BIO 388 Molecular Genetics
Spring 2016
Course Information

Professor: Colleen Jacks
Office: 233 NHS, X7326
Office hours: M 10:30 – 11:20 AM, WF 12:30 – 1:30 PM or by appointment. I am usually available immediately after class during chapel.
Class Schedule: MTWF 9:00-10:00 AM, NHS 305
Lab: Scheduled M 2:30-6:30 PM or T 1:30-5:30 PM, NHS 237, plus unscheduled time at your convenience.
Required:

- *Molecular Biology, Principles and Practice, 2nd edition* by Cox, Doudna and O'Donnell
- 1.5 – 2 inch 3-ring binder with tabbed dividers to use as your lab notebook. You will need 3 hole punched and lined theme paper for your lab notes.
- The lab manual will be available in modules on the course Moodle site. You will need to print out the protocols supplied; additional supporting material can be printed at your discretion.

Recommended Textbook: *Writing Papers in the Biological Sciences* by McMillan (This was required for BIO 201, 202)
Additional course information and materials: Course announcements will be sent by email to your Gustavus account; it is your responsibility to check for these. Course materials (PowerPoints, lab handouts, etc.) will be posted on the course Moodle site.

Course objectives:

- To understand how genes work at the level of DNA, RNA, and proteins.
- To enhance your working knowledge of molecular biological techniques and data analysis, both in theory and in practice.
- To obtain information and analyze data from the primary scientific literature in addition to your textbook. More broadly, to encourage your development as independent learners.
- To practice scientific writing skills in the form of journal-style lab reports.

Textbook readings:

Molecular Biology is a text book that provides an excellent overview of the discipline. We'll delve into more depth on some topics, using figures from papers that helped establish our current understanding of gene expression and regulation. Several of these papers are assigned in their entirety. Each chapter starts with a scientist describing a 'moment of discovery' to provide you a look into the human side of discovery; the chapters end with reports from specific research papers ("How We Know") to provide examples of the primary studies that are incorporated into our understanding.

Because my course is focused on gene expression and regulation, some chapters and chapter sections in the book will not be covered. I recommend that if you plan to work or continue in graduate school in this field that you keep the book and do some "summer reading" of these chapters. The experimental techniques used in cloning and studying genes and their expression will be introduced in both class and lab.

Papers:

These papers were selected to help you develop further your skill in reading the primary literature, as well as provide examples of the use of techniques relevant to the course and the data supporting many concepts in genetics today. They will be discussed in class with the appropriate lecture material, and will be covered in some form on the exams (for example, interpretation of data presented in a particular figure). There may also be written assignments covering specific papers. When papers are discussed in class everyone is responsible for having read the paper; individuals may be assigned specific figures to

describe to the rest of the class. The papers are listed below and will be available on the course Moodle site. Additional papers relevant to lab will also be posted; additional papers relevant to class may be added over the semester.

Noller, H. F., Hoffarth, V., and Zimniak, L. (1992) "Unusual Resistance of Peptidyl Transferase to Protein Extraction Procedures." *Science* 256:1416-1419.

R. K. Saiki, et al. (1988) "Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase." *Science* 239:487-491.

Ross, M.T. et al. (2005) "The DNA sequence of the human X chromosome." *Nature* 434:325-337.

Ross, W. et al. (1993) "A Third Recognition Element in Bacterial Promoters: DNA Binding by the α Subunit of RNA Polymerase." *Science* 262:1407-1413.

A. Fire, et al. (1998) "Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*." *Nature* 392:806-811.

Chen et al. (1994) "Assembly of Recombinant TFIID Reveals Differential Coactivator Requirements for Distinct Transcriptional Activators." *Cell* 79:93-105.

Problems:

The end-of-chapter problems in your text book and those provided in class are **recommended** to help you test your knowledge and understanding of the course material and apply this knowledge to new situations. Answers are provided at the end of the text book. I will select one or more of these problems to use directly (or with slight modification) on each exam for10 points of the 50 total points.

Exams:

Exams will be of the problem-essay-short answer variety. They will contain questions that require you to apply knowledge in addition to traditional "tell me everything you know about..." questions. I attempt to write the questions so that more than one approach is acceptable in the answer. For example, in the past the BMB majors have taken a more physical approach to the material than the biology majors on the exams.

We will go through a large amount of information during the semester and it would be unreasonable to expect you to be the 'expert' on every aspect of every topic. On the other hand, this is an upper level course and it is not enough for you to 'know it when you see it' or provide a one phrase summary ('DNA is the hereditary material.') My expectation is that you will demonstrate the depth and extent of your knowledge and your ability to apply what you learned in one context/example to a second, new situation. Questions on the exams may also come from the lab as specified on the course schedule; the techniques covered in lab are central to the discovery of information in the field and you will be held responsible for an understanding of how experiments were performed. Examples of past exam questions will be posted on the course Moodle site or used in class.

Exams are scheduled in the evenings on Wednesdays to allow you ample time to complete your answers to your satisfaction. You should allow yourself two hours - more if you know yourself to be a slow test taker. I am open to giving the exam earlier in the day to those students who have evening conflicts or prefer an earlier exam time. You must take the exam in a single sitting - I don't allow people to start and come back at a later time to complete the exam. The fourth and last exam is given during the scheduled final period but is a unit exam and not comprehensive.

Academic Integrity:

One of the objectives of Gustavus Adolphus College as stated in the mission statement is to "foster the development of values as an integral part of intellectual growth." In a community of scholars nothing is more valuable than the intellectual work or property of a member of the community. The college's honor code (*On my honor, I pledge that I have not given, received, nor tolerated others' use of unauthorized aid in completing this work.)* must be written and signed on the first exam and assignment of the semester; this will also indicate your pledge to comply with the code for all subsequent assignments during the semester. On exams, any assistance from individuals or sources except the course instructor (Jacks) is forbidden. On graded assignments (including lab work), discussion and collaboration is encouraged, BUT the final work/wording turned in for grading must be only that of the individual unless directed otherwise in class. Assignments that are essentially identical with only a few changes of words are not acceptable. It is unacceptable in this course to represent the work of another individual as your own. All cases of academic dishonesty including cheating on exams and plagiarizing written assignments will result in penalties up to and including automatic failure of the course and will be reported to the Provost for inclusion in your permanent file and disciplinary action as stated in the student code.

Academic Support Services:
Gustavus Adolphus College is committed to ensuring the full participation of all students in its programs. If you have a documented disability (or you think you may have a disability of any nature) and, as a result, need reasonable academic accommodation to participate in class, take tests or benefit from the College's services, then you should speak with the Disability Services Staff, for a confidential discussion of your needs and appropriate plans. Course requirements cannot be waived, but reasonable accommodations may be provided based on disability documentation and course outcomes. Accommodations cannot be made retroactively; therefore, to maximize your academic success at Gustavus, please contact Disability Services as early as possible. Disability Services (https://gustavus.edu/advising/disability/) is located in the Academic Support Center. Disability Services Coordinator, Kelly Karstad, (kkarstad@gustavus.edu or x7138), can provide further information.

Support for English learners and multilingual students is available through the Academic Support Center's Multilingual Learner Academic Specialist Jody Bryant (jbryant2@gustavus.edu or x7197). The MLAS can meet individually with students for tutoring in writing, consulting about academic tasks, and helping students connect with the College's support systems. When requested, the MLAS can consult with faculty regarding effective classroom strategies for English learners and multilingual students. The MLAS can provide students with a letter to a professor that explains and supports appropriate academic arrangements (e.g., additional time on tests, additional revisions for papers). Professors make decisions based on those recommendations at their own discretion. In addition, English learners and multilingual students can seek help from peer tutors in the Writing Center (www.gustavus.edu/writingcenter/).

Title IX and Cleary Act Compliance:
Title IX legislation states that violence and harassment based on sex or gender are civil rights violations. As a faculty member, I am mandated to report incidents of sexual misconduct or sexual harassment. This requirement is to make sure the College can support and protect students. For more detailed information please see: https://gustavus.edu/deanofstudents/policies/gustieguide/

I also am required by federal and state regulations to report campus crime to Campus Safety. Disclosure to me is voluntary, but not sharing information will hinder timely warnings to the campus, the ability to respond to crime and accurate disclosure of campus crime statistics.

Thanks in advance for any situation you want to share and for trusting in me to support students.

Electronics:

As a courtesy to your instructor and classmates, please turn your cell phones, pagers or other noisy devices OFF during class and refrain from texting. I prefer that laptops be used only for announced in-class assignments. Cell phones and other electronics are not allowed on your desk or turned on during tests – only calculators are allowed. If you forget your calculator one will be available for your use.

Policy on Late Work:

I will accept most late work for appropriate reasons if negotiated with me in advance. In cases where your work is to be peer reviewed, due dates are not negotiable. There will be a penalty for work turned in late – more extreme if we haven't discussed the situation in advance. Typically you will lose 10-20% of the point value of the assignment for each day it is late.

Labs:

The lab this semester will focus on work to characterize two ribosomal protein (*RP*) gene families in the plant *Arabidopsis thaliana*, introducing you to, or giving you additional experience with, common experimental methods in molecular genetics. Each group this year will have one *RP* gene to investigate.

Biological experimentation is messy and unpredictable. Lab is scheduled for four hours on Monday or Tuesday afternoons; two lab periods (Weeks of March 21 and May 9) have not been schedule and will be used only if necessary. The major amount of work on an experiment can be accomplished in the four hours, but you will sometimes run over the lab period, or need to come back for several hours during the week, for example, to start a bacterial culture necessary for the next day's work. Since you will be working in groups of 2-3 people this should not be a problem.

The attached planned lab schedule outlines my best estimate of what we will accomplish each week, but as successful results may be necessary to move your project forward, it's possible that we will be altering the schedule as needed. Note that you will typically be "multi-tasking" – performing steps for usually two of the different investigative threads. The protocols for these labs will be posted on the course Moodle site. They are generally very detailed to help you efficiently use your time in lab (including 'helpful hints' on how to increase the probability of 'good' results). Some lab methods you use will require you to follow protocols provided by manufacturers of our reagents; these make the assumption that you have familiarity with this type of lab work. **It is important that you think about what you are doing in the lab and why** - blindly following the protocol sentence by sentence can result in errors that will require you to back track and redo parts of your work, leading to much longer lab days than you'll want. Some of your class assignments will be focused on pre-lab preparation.

WRIT D for Lab Reports:

The writing that allows this course to be a WRIT-D will come from the lab. There will be three report opportunities to present your group's lab results. In many research labs, the writing of a paper for journal submission is not performed by one individual - different lab members are assigned to draft specific sections or subsections of a paper depending on how and where they contributed to the work to be reported. The drafts are pasted into a single document and one individual (oftentimes the lab head) takes the responsibility of editing and "massaging" the sections into a cohesive manuscript, smoothing out the stylistic differences of the contributors. This is the model we will use for writing this semester.

The writing of each paper will be divided among the two or three members of the lab group so that each group member will have personally written all sections of a paper. (I will talk with any groups with two members on how we will alter the assignments to accommodate their smaller group.) Groups will meet initially to discuss what should be reported in each section and perform any data analysis together, and

then separately write their specific section. I will grade each of the sections individually to provide you feedback on your specific section; the sections will be shared among the group members at the same time to provide peer review of the sections too. The reviews will be turned into me (to document completion of the review) and returned by me to the authors; the 3 members of the lab group will then 1) revise their section based on review comments (these will be resubmitted separately to me); 2) place all three pieces into a single document which will be reviewed by each member of the group; and 3) meet to discuss the paper as whole. Based on the discussion, one person (different each time) will be responsible for editing the final version of the paper into a cohesive product, performing any additional writing, etc. necessary. All members of the group will individually evaluate each member's participation in the development of the paper.

The labs we are performing take a number of weeks to complete. To start things off and provide you early feedback on your writing, everyone will write a "generic" introduction which will be due on **February 24**. I'll return these with comments for you to rewrite for inclusion in the lab report for which you are responsible for the introduction.

First draft assignments:	**Lab Report 1**	**Lab Report 2**	**Lab Report 3**
Introduction			
Materials and Methods			
Abstract, Results and Discussion (This group member will serve as the "editor" of this paper)			

Note: Each group member is expected to provide appropriately cited and referenced sources for their section; the paper title is for the group to discuss and the "editor" to determine.

Plagiarism of reports is totally unacceptable in any form. Please acquaint yourself with what constitutes plagiarism by reading pages 29-30 and 124-125 in McMillan (4th edition). Cases of plagiarism that are discovered will result in penalties up to and including an automatic F in the course. As this is a WRIT D course, you must turn in the required lab reports to receive credit for the course.

Target Schedule for Full Papers

Full descriptions of each report will be given in class approximately one week before the first draft is due. The dates shown are target dates – they are open to group negotiation. For each report there will be a group work evaluation that **must** be turned in by each group member at the time of the "complete paper submission" to earn the points associated with the rewrite; group evaluations will be considered in determining final grades in the course. The "complete paper submission points" noted below are only earned for your editing turn.

Lab Report 1: Southern Blot	**Target Date**
• Initial report planning meeting	March 9-12
• Submit original draft of your section	March 16
• Graded draft returned (10 points)	March 22

• Revised draft of your section due (20 points)	April 8
• Assembly of complete report	April 8-10
• Complete paper submitted by editor (15 points)	April 15
Lab Report 2: pENTR to pMDC Cloning	
• Initial report planning meeting	April 5-8
• Submit original draft of your section	April 18
• Graded draft returned (10 points)	April 25
• Revised draft of your section due (20 points)	April 29
• Assembly of complete report	April 29-May 2
• Complete paper submitted by editor (15 points)	May 6
Lab Report 3: GFP or qRT-PCR Gene Expression Analysis	
• Initial report planning meeting	April 27 – May 2
• Submit original draft of your section	May 6
• Graded draft returned (10 points)	May 11
• Revised draft of your section due (20 points)	May 16
• Assembly of complete report	May 16-18
• Complete paper submitted by editor (15 points)	Due by May 23 at 5 PM

Lab Notebooks:

Learning good record keeping skills is an important part of your professional development. Many cases of alleged scientific fraud result because of sloppy record keeping on the part of the investigator. You must keep complete notes during every lab as you will need to recall details of several weeks of work to write the required lab reports - the lab notebook is your source for this information. **Lack of appropriate records in your lab notebook will negate any grade you received on your lab reports.** If a record of an experiment, the data collected and its analysis is not found in your notebook, there is no documentation that the experiment was conducted or that the data was actually collected.

Francis Macrina in *Scientific Integrity: An Introductory Text with Cases* states that useful data books explain:

1) Why you did it. What were your objectives on that date? How did your work relate to the week before or the work that would follow?
2) How you did it. Detailed protocols that would allow you or another person to replicate the experiment. This might contain notes on how to use a particular piece of equipment if it was new to you.
3) Where materials are. You will generate materials one week that need to be used in a following week. Did you generate or isolate something that was stored in the -20 C freezer? The refrigerator? In your lab drawer? How was it labeled?
4) What happened. Did everything go as expected? If not, what happened? What results did you obtain?
5) Your interpretation. This is your data analysis and your conclusion(s).
6) What's next. Do you now go on to the next step/experiment or do you need to redo something to be able to continue?

Macrina goes on to write that good data books 1) are legible; 2) are well organized; and 3) allow repetition of the experiment.

For this course, your notebook will be a three ring binder. You may print out the supplied protocols or rewrite them for inclusion in the binder. Your notes can easily be added on separate sheets of paper and placed with the printed protocol or written on the protocol itself saving you the effort of rewriting each protocol. Important supporting documents such as plasmid maps, DNA sequences, etc. can be added easily to the place they were used or needed. The idea is to collect all the documents pertinent to a single experiment in one section of your notebook. The three ring binder makes appropriate organization of related materials easy.

Additional guidelines:

- Lab notebooks contain all your data and data analysis. Primary data and data analysis is required – **pages included in your notebook from a completed lab report do not count.**
- Notebooks are NOT written the night before they are due - some items are written in advance of an experiment, others during the experiment, and still others (analysis) after the experiment is completed. **I reserve the right to spot check or collect lab notebooks at any time.**
- Records should always be kept in ink (although editorial notes can be written in margins in pencil).
- Every member of a group should have a complete set of methodology, data and data analysis. Do not refer to someone else's notebook as a source.
- In addition to Macrina's list of entries above, your notebook should always contain: 1) the actual date (not "Week 1"); and 2) a title that will allow recognition by you and other readers of your experiment.

Course Grading:

Planned Point Assignments

4 lecture exams, each 50 points = 200
Lab reports (WRIT D) = 105
Lab Notebook = 40 (2 x 20 points)
Other assignments - TBD

Planned Grading Scale

90-100%	A range
80-90%	B range
70-80%	C range
60-70%	D range
Below 60%	F range

Total points expected ~ 370

		BIO 388 Molecular Genetics	
		Spring 2016	
Date		**Planned Lecture Topic**	**Reading Assignment**
			Note: Except for section 3.1, chapters 3-5 covering some chemistry basics and protein structure/function will not be covered in class or assigned. I do expect you to have some knowledge of these concepts from BIO 201 so you should read these chapters as needed.
8-Feb	M	Course and lab overview	Cha. 1
9	T	Lab sequence background	Cha. 18.1 (pp. 617-626); Arabidopsis (A14-15); Noller, H. F., Hoffarth, V., and Zimniak, L. (1992)
10	W	Lab background (cont.)	
12	F	Pre-lab	
15	M	Genetics review	Cha. 2; Cha. 12.1
16	T	Genetics, cont.	
17	W	Nucleic acid structure and properties	Cha. 3.1, Cha. 6, Cha. 13.3
19	F	Pre-lab	
22	M	Nucleic acid structure, cont.	
23	T	Focus on PCR	Cha. 7 (pp. 221-226); R. K. Saiki et al. (1988)
24	W	Catch up	
26	F	Pre-lab	
29	M	Techniques for studying genes	
1-Mar	T	Gene Study, cont.	Cha. 7
2	W	**EXAM 1, NHS 222, 7 PM**	
4	F	Pre-lab	
7	M	Gene Study, cont.	
8	T	Gene Study, cont.	
9	W	Genomics and other -omics	Cha. 8; Ross, M.T. et al. (2005)
11	F	Pre-lab	
14	M	Genomics, cont.	
15	T	Genomics, cont.	
16	W	Chromosomes: DNA topology and chromatin structure	Cha. 9, Cha. 10
18	F	Chromosomes, cont.	
21	M	TBA	
22	T	Chromosomes, cont.	
23	W	**EXAM 2, NHS 222, 7 PM**	
25	F	**SPRING BREAK: NO CLASS**	

		SPRING AND EASTER BREAK	
4-Apr	M	Transcription	Cha. 15, Ross, W. et al. (1993)
5	T	Transcription, cont.	
6	W	Transcription, cont.	
8	F	Pre-lab: Confocal microscopy -Jeff Dahlseid	
11	M	Confocal microscopy -Jeff Dahlseid	
12	T	RNA Processing	Cha. 16
13	W	RNA Processing, cont.	
15	F	Pre-lab	
18	M	RNA Processing, cont.	
19	T	Gene regulation overview	Cha. 19
20	W	Gene regulation overview	
22	F	Pre-lab	
25	M	Catch up	
26	T	Gene regulation in bacteria	Cha. 20
27	W	**EXAM 3, NHS 222, 7 PM**	
29	F	Pre-lab	
2-May	M	Gene regulation in bacteria	
3	T	Eukaryotic transcriptional regulation	Cha. 21, Chen et al. (1994)
4	W	Eukaryotic transcriptional regulation	
6	F	Eukaryotic transcriptional regulation	
9	M	Posttranscriptional regulation in euks	Cha. 22, Fire et al.
10	T	Posttranscriptional regulation in euks	
11	W	TBA	
13	F	TBA	
16	M	TBA	
17	T	TBA	
18	W	Wrap up and course evals	
19	R	READING DAY	
20	F	**EXAM 4, NHS 305, 3:30 - 5:30 PM**	
25	W	SENIOR GRADES DUE AT NOON	

BIO 388 Molecular Genetics
Planned Lab Schedule Spring 2015

Note: There will likely be changes to this schedule as going forward each week depends on results from previous weeks and the cooperation of the plants. The lab schedule is "front-loaded" for this reason.

Experimental threads:

1. Gene Cloning and Structure Analysis (First half of semester)
    1) Southern blot analysis of BAC-cloned *RP* genomic DNA (SBLOT)
    2) Cloning of your *RP* gene with its promoter region from a Gateway Entry vector (pENTR/D-TOPO) into a pMDC expression vector to generate a *RP*::GFP hybrid. (CLONE)
2. *RP* Gene Expression Analysis (Second half of semester)
    1) Characterization of your *RP* sequence in a pMDC GFP reporter vector in transiently transformed *N. benthamiana* leaves. To detect fluorescent protein expression we'll use the Zeiss LSM 700 laser confocal microscope. (GFP)
    2) qRT-PCR analysis of your *RP* gene expression. (QRT)

Lab Week of:	
Feb. 8	• Form your group and find a spot in the lab • Bioinformatic analysis of your assigned gene – laptops available for groups in lab, but you may want to bring your own computer to save work. • Isolate *RP* genomic DNA cloned in a BAC vector (SBLOT) and pENTR containing your RP gene and its upstream sequences (CLONE). • Perform PCR on BAC cultures and provided DNAs
Feb. 15	• Electrophorese isolated *RP* BAC DNAs and PCR products (SBLOT; CLONE) • Perform restriction enzyme digestions of BAC genomic DNA (SBLOT) • Label *RP* fragments (provided) to use as probe for Southern blot. (SBLOT)
Feb. 22	• Electrophorese restriction enzyme digested BAC *RP* genomic DNA and perform Southern blot transfer. (SBLOT) • Perform LR recombination to transfer your cloned DNA from pENTR into the pMDC destination vector (Su/M). (CLONE) • Transform *E. coli* with your LR recombination reaction (M/T). (CLONE)
Feb. 29	• **Group A:** Hybridize *RP* probe to Southern blots (Su; M) and detect hybridization (M; T). (SBLOT) • **All:** Isolate plasmid from pMDC transformed *E. coli* cells (CLONE) • **All:** Transform *A. tumefaciens* C58C1 with isolated plasmid. (CLONE)

Mar. 7	• **Group B:** Hybridize *RP* probe to Southern blots (Su) and detect hybridization (M). (SBLOT) • **All:** Start C58C1 cultures from transformed colonies. (Sa/Su) (CLONE) • **All:** Perform PCR and isolate plasmid DNA from cultured cells. (CLONE)
Mar. 14	• Analyze Southern blot results. (SBLOT) • Electrophorese PCR of C58C1 transformed cells to determine *RP* presence. (CLONE) • Send DNA for sequencing. (CLONE) • Isolate RNA from *Arabidopsis* seedlings. (QRT) • Perform control PCR with known *RP* primers on isolated RNA to check for contaminating genomic DNA. (QRT)
Mar. 21	• TBA; Lab as needed.
Mar. 28	SPRING AND EASTER BREAK
Apr. 4	• Analysis of returned *RP* pMDC sequence. (CLONE) • Electrophorese RNA samples to determine RNA integrity and previous PCR to determine genomic DNA contamination. (QRT) • Reverse transcribe RNA to produce cDNA for PCR amplification (QRT)
Apr. 11	• **Group A**: Perform qPCR reactions on cDNA (QRT) • **Group B:** Introduction to CLSM. Analysis of provided seedling roots for fluorescent protein expression using laser scanning confocal microscope. (GFP)
Apr. 18	• **Group B**: Perform qPCR reactions on cDNA (QRT) • **Group A:** Introduction to CLSM. Analysis of provided seedling roots for fluorescent protein expression using laser scanning confocal microscope. (GFP)
Apr. 25	• **Group A**: Determine *RP* expression levels from qRT/PCR data. (QRT) • **Group B**: Image *N. benthamiana* leaves transformed with pMDC RP::GFP by agroinfiltration on previous F or Sa. (GFP)
May 2	• **Group B:** Determine *RP* expression levels from qRT/PCR data. (QRT) • **Group A**: Image *N. benthamiana* leaves transformed with pMDC RP::GFP by agroinfiltration on previous F or Sa. (GFP)
May 9	TBA; Lab as needed.
May 16	COMPLETE REMAINING WORK AND CLEANUP LAB

2016 Planned Lab Schedule				
	Gene Cloning and Structure Study		**Gene Expression Study**	
Week:	Genomic Southern	pMDC cloning	GFP Analysis:CLSM	qRT-PCR
2/8	Isolate BAC DNA	Isolate RP pENTR DNA. PCR check for RP insert		
2/15	RE digestions of DNA Probe reactions	Electrophorese isolated DNAs and PCRs		
2/22	Electrophorese Re-digested DNA and transfer	LR recombination of pENTR and pMDC (Su/M). Transformation of *E. coli* (M/T)		
2/29	Hybridize and detect (Group A)	Isolate batched plasmid from transformed *E. coli*. Introduce into *A. tumefaciens* C58C1.		
3/7	Hybridize and detect (Group B)	Grow C58C1 cultures from positive colonies (Sa/Su). Isolate DNA (M/Tu). PCR of colonies.		
3/14	Analysis	Electrophorese PCR. Send DNA for sequencing.		RNA isolation. PCR for genomic DNA contamination
3/21	TBA	TBA	TBA	TBA
3/28	NO LAB - Break	NO LAB – Break	NO LAB - Break	NO LAB - Break
4/4		Analysis of *RP* pMDC sequence.		Electrophorese RNA (integrity) and PCRs (contamination). RT reactions.
4/11			Introduction to CLSM (Group B). Imaging of stably transformed *Arabidopsis* plants.	qRT-PCR (Group A)
4/18			Introduction to CLSM (Group A). Imaging of stably transformed *Arabidopsis* plants.	qRT-PCR (Group B)
4/25			CLSM analysis of N.b. for transient expression (Group B)	qPCR analysis (Group A)
5/2			CLSM analysis of N.b. for transient expression (Group A)	qPCR analysis (Group B)
5/9	TBA	TBA	TBA	TBA
5/16	LAB CLEANUP	LAB CLEANUP	LAB CLEANUP	LAB CLEANUP