Syllabus

Time: Tuesday and Thursday, 1:00-3:50
Place: SLB 337, and JH 216 as scheduled

Instructor: Andres Vidal-Gadea, Ph.D.
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Office hours: drop in, or by appointment

TA: Chance Bainbridge
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Required materials: one composition notebook, one 2-inch three-ring binder for protocols and notes, safety glasses, and be ready to get some great work done!

Protocols and hand-outs for the course: NEB Catalogs and Lab manual found under the Instructor's folder

Overview

Biotechnology I, BSC 353, is a lab-based course with three distinct goals:
1) The instruction of state of the art molecular techniques currently used in most research labs across the world. You will learn cutting-edge techniques such as: PCR, Cloning, RNAi, PCR-fusion, CRISPR/CAS9, Golden Gate and Gibson Assemblies, RT-PCR, etc. This will position you technically for the next steps of genetic analysis (i.e., Biotech II) but also will provide you with the tools that will make you a proficient molecular biologist ready to walk into any molecular lab!

2) Whatever your background, by the end of this class you will also be a molecular biologist. You will learn when to deploy different techniques, and how to troubleshoot them in order to answer different scientific questions.

3) You will learn cutting-edge techniques while working on actual scientific research questions, which will contribute to our understanding of biological processes or disease. Students in previous iterations of this class presented their work at scientific meetings, and there is a clear possibility for your contribution to be included in peer-reviewed papers!

The purification and manipulation of DNA is one of the mainstays of modern molecular biology. In this course you will have the opportunity to learn DNA techniques commonly applied in academic research, government, industry, forensic science, and medical testing. After successfully completing this class, you will be ready to perform all these techniques.

Prior experience with laboratory work or molecular techniques is not a requirement but would help you get the most out of this class. You will need a good foundation in chemistry, cell biology, and genetics. Prior coursework in biochemistry and molecular biology is a plus, but not required. Prerequisites: BSC 219; CHE 220 or CHE 230-232.

Course philosophy and structure

This course is designed so that any student with basic biology background can walk in with a positive, hardworking, attitude and by the end of the semester leave the class having learned the most useful (and used) molecular techniques currently used in labs around the world. You will learn these techniques while working on real scientific questions, and will generate new and useful data. By the end of the semester you will have ceased to think as a student, and will have started to think like a scientist. As an advanced laboratory course, our emphasis will be on molecular mechanisms rather than the memorization of facts. I will guide you through the concepts behind our techniques and the questions they can address. However, your ultimate success **will be up to you and will require your investment outside of class time** (e.g. studying ;-). Even though you will work in groups, there will be a level of independent learning and initiative required in this class that you probably have not experienced in previous courses.

Organization

This class focuses on the manipulation of recombinant DNA. In the first months, you will learn how DNA fragments are purified, altered, and analyzed. You will then apply these acquired skills to a real project of your choice. I will introduce

you to new topics with a short presentation, which followed by bench work. Your TA will serve as a resource during the experimentation portion of the class. You will work in groups, but results and their interpretation will be done on your own, and recorded by you in your lab notebook. Small groups will allow for collaboration and the potential to share reagents. Your own efforts and desire for knowledge will drive your success in the course (and ultimately in graduate school or professional career).

Lab Safety

Please refer to **Appendix A** for important information regarding laboratory safety.

Lab protocols and lecture notes

There are many kits, protocols, and companies to choose from for the manipulation of DNA, and manufactures nearly always provide guidance. The majority of the experiments conducted in this class will rely on protocols and supplies provided by **New England BioLabs** and their generous Educational Program. You can download this manual and associated protocols from the CAS IT server (see above).

It is your responsibility to print this material off, review protocols prior to class, and keep data sheets/protocols in a 3-ring binder.

Due to the high cost of molecular reagents and the collaborative nature of group work, YOU WILL NOT BE PERMITTED to conduct a lab experiment for which you have not prepared (which will affect class grade). Please sign ion the dotted line to acknowledge that you understand these terms: ……

Grading

Success in this class depends upon you. Emphasis is not based on exam performance but is rather goal oriented. You should gain an understanding of experimental design, data analysis, experimental techniques in DNA analysis, and an appreciation for the presentation of results. It is on that set of principles that you will be evaluated.

You will earn a grade based on performance at the lab bench, participation in the class, lab notebook, lab reports, pre-lab assignments, and a short presentation. Below is the grade breakdown followed by a description of each item.

Grades	**Points**
Pre-lab assignments	20
1st Lab report	10
2nd Lab report	20
Lab notebook	10
Project and Presentation	20
Participation	10
Bench work	10
Total	**100**

Grades:	100-90% = A
	80-89% = B
	70-79% = C
	60-69% = D
	<59% = F

Pre-lab assignments

This laboratory course consists of over forty scheduled experimental protocols chosen to provide you with a solid foundation for laboratory work. After successfully completing this course, you will have skills that would make you a competitive candidate in any scientific laboratory relying on molecular techniques (whether as a master student, private sector, or medical school labs). To accomplish this, we will consume three precious resources: ATP (**you** and **I** will **work hard**), time, and expensive molecular reagents/kits. To make sure that we accomplish our goals students will be required to complete a pre-lab assignment consisting of an experimental flowchart for each of the activities to be performed in each class. These **flow charts must be in your lab book prior to each relevant lab**. Students without a flow chart will not be permitted to conduct the relevant experimental activity. All the information needed for the flow charts can be obtained from the handouts and protocols present in this manual. Each accurate flowchart will be awarded **0.5 points**. Students arriving without a flowchart at the beginning of the class must read the manual and complete one in order to participate in the class, however **NO points will be awarded for late pre-labs**. A Description of the construction of a flowchart and an example are available at the end of this manual in **Appendix B**. Throughout the manual, we have inserted (and highlighted) links to online videos that should help you understand the protocols or techniques to be

performed in lab. These are a very easy way for you to obtain the information and understanding that you need in order to construct your pre-lab assignments.

1st Lab report

After finishing the first part of the class, you will have completed a set of molecular experiments and obtained data to answer some important scientific questions. For your first lab report you will be responsible for submitting a **typed** two-page document consisting of: a title (**2 points**), an abstract (**3 points**), and a methods sections (**5 points**) for a total of **10 possible points**. Please refer to **Appendix C** for instructions on constructing a lab report. The two-page is the maximum but you might not need that much space.

2nd Lab report

After finishing the second part of the class, you will **type** a lab report (up to a maximum of four pages in length) on the experiments you performed since the first lab report. For this report, you will need to include a title (**2 points**), an abstract (**3 points**), a methods sections (**5 points**); a results section (**5 points**); an introduction section (**3 points**); and a discussion section (**2 points**) for a total of **20 possible points**.

Lab notebook

Each student will keep two different books: a research notebook, and a protocol binder. The research notebook should be a bound composition-style book. Students will record activities, notes, and results within this notebook in such a fashion that a reader would be able to repeat the experiments and accurately interpret the data collected, even without the lab manual (pretend the lab manual doesn't exist). Notebooks do not need to be written in passive voice, since you are describing what you actually did. Don't write generalized protocols, **record your exact procedure and results**. Accurate labeling of experiments is important. Each new experiment should start at the top of a new page. All work should be dated. For gel photos, each experimental lane must be descriptively labeled ("pUC19/Bam" rather than "lane 6"). State the conclusions of each experiment (e.g., you obtained the necessary fragment and stored it at -20°C; the PCR failed because...; etc.). Any relevant comments on an experiment, including questions, uncertainties, and mistakes, are appropriate for the notebook; these comments may help you interpret anomalous results at a later date. Record experiments as results are obtained, and never rip out pages from your notebook. Grades will be based on your ability to keep your notebook complete and up to date, accurate record keeping, dating all experiments, inclusion of major notebook categories for each experiment (such as **goals**, **methods**, **results**, and **discussion**), and the maintenance of a protocol notebook. Your protocol notebook will be yours to keep, and should include all method-related handouts, all relevant manufacturer protocols, and notes/techniques that you write as notes. The notebooks are an important part of the course, and adds up to **10%** of your grade.

Presentation

For this part of the class, you will put to use the cumulative knowledge and techniques you acquired. Your group will develop a question that you will then investigate using what you have learned. It will take time and work to develop these questions so I encourage you to start early and meet with me often. The questions can come from a variety of sources such as: 1) a scientific question in your own graduate project (for graduate students); 2) a basic science question introduced in the lab; 3) a medically-relevant project introduced in the lab.

After completing your experiments, your group will create and deliver a presentation describing the chosen research problem; the designed experimental approach incorporating the techniques learned in the lab to answer this question (including relevant primer sequences for the creation of needed reagents); your prediction of possible outcomes along with their potential meaning; the results that you obtained; and your interpretation of these results. Your group will need to develop a question that will employ one or more of the advanced taught in this class. The presentations will last 25 minutes with an additional five minutes for questions. Please note that each student will be responsible for every aspect of the presentation. Refer to **Appendix D** for a collection of guides when preparing and delivering scientific presentations. Oral presentations will be scored out of a possible maximum of **20 points**.

Participation

Participation refers to your engagement in the class material. For example, while **being able to perform** a protocol is part of what is considered your **bench work ability**, participation involves your ability to understand **why** a protocol works, **when** you would select a protocol, or **how** you would trouble shoot a failed experiment. In other words, *Participation* measures your intellectual engagement in the material taught. Participation and bench work are evaluated on an ongoing basis throughout the semester by the instructor and TA. You are encouraged to obtain feedback on your performance on these parameters by appointment or during office hours.

Bench work

Bench work is an important part of this course. It incorporates **how** you perform the protocols that you are learning (e.g. do you **know what you are doing and why?**). Bench work also incorporates your practices as far as techniques, safety, constructive interactions with lab-mates, etc. **You will not be graded on the success of your experiments**; however, you will be evaluated on your honest attempt at the different protocols and experiments. After each lab, each group will be responsible for cleaning up the bench where they conducted work so that other classes will find the lab as clean as you did. To the effect of keeping a record of this practice, you will need to complete a checklist provided in **Appendix E**.

Exams

We apologize for the inconvenience but there will be no exams in this class. Since this is a laboratory course, you will be evaluated in your participation in lab activities. Your grade will result from your success at designing and performing experiments, writing lab reports, lab preparation, performance, participation, and the presentation of your finding.

Graduate students

*Graduate credit will be awarded to graduate students upon completion of the above exams and assignments, **PLUS** an additional write-up: You will produce a write up of your group's results following the **Author Guidelines** provided by the journal **Genes, Brain and Behavior**. This will be focused on the techniques learned in the lab to research questions in your own project (unless authorized otherwise by the instructor). It will be graded as a part of the notebook grade. We expect graduate students to participate actively in class, and to excel in the understanding of the techniques learned.*

Expectations for this course

You must be prepared for class, and **it is your responsibility to read to protocols ahead of the lab**. Failure to be prepared will affect your ability to learn the techniques and will: **i)** add unnecessary time to laboratory exercises; **ii)** put a burden on your lab group; **iii)** waste valuable and expensive reagents; and therefore **iv)** impact your grade considerably.

Print all relevant material before coming to class and bring them with you. It is your responsibility to check ***your email*** and the ***Instructor folder*** for updates from your instructors (including the TA) prior to class. We expect that you will work effectively with your assigned lab team. Poor behavior will not be tolerated and you will be asked to leave if it becomes a distraction from learning or a safety issue.

Minimum expectations

- Please read and strictly follow Equipment and Safety rules on the following page. It is essential to follow these rules and the instructions specific to the individual lab exercise. This is one of your responsibilities in this class.
- Excellent attendance is expected. Completion of research projects will require your attendance. Contact the instructor if illness or unforeseen circumstances influence your ability to be on time for class. **Make up labs will not be given for absences**.
- You will need to come to the lab during the week on days other than Tuesday and Thursday. As a balance, some classes will end early. Similarly, there will be down times in class where experiments incubate, spin, or resolve, etc. It is perfectly acceptable to leave during down times or even leave class early, if appropriate.

- Be a good lab citizen. This factors into your participation points.
- Clean up your lab bench after each experiment, and at the end of the class period. Poor lab-bench maintenance is an indicator of weak participation in the course.
- Maintain lab notebooks, be an active participant.

Absences

Absence or failure to submit class work due to a family emergency may be rescheduled, but this will be at the instructor's discretion and consistent with the University's bereavement policy, which can be found at http://policy.illinoisstate.edu/students/2-1-27.shtml. Should a weather event or university-wide emergency result in closure of the university on a due date, the new due date will be rescheduled for the next class day when the university is open. In these situations, students are advised to check their university emails for updates and any relevant instructor or university correspondence.

Electronic devices

Out of respect for us and your classmates, please **refrain from using your cell phone in class** (i.e., texting, calling, googling, etc). These devices are a distraction from learning. If you absolutely must make a call or text during class, please excuse yourself to the hallway. During lectures, please mute your phone. Ideally, you should turn them off.

Policy on cheating

You must do your own work for this class. A degree of collaboration is inherent to (and important for the success of) laboratory science. Nevertheless, experiments and lab notebook are to be done on your own and are to represent your efforts in the class and not the work of others. Be sure to credit any images used in your presentation. For complete ISU policy on academic integrity, see the current ISU Catalog. **ANY** documented case of plagiarism will result in an automatic grade of **F** for the course and referral of the case to University authorities. If in doubt about what constitutes cheating or plagiarism, please talk to one of the instructors.

Honors credit

I am happy to arrange honors projects.

NEB Website

Most of the materials used this semester are provided free of charge by New England Biolabs, one of the leading companies in molecular reagents, through their unique Educational Program. NEB has expressed their interest in receiving photographs of our lab and experiments as a way to publicize their educational program. If you do not object to having photographs of ongoing lab activities displayed in NEB's website, please complete and return the form attached in **Appendix F**.

Tentative schedule

We will follow the basic outline below. It is very likely that there will be deviations from this schedule due to unavoidable experimental set-backs. Do not be concerned with schedule modifications. You will find that you will get better and faster at these techniques as the semester progresses.

Because we are training you to be a proficient molecular biologist, the most valuable lessons you will learn in the lab will not be how to follow the detailed instructions that you can easily find on the internet. Rather, our goal is to enable you to troubleshoot when things go wrong (and they often do in real life!). We aim to provide you with sufficient understanding about the techniques and the differences between them in order to enable you to select the ones most suited for your goals.

These are lofty goals, and I will work very hard to make sure you have everything you need to achieve them!

Date	Lab Topic	Lab Activity
08/22	Safety, Lab Essentials;	Introduction; safety; dilutions (**act 1**); common solutions (**act 2**); sterile technique
08/24	Genomic and Plasmid DNA Purification	Miniprep (**act 3**); transformation (**act 4 and 5**); genomic extraction (**act 6**); worm DNA (**act 7**)
08/29	Electronic Constructs	Primer design (**act 8**); enzyme cutters; ligation design (**act 9**); restriction (**act 10**)
08/31	Restriction Digest Analysis	Ladder design (**act 11**); DNA digestion (**act 12**); ladder construction (**act 13**); keeping worms (**act 14**); moving worms (**act 15**)
09/05	Polymerase Chain Reaction	DNA amplification (**act 16**); electrophoresis (**act 17**)
09/07	Bio-Informatics PCR-Fusion: translation and transcription	**Computer Lab:** BLAST; NCBI tools; WormBase PCR of gene promoter (**act 18**); GFP (**act 19**); and PCR fusion (**act 20**)
09/12	Gene Cloning I	Evaluation of PCR fusion (**act 21**); Topo cloning (**act 22**); blunt-end cloning (**act 23**)
09/14	Gene Cloning II	Miniprep (**act 24**); enzyme digest (**act 25**); electrophoresis (**act 26**); cleanup (**act 27**)
09/19	Gene Cloning III/ RNA interference I	Gibson assembly (**act 28**); Golden gate assembly (**act 29**); transformation of products (**act 30**)
09/21	RNA Interference II	RNA plasmid isolation (**act 31**); and transformation (**act 32**) into H115 cells
09/26	RNA Interference III	Test of RNAi animals (**act 33**)
09/28	DNA Sequencing	Sequencing facility (**act 34**); sequencing PCR (**act 35**), cleanup reaction (**act 36**)
10/03	RNA isolation	RNA isolation (**act 37**) from silenced and ctrl worms; purity quantification (**act 38**)
10/05	cDNA synthesis	RNA cleaning (**act 39**); cDNA synthesis (**act 40**)
10/10	RT-PCR	Electrophoresis (**act 41**); and SybrGreen RT-PCR (**act 42**)
10/12	Site Directed Mutagenesis	Primer design: creation of Cas9 plasmid (**act 43**)
10/17	CRISPR/Cas9	Construction of SEC repair plasmid (**act 44**)
10/19	CRISPR/Cas9	Confirmation of sgRNA (**act 45**) and SEC repair (**act 46**) plasmids
First Lab report due 10/19 by start of class		
10/24	Group Projects	Lab 1/week 1 of independent group projects
10/26	Group Projects	Lab 2/week 1 of independent group projects
10/31	Group Projects	Lab 3/week 2 of independent group projects
11/02	Group Projects	Lab 4/week 2 of independent group projects
11/07	Group Projects	Lab 5/week 3 of independent group projects
11/09	Group Projects	Lab 6/week 3 of independent group projects
11/14	Group Projects	Lab 7/week 4 of independent group projects
11/16	Group Projects	Lab 8/week 4 of independent group projects
11/21	Group Projects	Lab 9/week 5 of independent group projects
Turn in Laboratory books for evaluation 11/21 by start of class		
11/23	Thanksgiving, no class	Thanksgiving, no class
11/28	Thanksgiving, no class	Thanksgiving, no class
11/30	Group Projects	Lab 10/week 6 of independent group projects
12/05	Group Presentations	Group Presentations
Final Lab report due 12/5 by start of class		
12/08	Lab Cleanup	Cleanup bench and lab