BOT 210 PHYTOBIOTECHNOLOGY, Fall 2012

Number of Credits: 4 hour lecture/lab course

Monday-Friday, 1:00pm to 3:30pm, Hale ʻImiloa 106, CRN: 61387

INSTRUCTOR: Brad Porter, PhD
OFFICE: Hale ʻImiloa 107
OFFICE HOURS: Thursdays 5:15pm – 6:15pm
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EFFECTIVE DATE: Fall/2012

WINDWARD COMMUNITY COLLEGE MISSION STATEMENT

Windward Community College offers innovative programs in the arts and sciences and opportunities to gain knowledge and understanding of Hawaiʻi and its unique heritage. With a special commitment to support the access and educational needs of Native Hawaiians, we provide Oʻahu's Koʻolau region and beyond with liberal arts, career and lifelong learning in a supportive and challenging environment — inspiring students to excellence.

COURSE DESCRIPTION

This course provides an introduction to plant molecular biology/biotechnology techniques used for applications ranging from gene functional characterization to crop improvement. The class begins with a review of the central dogma of molecular biology and then transitions to the laboratory where nucleic acid (DNA and RNA) extraction and gel electrophoresis are conducted. Lectures and laboratory activities address PCR (including primer design and ordering), cloning, the use of restriction enzymes, sequencing, and sequence analysis. Next-generation sequencing, whole transcriptome shotgun sequencing, and microarray analysis are also discussed. Because of its rapid life cycle, the model plant *Arabidopsis thaliana* will be used to introduce the concept of gene functional characterization, including transformation strategies used for the generation of gene knockouts and protein localization. Finally, protein extraction will be conducted before evaluating micropropagation techniques including plant tissue, cell, and protoplast cultures.

STUDENT LEARNING OUTCOMES

By the end of this course, you should:

1) Be able to extract nucleic acids (DNA and RNA) and evaluate DNA and RNA concentration and purity.
2) Understand the principles of PCR and the applications for which PCR can be used.
3) Understand the principles of molecular cloning using *E. coli*, including ligation reactions and the use of restriction enzymes.
4) Be able to describe sequencing by capillary electrophoresis, next-generation sequencing, and whole transcriptome shotgun sequencing.
5) Be familiar with protein localization techniques using green fluorescent protein (GFP) and beta-glucuronidase (GUS).
6) Understand strategies used for gene characterization, including transfer DNA (T-DNA) knockout lines.
7) Be familiar with the model plant *Arabidopsis thaliana* and understand its role in plant biology.
8) Understand micropropagation techniques (plant tissue, cell, and protoplast cultures).

9) Be able to document experiments in a laboratory notebook is sufficient detail to repeat experiments or report the methods and results in a scientific journal.

Learning outcomes will be achieved through the aid of the following activities:

1) Classroom discussions, lectures, and presentations
2) Reading assigned hand-outs
3) Conducting laboratory exercises and activities and maintaining a laboratory notebook
4) Writing a research paper, and presenting the same research topic as an oral presentation

ASSESSMENT TASKS AND GRADING

Grading:
Laboratory participation and a laboratory notebook documenting activities and the methods, results, and discussion for all experiments will be graded. A six page research paper (with references) covering the background and recent discoveries of a topic related to plant molecular biology/plant biotechnology will be required, as well as an oral presentation of the paper's topic.

The total possible points:

1.	Laboratory participation and performance	100
2.	Laboratory notebook	200
3.	Plant molecular biology research paper (term paper)	100
4.	Final exam (oral presentation of research paper)	100
	Total Points	**500**

Letter grades will be assigned as follows:

A – 90% or above in total points
B – 80-89% of total points
C – 70-79% of total points
D – 60-69% of total points
F – 59% and below of total points
I – Incomplete; given at the instructor's option when you are unable to complete a small part of the course because of circumstances beyond your control. It is your responsibility to make up incomplete work with a minimum level (or better) of achievement. Failure to satisfactorily make up incomplete work within the appropriate time period will result in a grade change for "I" to the contingency grade identified by the instructor (see Windward Community College Catalog).
CR- 70% or above in total points; you must indicate the intent to take the course as CR/NC in writing by the end of the 10^{th} week of classes (see catalog).
NC – Below 70% of total points.
N – Not given by this instructor except under extremely rare circumstances (e.g., documented serious illness or emergency that prevents you from officially withdrawing from the course); never used as an alternative for an "F" grade.
W – Official withdrawal from the course. Refer to the Academic Calendar for withdrawal deadline.

POLICY ON LATE WORK

Late work will only be accepted with a valid reason (medical emergency) and at the discretion of the instructor. In such circumstances, you should contact the instructor as soon as possible.

TEXTBOOK

The following reference will be made available in the laboratory: Molecular Cloning: A Laboratory Manual (Fourth Edition): Three-volume set by Michael R. Green and Joseph Sambrook
ISBN-10: 1936113422 | ISBN-13: 978-1936113422

LAB COATS, SAFETY GLASSES, and CLOSED TOED SHOES

Students must purchase a lab coat and a pair of safety glasses for this class. Closed toed shoes are also mandatory while working in the laboratory.

RECORDING DEVICES

The use of any device to record audio or video in the classroom or laboratory is prohibited.

DISABILITIES ACCOMMODATION

If you have a physical, sensory, health, cognitive, or mental health disability that could limit your ability to fully participate in this class, you are encouraged to contact the Disability Specialist Counselor to discuss reasonable accommodations that will help you succeed in this class. Ann Lemke can be reached at 235-7448, lemke@hawaii.edu, or you may stop by Hale ʻAkoakoa 213 for more information.

LECTURE/LAB CONTENT

Date	Lecture/Lab Topic
Aug 20	A review of the central dogma. Ordering supplies and stocking a molecular biology lab.
Aug 24	Learning to use micropipettes, microcentrifuge tubes, and laboratory reagents
Aug 27	Making TAE and conducting agarose gel electrophoresis
Aug 31	Plant DNA extraction and evaluation of concentration and purity
~~Sep 3~~	Labor Day
Sep 7	PCR and TA cloning (ligation reaction)
Sep 10	*E. coli* heat shock transformation, incubation, and plating (w/IPTG and Xgal)
Sep 14	Picking transformed *E. coli* colonies and starting cultures (LB media, 37°C)
Sep 17	Plasmid DNA isolation. Evaluation of concentration/purity and preparing for sequencing.
Sep 21	Interpreting sequencing results.
Sep 24	RNA isolation and evaluation of concentration and purity. **Laboratory Notebook Due**
Sep28	RNA gel electrophoresis and reverse transcription (RT) reactions
Oct 1	RT-PCR (w/controls) and gel electrophoresis
Oct 5	Restriction enzymes
Oct 8	Introduction to *Arabidopsis thaliana* as a model system
Oct 12	Crossing *A. thaliana*
Oct 15	Making MS media and plating *A. thaliana* seed
Oct 19	Observing *A. thaliana* mutants
Oct 22	*A. thaliana* transformation using *Agrobacterium*. **Laboratory Notebook Due**
Oct 26	Next-generation sequencing
Oct 29	Whole transcriptome shotgun sequencing
Nov 2	Microarray analysis
Nov 5	Basic bioinformatics – 1
Nov 9	Basic bioinformatics – 2
~~Nov 12~~	Veteran's Day
Nov 16	Protein extraction
Nov 19	Protein gel electrophoresis. **Laboratory Notebook Due**
~~Nov 23~~	Non-Instructional Day
Nov 26	Plant tissue, cell, and protoplast culture
Nov 30	**Research papers due**
Dec 3	Plant tissue, cell, and protoplast culture
Dec 7	**Presentations**
Dec 10	**Presentations/Laboratory Notebook Due, 12:00 pm to 2:00 pm (Final exam schedule for Fall 2012.)**