails. How you conduct the experiment is more important than the outcome.

Lab courses involve a great deal of planning and preparation and also consume expensive materials. There will be no make-ups for any labs, exams, or reports. Any unexcused absences will result in deducted points. You must provide yourself with a lab coat and with marker pens that write on glass and plastic.

Laboratory Rules

Safety is our main concern in the lab. Every student must wear a lab coat at all times. No sandals or open-style footwear is allowed (fragments of broken glass are often found on lab floors – and can be extremely painful!). Always follow directions carefully and especially be aware of the **CAUTION** notes in your lab manual. You will be told in your lab manual to wear gloves and/or eye goggles during certain procedures -- do so. There is to be absolutely no eating, drinking, gum chewing, or applying of cosmetics in the lab at any time. Anyone not following these directions will be asked to leave immediately -- there are no exceptions.

One of the instructors will be present during each lab period to explain what is to be done that day, discuss concepts, and answer any questions or problems. We may occasionally exit the lab – this is a senior level lab course and you are expected to develop some independence and to work cooperatively with your lab partners. You will always be able to find us during lab periods either in our offices or labs. Please feel free to come and talk to us, especially if you are experiencing difficulties during the lab period (when you aren't actually in the middle of an

experiment, of course). Remember, we will not know that there is a problem and will be unable to address it unless you inform us. We also welcome well-thought-out suggestions or comments on the lab or about the concepts covered in the lab manual.

As mentioned above, how you conduct the experiment is more important than the outcome. Keep this in mind during those times (and there are bound to be a few) when things don't seem to be working. If you conscientiously follow directions and think about what you are doing (both before and during the experiment), you should master those skills expected of you in this lab. But if you're sure you did everything right and your experiment still doesn't work, don't despair. There is such a thing as the fickleness of lab experiments and it happens at some time to everyone. That is why you are graded on your effort and not necessarily on your outcome. If, however, none of your experiments work, some reexamination of your lab technique may be in order.

Units & Conversion Factors etc.

Metric Prefixes

M = mega = 10^6 k = kilo = 10^3 m = milli = 10^{-3} µ = micro = 10^{-6}
n = nano = 10^{-9} p = pico = 10^{-12} f = femto = 10^{-15} a = atto = 10^{-18}

Conversions

1 µg of a 1-kb DNA fragment = 1.5 pmol; 3.0 pmol ends
1 pmol of a 1-kb DNA fragment = 0.66 µg
10 kDa protein ≈ 270 bp DNA
1 kb dsDNA = 6.6×10^5 Da

Nucleic acid	Concentration (µg/ml) for 1 A_{260} unit
dsDNA	50
ssDNA	33
ssRNA	40
oligos	20

Dye migration in polyacrylamide denaturing gels

Size of DNA fragments (in bp) with which the dyes would migrate

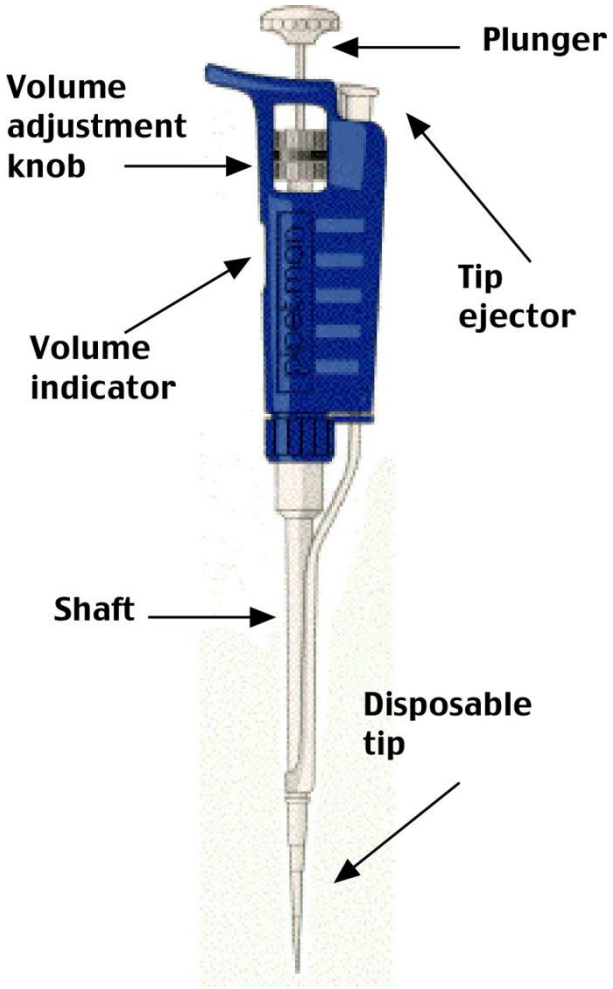
% Gel	BPB	Xylene Cyanol
6	26	106
8	19	75
10	12	55

Using a Pipetman

Molecular biology experiments often require accurate and precise transfer of very small volumes of liquids. Many laboratories use an adjustable pipeting device called a Pipetman (Rainin Instrument Co.) for these transfers. There are several different models, each having a specific range of volumes. Use the Pipetman appropriate for the volume you want to transfer for the greatest accuracy. Different plastic disposable tips are used for different models. Tips must seal properly on the shaft for an airtight seal in order to get the desired volume. Proper technique must also be used.

Instructions for Use

Model	Volume Range	Tip Color
P-2	0.1 – 2 µl	clear
P-10	0.5 –10 µl	clear
P-20	2 – 20 µl	yellow
P-100	10 – 100 µl	yellow
P-200	50 – 200 µl	yellow
P-1000	100 – 1000 µl	blue



1. Hold the Pipetman vertically in one hand. With the other hand, turn the volume adjustment knob until the volume indicator is 1/3 turn above the desired volume, then adjust the volume down to the desired value. The volume indicator shows µl, with the black numbers showing whole µl and the red numbers showing 10ths (some also show 100ths of µl). For example, to transfer 5.5 µl using a P-10 Pipetman, the volume indicator should show: 0 (black) 5 (black) 5 (red)

2. Place a clean and/or sterile pipet tip securely on the shaft.

3. Smoothly press the plunger down with your thumb to the FIRST STOP.

4. Immerse the tip below the surface of the sample.

5. Allow the plunger to return smoothly and slowly to up position. Do not let the plunger snap up.

6. Look at the sample in the tip, there should be no air bubbles present.

7. Withdraw the tip from the sample. If liquid clings to the outside of the tip, remove it by gently touching the tip to the inside of the sample container. Keep the Pipetman vertical.

8. To dispense the sample, touch the tip end against the side wall of the receiving vessel and depress the plunger slowly to the first stop, wait 1-2 seconds (longer for viscous solutions), then depress the plunger completely to the second stop to expel any residual liquid in the tip.

9. Keep the plunger fully depressed and withdraw the tip from the vessel carefully, keeping the tip against the vessel wall.

10. Allow the plunger to return to the up position, then discard the tip by depressing the tip ejector button.

Laboratory Reports

As indicated in the scoring for the course, your performance on the laboratory reports/assignments constitutes more than 40% of your grade. Laboratory reports and assignments are considered individual projects; that is, although you will be working in groups during lab, you must write your own lab report.

All lab reports must be typewritten, single-spaced, in 12 point Arial font. Graphs and tables may be hand-drawn or computer-generated. Reports must be received by no later than **5:00 p.m. on the due date. Late reports will be assessed a point penalty – 5 points will be deducted from the score for every day past the deadline.**

Laboratory reports are due on the following dates and worth the points listed:

Laboratory Report/Assignments	**Due Date**	**Points**
Beta Galactosidase Assay	Sept 14	15 – short
Growth Rate & Cell Constituents	Sept 28	25 – complete
Estimated Dates for Later Write Ups		
Rader lab report 1	Oct 19	10 – short
Rader lab report 2	Nov 2	30 – complete
Fix lab write up	Dec 7	40 – complete

Short laboratory report (10 points for results, 5 points for discussion). Include only the results and a brief discussion, which should be no more than one page.

Complete laboratory reports will be formatted as in standard scientific publications (see description below and the handouts) and will include the following sections:

Conclusion = Summary = Abstract: One paragraph summarizing **YOUR** results. Did your experimental approach successfully test your hypothesis? Summaries of scientific papers are often distributed without the rest of the paper. **The reader should therefore be able to understand your summary on its own.**

Introduction: 2-4 concise paragraphs that describe the experiment and its objectives (*i.e.* the questions you are asking, the hypotheses you are proposing *before* you actually start the experiment). Not to exceed one page.

Materials and Methods: Include any additions to or changes from the procedure in the Lab Manual. Apart from this just say: "Please refer to MICR 480 Laboratory Manual".

Results: 4-5 paragraphs describing the results (either for your group or the entire class, depending on the experiment). Where possible, summarize results in tables or graphs. Number and provide a title for each table or graph. Refer to them by their number or title in the text. Provide a legend for each figure describing what the data refer to and a key for any abbreviations or symbols used. Footnotes may be used below tables. Graphs may be plotted by hand or computer-generated. Table titles should be placed at the top of the table. Figure titles and legends should be placed below or immediately adjacent to the figure (not above the figure).

Discussion: Discuss the principles of the experimental approach. Explain **YOUR** results with reference to these principles and any hypotheses you proposed in your Introduction. The Discussion should also include possible explanations for any problems you encountered, e.g. if you got only negative results. Not to exceed 2 pages.

Example Summary:

Under anaerobic conditions *Escherichia coli* converts pyruvate to D-lactate by an NADH-linked lactate dehydrogenase (LDH). The *ldhA* gene, encoding this LDH was cloned using λ10E6 of Kohara's collection as the source of DNA. The *ldhA* gene was sequenced. The *ldhA* gene of *E. coli* was highly homologous to genes for other D-lactate specific dehydrogenases, but unrelated to those for L-lactate specific enzymes. We disrupted the *ldhA* gene by inserting a kanamycin resistance cassette into the unique *KpnI* site within the coding region. When transferred to the chromosome, the *ldhA*::Kan construct abolished the synthesis of the D-LDH completely. When present in high copy, the *ldhA* gene was greatly over-expressed suggesting escape from negative regulation.

Example Table:

TABLE 4. LDH Activity of Regulatory Mutants

	LDH (specific activity*)			
Strain	No buffer	HEPES pH8.0	TAPS pH8.5	AMPSO pH9.5
NZN130 (Parent)	640	310	250	71
NZN131 (Mutant)	1690	850	1050	1050
NZN132 (Mutant)	690	780	710	190

*Specific activity in nmoles of NADH min^{-1} mg^{-1} protein.
All cultures were grown anaerobically in rich broth plus 0.4% glucose for 6 hours at 37°C. Buffers were used at 100 mM.

Note: Put units in the headings of the table or of individual columns (or in footnotes if too long to fit in headings) instead of repeating them on each line of the table. Round off numbers to 3 or 4 significant figures.

Schedule of Experiments, Fall 2016

General Comments

You will sometimes be required to come in at non-scheduled times to perform various chores such as taking your plates out of the incubator or putting your overnight cultures in the cold room. You may lose points if your experiment is ruined because you forgot to come in to lab to check on something, so don't forget.

Keep good records of all of your results, including culture densities, plate counts, etc. Also note any unusual results in your manual (for example, if your culture seems to take much longer than anyone else's to double). Many discoveries rely on *seemingly trivial details*.

All dilutions are to be done in sterile saline unless otherwise instructed. Incubate all liquid cultures in a shaking water bath and incubate plates upside down in the incubator. Centrifugation is to be done only by the instructor. On a regular basis remove and discard plates or cultures in the cold room that you no longer need.

Detailed Timetable

Each week of classes has FOUR lab days. Of these, Monday and Wednesday are the formal lab periods and Tuesday and Thursday are the intervening days when you may have to inoculate cultures, examine plates, etc.

Week 1: Dr. Fisher's Section: Basic Techniques (BT) and Genotypes and Phenotypes (GP)

Mon 22 Aug	•Dilutions, streaking, and spreading (BT)
Tue 23 Aug	•Count colonies (BT)
Wed 24 Aug	•Genotypes and phenotypes (GP)
Thu 25 Aug	•Record results (GP)

Week 2: Dr. Fisher's Section: Spontaneous and Chemical Mutagenesis (SCM)

Mon 29 Aug	•Select mutants by several approaches
Tue 30 Aug	•Count colonies (SCM)
Wed 31 Aug	•Analyze mutants for growth defects (SCM)
Thur 1 Sep	•Check plates for growth, record results and let incubate over break

Week 3: Dr. Fisher's Section: Assay of Beta-Galactosidase (BG)

Mon 5 Sep	**Labor Day**
Tue 6 Sep	•Check results of mutant analysis (SCM)
Wed 7 Sep	•Induce *lac* operon and assay beta-galactosidase (BG)

Week 4: Dr. Fisher's Section: Growth Rate and Cell Constituents (GRCC)

Mon 12 Sep	•Growth rate determination by optical density and viable counts (GRCC)
Tue 13 Sep	•Store viable count plates in cold room (GRCC)
Wed 14 Sep	•Chemical assays for protein and RNA (GRCC)

First Lab Report Due (15 points - SHORT report) Wed Sep 14

Week 5: First Exam and Start of Dr. Rader's Section: Exercise 1 and Article Assignment

Mon 19 Sep First Exam

Wed 21 Sep •Experimental Design Discussion
•Media preparation (Ex. 1)

W**eek 6: Dr. Rader's Section: Exercises 2-3**
Mon 26 Sep •Conjugation of *Serratia* and the *E. coli* WM20767/pRL27 transposon (TN) donor (Ex. 2)
Wed 28 Sep •Selection of transposon induced *S. marcescens* mutants (Ex. 3)

Second Lab Report Due (25 points - COMPLETE report) Wed 28 Sep

Week 7: Dr. Rader's Section: Exercises 4-8
Mon 3 Oct •Calculation of TN insertion frequency and phenotypic analysis of pigment mutants (Ex. 4)
•Extraction of gDNA from *S. marcescens* TN pigment mutants (Ex. 5)
Tue 4 Oct •Restriction enzyme digestion of *S. marcescens* TN mutant gDNA (Ex. 6)
Wed 5 Oct •Gel electrophoresis analysis of digested TN mutant DNA (Ex. 7)
•Precipitation of digested DNA and ligation (Ex. 8)

Week 8: Dr. Rader's Section: Exercises 9
Mon 10 Oct Fall Break
Wed 12 Oct •Transformation of ligations into *E. coli* DH5α λpir cells (Ex. 9)

Week 9: Fall Break and Dr. Rader's Section: Exercise 10-12
Mon 17 Oct •Selection of *E. coli* transformants for plasmid preparation (Ex. 10)
Tue 18 Oct •Plasmid preparation of DNA for sequencing (Ex. 11)
Wed 19 Oct •DNA sequence analysis and chromosomal location of TN insertion determination (Ex. 12)

SHORT report for third lab (10 points) due Wed Oct 19

Week 10: Dr. Rader's Section: Second Exam
Mon 24 Oct Second Exam

Week 11: Start of Dr. Fix's Section: Exercise 1
Oct 31-Nov 3 •Exercise 1: Chemical Transformation and Electroporation of Competent *E. coli* Cells with Plasmids (pUC19 and pGFP)

COMPLETE report third lab (30 points) due Wed Nov 2

Week 12: Dr. Fix's Section: Exercise 1 and 2
Nov 7-10 •Exercise 1 continued
•Exercise 2: Biotechnology-Biosynthesis of Indigo by Recombinant *E. coli*

Week 13: Dr. Fix's Section: Exercise 3
Nov 14-17 •SDS-PAGE

Week 14: Dr. Fix's Section: Exercise 3 and Thanksgiving Break

Nov 21-22 •Exercise 3 continued

Nov 23-27 Thanksgiving Break

Week 15: Dr. Fix's Section: Exercise 4

Nov 28 - Dec 1 •Forensic DNA Analysis

Week 16: Dr. Fix's Section: Third Exam

Mon 5 Dec Third Exam

Wed 7 Dec Final Lab Report Due (40 points-complete report)