Biology 216 - Fall 2018 - Biology Second Year Laboratory

Laboratory Sections. BIOL 216-04, BIOL 216-05, BIOL 216-06,
M 1:30 pm – 4:20 pm ISC 302, 147, 304

Instructors: ***Joshua Baecker***, Office ISC 332 A: Genetics Instructor, Room 306
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Overall Goals for this Course.

1. To give you the intellectual, physical and technical skills that will enable you to succeed in more advanced Biology labs (Developmental Biology, Genetics, Cell, Physiology etc.), in independent research with Biology faculty members, in summer research experiences and in technical jobs.

2. To introduce you to selected technical and intellectual approaches used by biologists in Genetics, Cell Biology and Developmental Biology.

General structure of Course:

This course is designed to introduce second year students to three core areas of the Biology curriculum. It will be structured in three 4-week modules, with one module each representing Cell Biology, Genetics and Molecular Biology and Developmental Biology. Each module will be taught in a different room by faculty member with interests in the field being covered. After a month in one laboratory, students will rotate to another part of the course.

The modules are not meant to cover all of their respective fields, but rather to introduce you to one or two in-depth examples of modern approaches to answering contemporary questions in each. This lab does not replace the 1-credit laboratories that are offered in Ecology, Genetics and Cell, and Developmental Biology all of which will be available for students who wish to obtain more laboratory exposure to those areas. In contrast to our first-year laboratory (which emphasizes process skills), the second year laboratory will introduce student to techniques used in the respective fields. We hope this lab will give you increased intellectual and technical skills

that allow you to excel in upper level lab laboratories, in summer research experiences and in the workplace.

Grading:

Cell Biology Section	33.3%
Genetics Section	33.3%
Developmental Biology Section	33.3%

SUNY Geneseo will make reasonable accommodations for persons with documented physical, emotional or learning disabilities. Students should consult with the Director in the Office of Disability Services (Tabitha Buggie-Hunt, 105D Erwin, tbuggieh@geneseo.edu) and their individual faculty regarding any needed accommodations as early as possible in the semester.

Tentative Schedule

Common Activities

Week	Date	Day	Topic
1	August 27	M	Introduction, Pre-lab for each section
12	December 10	M	Laboratory Practical Exam

Laboratory Modules

There are 4-week sections in three areas of Biology that will be done during Weeks 2 to 14 during the semester. Each laboratory group will move to different rooms to complete each module.

Cell Biology Module: Mitochondrial Isolation and Function

Genetics and Molecular Genetics Module: Bacterial Transformation

Developmental Biology and Bioinformatics Module: Examining mutation and learning in *Caenorhabditis elegans* by Chemotaxis Assay

Cell Biology Module: Mitochondrial Isolation and Function

Cells are complex both structurally and functionally. One way in which cells can carry out very different kinds of tasks is by compartmentalization, where some reactions and events are sequestered away from others that might interfere or inhibit them. Cell biologists therefore often start by separating organelles or other compartments so that they can study a less complex system. Such separations are often based on differential centrifugation – a technique where cells are broken up and the resultant homogenate is subjected to increasingly greater sedimentation forces in a centrifuge. In this module of the course, we will first isolate mitochondria from cauliflower florets. Using an assay of the mitochondrial specific enzyme succinate dehydrogenase (SDH), we will measure the relative levels of mitochondria in our isolated fractions. We will also measure the protein levels in each isolated fractions so that we can better determine the characteristics of our isolated mitochondria.

Given that we now have the ability to isolate a fraction of mitochondria and do an enzyme assay, we will then use these techniques to examine the kinetics of the SDH reaction. You will be introduced to the Michalis-Menten equation which describes the relationship between the rate of an enzyme reaction and concentration of enzyme substrate. We will then actually measure the kinetic parameters K_M and V_{MAX} for SDH. Finally we will learn something about enzyme inhibitors and examine the effects of different inhibitor types on the SDH reaction.

Week	Topic
1	Mitochondrial Fractionation, Enzyme Assay
2	Protein Assay, Enzyme rate calculations, Introduction to Enzyme kinetics
3	Mitochondrial isolation, Determining SDH enzyme kinetics, K_M, V_{MAX}, Introduction to enzyme inhibitors
4	Characterizing inhibitors of Mitochondrial SDH

Expected Learning Outcomes

After successfully completing this four-week module you should be able to:

- use differential centrifugation to isolate cellular organelles;
- use spectroscopy to follow the progress of enzymatic reactions;
- graph data to promote effective analysis and interpret graphical data to solve questions;
- understand the basic properties of enzyme kinetics and be able to determine the kinetic parameters K_M and V_{MAX} experimentally;
- understand nature of enzyme inhibitors, and use an enzyme assay to characterize inhibitors

Genetics and Molecular Genetics Module. Bacterial Transformation

Introducing DNA molecules into organisms is at the core of both molecular genetics and genetic engineering, and is one of the most common "techniques" used by biologists. There are numerous reasons why an investigator will introduce a DNA molecule into a microorganism such as generating multiple copies of the DNA molecule for applications such as cloning or

sequencing and engineering a microorganism to produce a specific protein for biochemical studies. In this section, you will transform *E. coli* XL1-Blue strain with the plasmid pBluescript II KS+. XL1-Blue is sensitive to ampicillin (and most other antibiotics) but the plasmid contains the gene for ampicillin resistance. After obtaining strains which we tentatively believe to have taken up the plasmid, we will do a plasmid DNA isolation. You will learn to measure the amount and purity of the DNA that you have isolated. But how can we convince ourselves that the transformants contain the predicted plasmid and is not simply a random mutant to ampicillin resistance? One way is determine if the plasmid is truly pBluescript II KS+ via a restriction mapping experiment. Another way is to analyze the isolated plasmid for the presence of the ampicillin resistance gene using PCR.

Week	Topic
1	Bacterial Transformation
2	Plasmid Isolation and DNA Measurement
3	Restricting Mapping I- Restriction Digest and PCR
4	Restriction Mapping II- Agarose Gel Electrophoresis

Expected Learning Outcomes

After successfully completing this four-week module you should be able to:

- Explain the parameters relevant for design of a transformation experiment including strain genotype and selection/screening strategies;
- Describe how DNA yield and DNA purity are determined following plasmid DNA isolation;
- Differentiate genetic transformation from gene mutation;
- Develop experience in microbial culturing techniques, plasmid isolation and transformation;
- Understand the principles behind the restriction digest, polymerase chain reaction (PCR), and agarose gel electrophoresis.

Developmental Biology and Bioinformatics Module. Examining mutation and learning in *Caenorhabditis elegans* by Chemotaxis Assay

In humans, the study of complex development at the molecular level is extremely challenging, expensive and often unethical. Instead, the microscopic and harmless nematode worm *C. elegans* and other simpler animals have been exploited as key models. Remarkably, *C. elegans* has a simple body plan with exactly 959 body cells (plus varying numbers of eggs and sperms) arising from a fixed number of cell division and precise development into specific cell types. Additionally, it has a short (few days) and unique life cycle making it particularly suited for genetic manipulation, can be flash-frozen alive, easily cultured, and possesses a transparent body wall that permits live microscopic observation of its individual cells. Most importantly, it's biochemistry, cell biology, and development largely reflect those of humans. The huge contribution of *C. elegans* in advancing fundamental biological understanding is underscored by Nobel Prizes in Physiology and Medicine awarded to *C. elegans* researchers in 2002 (genetics

of organ development and programmed cell death), 2006 (RNAi interference), and 2008 (GFP expression).

In this module, we will learn and appreciate how a mutation of a single gene can cause a complex change in learning behavior in *C. elegans*. A thorough examination and documentation of the phenotype of the normal (wildtype) and mutant worms using a chemotaxis (chemical stimulus response) assay combined with microscopic observations, photos and video recordings should allow us to deduce the likely function(s) of the mutated gene in *C. elegans* neurological development and learning capacity. We will quantify observed behavioral phenotypes, present them graphically and apply a statistical analysis to assess their significance.

To extend our understanding of the possible functions of the mutated gene in human physiology and disease, we will first perform bioinformatic analyses using the *C. elegans* online database, called *Wormbase*, to determine its protein product(s) and use this information to uncover its biochemical function(s). And then we will explore the relatedness of the mutated gene and its corresponding protein to those of humans using the *in silico* Basic Local Alignment Tool (BLAST). Finally, we will search the Human Genome databases for information that might connect the developmental role(s) of the mutated gene between *C. elegans* and humans.

Note: *You will be working in groups of 3-4. But come to the lab individually prepared with a laptop computer and a smartphone. Be prepared to coordinate as a group to perform some laboratory tasks outside of the designated class period.*

Week	Topic
1	Growing *E. coli* in liquid culture, seeding them to solid media plates, and growing *C. elegans* in seeded plates
2	Chemotaxis assay and observation of phenotypes
3	Statistical and Bioinformatic analyses
4	Oral Presentation of Work

Expected Learning Outcomes

After successfully completing this four-week module you should be able to:

- Recognize the importance of using *C. elegans* as a model organism to gain insight into the functions of developmental genes in humans.
- Be familiar with the basic life cycle and chemotaxis behavior of *C. elegans*.
- Gain experience in designing and skillfully executing a chemotaxis assay in *C. elegans*, the ensuing collection of data, and the use of basic statistical technique to analyze data.
- Develop a basic understanding and skill in the use of bioinformatic methods and online genome databases to explore and discover functions of genes among related organisms.
- Refine your fundamental skills in growing microscopic organisms (bacteria and *C. elegans*) in culture, and the use of microscopes.

- Develop your scientific communication skills orally by presenting your completed work in a seminar format (Introduction, Methods, Results and Discussion) before your peers.
- Enhance your team collaboration and coordination skills critical for successful completion of a typical research project exemplified by this module.

GRADING SCHEME – Developmental Biology Module

Grades: The 33.3% of your final grade in the course will come from this module. It is broken down into the following categories of evaluation:

3 Quizzes	30 points
Group Scientific Paper	25 points
Group presentation	20 points
Participation and Attitude	10 points
Practical Exam	15 points
(BONUS)	(5 points)

Grades will follow the following point distribution:

≥93% = A	90-92.9% = A-	87-89.0%, = B+	83-86.9% = B	80-82.9% = B-
77-79.9% = C+	**73-76.9% = C**	**70-72.9% = C-**	**60-69.9% = D**	**<60% = E.**