

Molecular and Cell Biology Laboratory (BIOL-UA 223)

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Course Hours:

Section 1: Mon: 12:30 - 3:15
Section 2: Wed: 12:30 - 3:15

Course Description:

This laboratory course applies concepts learned in the Molecular and Cell Biology course (BIOL-UA 21) to a molecular biology research project. The research project will introduce students to standard genetic and biochemical techniques common in a molecular biology lab, such as DNA isolation, agarose-gel electrophoresis, and transformation. The project also will provide students with a hands-on understanding of how modern DNA-sequencing technology, along with bioinformatic tools, can be used to discover genetic differences and understand cellular function.

Course Objectives:

Students in this course will:

- (1) Learn fundamental aspects of experimental design.
- (2) Apply concepts and theory to a hands-on research project.
- (3) Learn the purposes of the experimental methods they use.
- (4) Learn to interpret and effectively communicate experimental results.

Course Policy and Grading:

- (1) Attendance is mandatory. Three or more unexcused absences will result in a grade reduction (e.g. A grade to an A-grade).
- (2) No make-up will be given for a missed quiz or final exam.
 - a) In the case of illness, a doctor's note will be accepted at the discretion of the course instructor.
- (3) Late submissions of assignments of one day or more, will result in a grade reduction (e.g. A grade to an A-grade).
- (4) Students will be evaluated based on:
 - a) Three Quizzes (15% Each)
 - b) Final Exam (20%)
 - c) Lab Notebook (15%) - See below for the format and the attached Rubric for details.
 - d) Class Participation/Presentations (10%) - See attached Rubric for details.
 - e) Assignments (10%)

Textbook and Suggested References:

- (1) There is no lab manual for this course. Handouts will be provided.

This laboratory course will follow the readings associated with the MCB lecture course as much as possible. Students are therefore strongly advised to follow the same reading assignments as found in

the MCB lecture syllabus, which will primarily be from the Lodish textbook. Additional readings specific for this course are indicated on this syllabus.

(2) Lab Notebook (Required) - Carbon-less.

- (3) References:
- (1) Molecular Cell Biology. 7th Ed. By Lodish, et.al. Freeman Publisher
 - (2) Recombinant DNA: Genes and Genomes -A Short Course. 3rd Ed.
By James Watson, et.al. Cold Spring Harbor Laboratory Press
 - (3) Molecular Biology: A laboratory Guide. By Alan Gerstein.
John Wiley Publisher

Lab Notebooks:

Each student's lab notebook should be *individual work, legible and organized*. It should be written so that other people can follow your work exactly to achieve the same result(s). Lab reports will be collected on a weekly basis. It is expected that students will write about 3 to 5 pages for each week's lab in the following format:

Title and Date: Provide a descriptive title of the week's lab and date the work.

Purpose: Briefly describe the purpose of the lab. (Min.: 2-3 sentences). You may also explain the significance of the procedure or the type of application that can be used with the procedure.

Materials and Methods: Specific information on materials used other than regular reagents.
-Example of information to be recorded: Specific activity, Company/Lot #, Concentration, Nucleotide sequence, type of DNA plasmid, DNA probes sequence, enzymes, label, etc.
- Procedure: Handouts will be provided. These handouts should be put into or the procedure written into your notebook. In some cases there will be some modification or changes in the procedure. This should be recorded to show the changes. Information on volume, concentration and time should be added when needed.

Results: Your results should be labeled and have enough information so that an outside reader can understand your results. The results should also be referenced appropriately (Fig. 1, ... or Table 1, ...) so that you can discuss the results in the discussion section.

Discussion: Summarize your result(s). Explain the outcome of the procedure. Was there anything you could have done to better your result(s)?

Outline of Lab Experiments:

I. DNA Barcode Experiment.

Purpose: Understand the process to identify and classify living things. Species identification will be through unique patterns of DNA sequences.

- 1) Collect plants, animals or products from local environment.
- 2) Extract and purify DNA from collected samples.
- 3) Use PCR to amplify specific regions from the chloroplast or mitochondrial DNA that

- are short but highly variable.
- 4) Analyze PCR product by agarose-gel electrophoresis.
 - 5) Use BLAST to identify sequences in database to taxonomically assign the sample.

Experimental Outline:

- Weeks 1-2: (1) Students collect samples for species identification.
- Week 3: (1) Isolate the DNA from samples.
- Week 4: (1) PCR using primers specific for identification of species.
- Week 5: (1) Gel electrophoresis of PCR products.
(2) Sequences sent out for sequencing.
- Week 8: (1) Sequence analysis of PCR products.

II. Hypothesis driven Genomic Experiment.

Purpose: Understand how molecular biology can be used to identify genetic differences and understand their cellular consequences.

- 1) Construct genomic DNA libraries from wild-type and mutant budding yeast (*Saccharomyces cerevisiae*).
- 2) Perform functional complementation by transforming a mutant (auxotrophic) yeast strain with the wild-type genomic library and selecting for prototrophic colonies. Isolate plasmid DNA from surviving clones and obtain DNA sequence to identify the mutated gene.
- 3) Use next-generation sequencing technology to sequence the genomic DNA of the mutant strain.
- 4) Use bioinformatics to determine sequence differences between wild-type and mutant strains, and compare to sequences recovered by functional complementation.

Experimental Outline:

- Week 2: (1) Each group will grow an auxotrophic yeast mutant on deficient media to determine what nutritional requirement is required by the mutant.
(2) Setup competent cells for week 3 transformation experiment.
- Week 3: (1) Each group will attempt to create a prototroph through a transformation experiment of the auxotrophic yeast mutants with the wild-type complement library.
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- Week 4: (1) Prototroph strains will be isolated and grown so that the DNA can be isolated in Week 5.

Week 5. (1) Mutant yeast genome will be isolated for Next Generation Sequencing. A reference wildtype genome sequence will be used for comparative purposes.

Weeks 6 to 9: (1) Library construction for sequencing on Ion Torrent

Weeks 11-12: (1) Analysis of sequence results using bioinformatic tools.

Tentative Syllabus

	Hypothesis Driven Project	Discovery Driven Project	Notes
Week 1	<p>- Introduction to Course. - Introduction to Genomic Experiment. (1) Distribute auxotrophic mutant yeast strain to each group. Genotypes of groups' mutant yeast are unknown to students.</p> <p>- Readings: (1) Auxotrophic Yeast Strains in Fundamental and Applied Research. Jack T. Pronk. 2002. Applied and Environmental Microbiology. 68(5):2095-2100. (2) Sequencing Technologies - The Next Generation. Michael Metzker. 2010. Nature Review: Genetics 11: 31-46.</p>	<p>- Introduction to Barcoding Experiment - Readings: (1) Biological Identifications through DNA Barcodes. Hebert, Cywinska, Ball, deWaard. 2003. Proc. R. Soc. London 270: 313-321 (2) DNA Barcoding: Potential Users. Peter M. Hollingsworth. 2007. Genomics, Society and Policy 4(2) 44-47. (3) The Promise of DNA Barcoding for Taxonomy. Hebert, Gregory. 2005. Systematic Biology 54(4) 852-859.</p>	
Week 2	<p>- Plate yeast strains (Type a) to determine what mutants they have (e.g. -His, -Leu, -Met,...) in appropriate minimal media -</p>	Lecture Topic: Primer designs for DNA Barcoding	
Week 3	<p>- Setup functional complementation transformation between mutant and wild type library to examine auxotrophic mutant. - Lecture Topic: Genomic vs Plasmid DNA libraries</p>	- Isolate DNA from samples students collected.	
Week 4	- Isolate prototrophic Colonies and Grow in Broth.	<p>- Amplify DNA by PCR. - Lecture Topic PCR, - Review MCB Lecture: Structure of DNA (#3) and reading material</p>	- Quiz # 1.
Week 5	DNA extraction and sequencing to identify the mutated gene.	<p>- Analyze PCR Products by Gel Electrophoresis. - Lecture Topic Gel Electrophoresis.</p>	
Week 6	<p>- Lecture Topic: Transformation - Review MCB Lecture: DNA Replication (#7, 8) and reading material</p>	- Sequence PCR Products: (Genewiz).	

Week 7	- Generate Wild-Type Yeast Genomic DNA Library. Part 1: Restriction Enzyme Digest - Lecture Topic: Library construction, R.E.	- Lecture Topic: Sequencing technology. - Review MCB Lecture (# 25, 26) reading material	- Quiz # 2
Week 8	Generate Wild-Type Yeast Genomic DNA Library. Part 2: Ligation - Lecture Topic: Ligation	- Analyze Barcoding Data. - Lecture Topic Blast search engine - Review MCB Lecture: Gene Structure (#7) and reading material.	
Week 9	- Construct the Library for NGS. (Ion Plus Fragment Library Kit # 4471252. 10 rxn/\$500) Part1: Isolation of Yeast Genomic DNA.		
Week 10	Construct the Library for NGS. Part2: Library - Lecture Topic: Sequencing Technology - PCR - Lecture Topic: Bioinformatics		- Quiz # 3
Week 11	- Template Preparation for NGS - Sequence the Library		
Week 12	- Analyze NGS Data for differences between mutant and wild-type yeast.		
Week 13	- Discussion of complementation and genomic results.		- Student Presentations - Group 1 and 2
Week 14	- Review.		Student Presentations - Group 3 and 4
Week 15	- Final Exam - Lab Notebook Due		

Student Writing/Report Assessment:

Biology Report Assessment Criteria

Criterion	Highly Proficient (3)	Moderately Proficient (2)	Developing Proficiency (1)	Below Expectations (0)
Title	(1) The title clearly describes the experiment.	(1) The title somewhat describes the experiment..	(1) The title vaguely describes the experiment.	(1) The title is NOT stated .
Purpose: <i>Content</i>	(1) This section provides a clear sense of why this knowledge is of interest. (2) The background information is complete and accurate . (3) Primary literature are relevant to this section.	(1) This section provides a reason of why this knowledge is of interest. (2) The background information is not complete and has minor inaccuracies . (3) Primary literature are relevant but few to this section.	(1) This section is vague as to why this knowledge may be of interest. (2) The background information is narrow or too general and has inaccuracies . (3) Primary literature is few and not relevant.	(1) This section does not address the question of why this knowledge may be of interest. (2) The background information missing . (3) Primary literature is absent .
Purpose: <i>Reason</i>	The testable hypothesis/objective is clearly stated. It is also novel and has significance to the understanding of the field.	The hypothesis/objective is clearly stated. It also provides a level of understanding to the field.	The hypothesis/objective is relevant . It may provide a level of understanding to the field.	No hypothesis/objective is stated or hypothesis is trivial or incorrectly stated .
Methods: <i>Design</i>	The use of appropriate methodologies was clearly described.	The use of appropriate methodologies was not clearly described.	The use of appropriate methodologies was vaguely described.	The use of appropriate methodologies was not described.
Results: <i>Data Presentation</i>	(1) Relevant data is presented in the correct format (table, graph) with no mistakes and shows the relationships between the data without the reader referring to the text. (2) The narration of the results was very clear and relevant to the hypothesis. (3) Statistical analysis is appropriate, correct and clearly explained.	(1) Relevant data is presented in the correct format (table, graph) with minor mistakes and shows the relationships between the data without the reader referring to the text. (2) The narration of the results was not clear . and relevant to the hypothesis. (3) Statistical analysis had some errors but reasonably explained.	(1) Relevant data is presented in an incorrect format (table, graph) with minor mistakes and shows the relationships between the data without the reader referring to the text. (2) There was no explanation of how or why the data was formatted. (3) Statistical analysis is not properly performed and not reasonably explained.	(1) Data is presented in an incomplete format with label or unit errors. Reader must read the text to understand the relationship between the data. (2) No narration of the results were provided. (3) No statistical analysis is performed.
Discussion: <i>Logical Conclusion</i>	(1) Conclusions are clearly formed from the stated hypothesis and collected data. (2) Primary literature are extensively used to make connections between the project and other research in the field.	(1) Conclusions are stated based on the stated hypothesis and collected data. (2) Primary literature are used to make connections between the project and other research in the field.	(1) Conclusions are stated with little basis on the stated hypothesis and data. (2) Some primary literature are used but do not make connections between the project and other research in the field.	(1) Conclusions have no basis on the stated hypothesis or collected data. (2) No primary literature was cited.
Discussion: <i>Implication</i>	The implications or application of the research is clearly stated.	The implications or application of the research is mentioned .	The implications or application of the research is vague .	The implications or application of the research is not stated
Writing Quality:	The paper is easily understood due to the excellent grammar, word usage, and organization.	The understanding of the paper is not hindered due to some mistakes in grammar, word usage, and organization.	The understanding of the paper is somewhat hindered due to mistakes in grammar, word usage, and organization.	The understanding of the paper is hindered due to many mistakes in grammar, word usage, and organization.

CLASSROOM PARTICIPATION RUBRIC

CRITERION	QUALITY				DATE
Degree to which student integrates course readings into classroom participation	often cites from readings; uses readings to support points; often articulates "fit" of readings with topic at hand (4 points)	occasionally cites from readings; sometimes uses readings to support points; occasionally articulates "fit" of readings with topic at hand (3)	rarely able to cite from readings; rarely uses readings to support points; rarely articulates "fit" of readings with topic at hand (2)	unable to cite from readings; cannot use readings to support points; cannot articulate "fit" of readings with topic at hand (1)	
Interaction/participation in classroom discussions	always a willing participant, responds frequently to questions; routinely volunteers point of view	often a willing participant, responds occasionally to questions; occasionally volunteers point of view (3)	rarely a willing participant, rarely able to respond to questions; rarely volunteers point of view (2)	never a willing participant, never able to respond to questions; never volunteers point of view (1)	
Interaction/participation in classroom learning activities.	always a willing participant; acts appropriately during all role plays; etc., responds frequently to questions; routinely volunteers point of view (4 points)	often a willing participant; acts appropriately during role plays; etc., responds occasionally to questions; occasionally volunteers point of view (3)	rarely a willing participant, occasionally acts inappropriately during role plays; etc., rarely able to respond to direct questions; rarely volunteers point of view (2)	never a willing participant, often acts inappropriately during role plays; etc., never able to respond to direct questions; never volunteers point of view (1)	
Demonstration of professional attitude and demeanor	always demonstrates commitment through thorough preparation; always arrives on time; often solicits instructors' perspective outside class (4 points)	rarely unprepared; rarely arrives late; occasionally solicits instructors' perspective outside class (3)	often unprepared; occasionally arrives late; rarely solicits instructors' perspective outside class (2)	rarely prepared; often arrives late; never solicits instructors' perspective outside class (1)	