Fall 2025

Idaho State University

Major Concepts in Biology I Laboratory

BIOL 1101L
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Prepared by:

Idaho State University

Jesssica Fultz
Senior Lecturer & Lab Coordinator

Department of Biological Sciences
921 South 8th Avenue, Stop 8007
Pocatello, ID 83209-8007

(208)282-3821
fultjess@isu.edu
https://www.isu.edu/biology/biolab/

Please note that this manual is a work in progress and was compiled specifically for the ISU Department of Biological Sciences. It changes each semester/session. It is a free and unpublished manual that has not seen reviewers or editors; there are errors.

Laboratory Rules

1. No food or drink allowed.

2. Coats, bags, purses, backpacks, etc. must be stored on the shelves provided on the side of the room. Do NOT keep them next to you at your workstation. Only your binder with syllabus, task sheets, pencil, colored pencils, and mini-stapler should be with you at the workstation.

3. Mobile electronic devices are NOT allowed in the lab (excluding the ISU lab computers and equipment). If a student has one on his/her person OR anywhere near their seat they will receive a zero on their quiz and/or task sheet. They must be placed in your bag or coat and stored on the shelves provided.

4. Standard lab attire is required:
   - work shirt that covers the upper torso and arms
   - lower body clothing that covers the leg to the knee (e.g., pants, skirt, coveralls, lab coat) and fully protects exposed skin, and
   - shoes that have a closed toe AND heel (i.e. NO flip flops or sandals.

5. Long hair must be restrained (i.e., braided, tied in a pony tail, etc.) for safety reasons.

6. Random assigned seating is required during each lab.

7. Before and after each lab, wipe down all student benches, computer keyboard and mouse, microscope knobs and eyepieces, and all other equipment that is touched during lab with the correct cleaning supplies.

8. Instructors must also wipe down the instructor benches, computer keyboard and mouse, microscope knobs and eyepieces, projector remote, as well as any other equipment they use during lab the correct cleaning supplies.

9. Participation in lab is required. If the lab instructor observes that a student is not actively participating in the completion of the task sheet, the student will receive a zero on the task sheet.

10. Notify your instructor of unsafe conditions such as broken glassware or water on the floor.

11. Never pour chemical reagents down the sink drain unless instructed to do so.

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Journal Articles (JA)

Objectives of the assignment are to learn about the;

- Scientific method
- Experimental design
- Technical and quantitative methods
- Relevance, utility, intellectual merit, and broader impact

Terms & Definitions

Axes of a graph:
A. x-axis is the horizontal axis of a graph; typically describes the predictor (independent) variable.
B. y-axis the vertical axis of a graph; typically describes the response (dependent) variable.

Biology - the study of life

Controlled experiment groupings:
A. Control - in a clinical trial, the group that does not receive the new treatment being studied. This group is compared to the group that receives the new treatment, to see if the new treatment works
B. Experimental - the sample in an experiment that is subjected to some type of variation that does not occur naturally.

Controls - constant and unchanging standards of comparison in scientific experimentation:
A. Negative - is not exposed to any treatment (experimental or otherwise) that is known to produce the expected effect.
B. Positive - is exposed to some other treatment that is known to produce the expected effect but not the experimental treatment.

Experimental design - the laying out of a detailed experimental plan in advance of doing the experiment. Well chosen experimental designs maximize the amount of "information" that can be obtained for a given amount of experimental effort.

Generative AI - artificial intelligence programming models that emulate the structure and characteristics of input data in order to generate derived synthetic content such as text, images, music, videos, code, and more, based on the input data and prompts; computational frameworks used to train machines to perform specific tasks by learning from data.

Genus - a taxonomic category ranking used in biological classification that is below family and above species.

Investigator error - is a type of systematic error caused by technical skills of the investigator and can result from measuring solutions inaccurately, not rinsing equipment well enough between tests (contamination), etc.

Observational / measurement error – the difference between a measured value of a quantity and its true value and can be the result of systematic error and random error. Systematic error always occurs, with the same value, when we use the instrument in the same way and in the same case and can be reduced with standardized procedures. Random error varies from one observation to another:
A. Accuracy - how close or far off a given set of measurements (observations or readings) are to their true value, accurate if their average (mean) is close to the true value.
B. Precision - how close or dispersed the measurements are to each other describes random errors; standard deviation is relatively small.

Organism - a living thing that maintains an internal order that is separated from the environment; descended from a single-celled ancestor that appeared almost 4 billion years ago: Consist of one or more cells, Contain genetic information, Use genetic information to reproduce themselves, Are genetically related, Covert molecules obtained from their environment into new biological molecules, Extract energy from the environment and use it to do biological work, Can regulate and internal environment.

Reasoning - the process of thinking about something in order to make a decision; logical thinking:
A. Inductive - a logical process that argues from specific instances to a general conclusion; deriving a generalization from specific details.
B. Deductive - making a prediction about the outcome of a test; generating a specific expectation from a generalization.

Science - the observation, identification, experimental investigation, and theoretical explanation of natural phenomenon.

Scientific method - a hypothesis-prediction approach to acquiring scientific knowledge about the natural world.
A. Observation - a note, record, of an occurrence, or phenomenon. Observations may be made directly or indirectly using tools.
B. Question - address something that can ultimately be measured. This means that the question has to be answerable – one that can be used to propose a set of hypotheses that can be tested and a set of predictions against which one can compare the results from the study.
C. Hypothesis - a tentative statement, derived from inductive reasoning, that proposes a possible explanation to the question and states a generalized relationship between two variables.
D. Prediction - a specific statement, derived from deductive reasoning, about what will occur (i.e. the outcome or pattern that will be observed) in a particular research investigation (e.g., an experiment).

Specimen - something shown or examined as an example

Species - a group of related organisms that share a distinctive form in nature and (for sexually reproducing species) are capable of interbreeding.

Species name – a formal system of naming species of living things by giving each a name composed of two parts (binomial nomenclature) and are italicized. The two words are as follows:
A. generic name – identifies the genus to which the species belongs and is capitalized.
B. specific name or specific epithet – distinguishes the species within the genus and is not capitalized.

Statistics - a branch of mathematics that estimates the reliability of data by dealing with the collection, analysis, interpretation, presentation, and organization of data:
A. Data - a collection of discrete values that convey information, describing quantity, quality, fact, statistics, other basic units of meaning, or simply sequences of symbols that may be further interpreted.
B. Descriptive statistics - quantitatively describe or summarize features of a collection of information.
C. Mean - is a descriptive statistical measure that reports the central location in a sample of data. A central value of a discrete set of numbers: specifically,

the sum of the values divided by the number of values.
D. Replication - the repetition of an experimental condition so that the variability associated with the phenomenon can be estimated
E. Standard deviation - a measure that is used to quantify the amount of variation.

Testable - possible to evaluate through observations of the measurable universe.

Theory - a broad explanation of some aspect of the natural world that is substantiated by a large body of evidence.

Variables - any characteristics, number, or quantity that can be measured or counted.
A. Predictor - causes or affects the response variable; are also known as explanatory or independent variables; are denoted by an X and are shown on the horizontal x-axis.
B. Response - influenced by the predictor variable; are also known as dependent variables; are denoted by a Y and are shown on the vertical y-axis.

Part 1. Software Install

☐ Bring your laptop and charging cord (please contact your instructor if you do not have a laptop).

☐ Load software onto your personal laptop:

- How to install Mircrosoft Office 365 Education.
- How to install Adobe Reader.

Part 2. Journal Article

Textbooks and websites are the most familiar form of educational media. University freshman and sophomore students use these for most of their coursework but as a student progresses to junior and senior level courses they are expected to rely on relevant research articles. Research articles in the sciences must be rigorously peer-reviewed against strict criteria if they are to be published in scientific journals. Students do not have the foundational or applied knowledge in their chosen field nor the quantitative background to accurately critique most articles. As a student progresses to their upper division courses, they will become more proficient at critically reading research articles but they have not acquired the advanced education and experience to be considered 'peers' of the principal investigators that conduct the research and publish the peer-reviewed articles.

Your lab instructor has chosen one journal article from a specific group of journals from a specific publication year that is an **experimental study done by the authors, NOT a review / note / comment.** Your entire lab section will be required to read the article and answer questions about the article **during lab 1**.

A. DOI

1. What is a DOI and what is its purpose (see the DOI Foundation website)?
2. What is the DOI of the article you instructor chose?

B. Questions

☐ Go to Biolab > Training > Learning Environment > Research > Scientific Method & Experimental Design (https://www.isu.edu/biology/biolab/instructor-training/learning-environment/research/#d.en.218210) and study the content at that web page.

☐ **Answer questions 1-6** concisely, thoughtfully, and in **complete sentences and paragraph form** as an individual. Remember that your audience does not include experts. You will not understand many, if not most, of the terms and acronyms in the articles; look them up, and then use the definition in your answer. You must **RE-WRITE** the ChatGPT AI response in **YOUR OWN WORDS and Handwriting**, no quoting. Be sure to check your spelling and grammar. Then make sure you have completed **question 7**.

3. What is the **species** name of the model/study organism? If the study is looking at human disease choose the disease causing organism. If the study is on some aspect of human physiology or anatomy, the model organism is a human. You may need to find this information using another resource.
4. Intellectual merit and broader impact:
   A. Intellectual merit - Why is this research important enough to spend time and money doing it? Does it advance knowledge and understanding within its own field or across different fields?
   B. Broader impact - What were the economical, environmental, medical, health, or a combination of reasons that make this research valuable? Does it benefit society or advance desired societal outcomes?

5. What is the major question and/or hypothesis studied in the journal article?
6. Sampling for the study:
   A. Where did they sample?
   B. How did they sample?
   C. Number of samples taken?
7. Choose **one experiment** from the study:
   A. Describe the experiment.
   B. What are the predictor and response variables for this experiment?
   C. What evidence did the experiment produce?
   D. Describe how the evidence supported/answered the major hypothesis/question?
8. What was the major conclusion of the study?
9. Reference your resources **in**cluding the article under study and the Generative AI program.

✓ Author, A. A., Author, B. B., & Author, C. C. (Year). Title of article. *Title of Journal*, *volume number*(issue number), pages. http://dx.doi.org/xx.xxx/yyyyy

✓ OpenAI. (2025). ChatGPT 5 (August 14 version) [Large language model]. https://chatgpt.com/

Part 3. Calculate AI Footprint

10. Open the calculator and use the “Text Queries” cell to calculate the impact:

Table JA-1. AI Footprint.

	Individual (n =1)	Individual per year (n =1)	Section (n=____)	Entire Lab Course (n=___)
Energy Consumption kWh				
CO_2 Emissions kg				
Water Usage L				
Smartphone charges				
Laptop hours				
Car equivalent km				
Trees needed for offset				

Introduction

Objectives

- Learn about and practice microscopy.
- Learn about cells.
- Identify and classify organisms in a pond community

Terms & Definitions

Animalia – a eukaryotic kingdom of the domain Eukarya that encompasses those organisms called animals. Animals are multicellular heterotrophs with cells that lack cell walls.

Autotroph – an organism that has a metabolic pathways that use energy either from inorganic molecules or light to make organic molecules.

Archaea – one of the three domains of life that encompasses those one-celled organisms called archaeans.

Bacteria – one of the three domains of life that encompasses those one-celled organisms called bacteria.

Bacterial morphology – are the shapes that are characteristic of various types of bacteria and often key to their identification. Their direct examination under a light microscope enables the classification of these bacteria:

A. Coccus – spheres
B. Coccobacillus – oval and similar to coccus
C. Diplococci – pairs of cocci
D. Fusiform – spindle-like shape that is wide in the middle and tapers at both ends.
E. Rod - round-ended cylinders
F. Sarcina – pack-like cuboidal arrangement of eight cocci
G. Spirochetes – helically twisted cylinders
H. Streptococci – chains of cocci
I. Staphylococci – irregular (grape-like) clusters of cocci
J. Tetrads – clusters of four cocci arranged within the same plane

Biological classification – is a system for comparing and grouping organisms, and the naming of those groups.

Cell – the simplest unit of a living organism:

A. Cell wall - a relatively rigid, porous, structure located outside the plasma membrane of prokaryotic plant, fungal, and certain protists cells; provides support and protection.
B. Cilium(a) - membrane-bound organelle attached to the cell membrane that can be motile or non-motile.
C. Chloroplast - plastids found in plant and algal cells that carry out photosynthesis.
D. Cytoplasm - the region of the cell that is contained within the plasma membrane.
E. Cytoskeleton - in eukaryotes, a network of three different types of protein filaments in the cytosol called microtubules, intermediate filaments, and actin filaments.
F. Cytosol - the semifluid portion of the cytoplasm.
G. Desmosomes - intercellular junctions/bridges that mediate cell–cell adhesion and anchor the intermediate filament network to the plasma membrane, providing mechanical resilience to tissues and act as mediators of cell signaling important for proper cell and tissue functions.
G. Endoplasmic reticulum: the transportation system of the eukaryotic cell, and has many other important functions such as protein folding. Smooth - lacks ribosomes and helps synthesize and concentrate various lipids, phospholipids as in plasma membranes, and steroids needed by the cell. Rough - studded with protein-manufacturing ribosome.
H. Flagellum(a) - relatively long cell appendages that facilitate cellular movement or the movement of extracellular fluid.
I. Golgi apparatus - a complex of vesicles and folded membranes within the cytoplasm of most eukaryotic cells, involved in secretion and intracellular transport.
J. Lysosome - a membrane-bound organelle found in many animal cells. They are spherical vesicles that contain hydrolytic enzymes that can break down many kinds of biomolecules. A lysosome has a specific composition, of both its membrane proteins, and its lumenal proteins.
K. Microtubule - protein structure that moves chromosomes around during mitosis and meiosis.
L. Mitochondrion(a) - organelle found in eukaryotic cells that supply most of a cell's ATP.
M. Nuclear envelope - a double-membrane structure that encloses the cell's nucleus.
N. Nucleolus - a prominent region in the nucleus of nondividing cells where ribosome assembly occurs.
O. Nucleus - cell structure that houses DNA; found in eukaryotes.
P. Organelle - a subcellular structure or membrane-bound compartment with its own unique structure and function.
Q. Peroxisomes - small, membrane-enclosed organelles found in the cytoplasm of virtually all eukaryotic cells that contain enzymes involved in a variety of metabolic reactions, including several aspects of energy metabolism.
R. Plasma (cell) membrane - the biomembrane that separates the internal contents of a cell from its external environment.
S. Pseudopod - a part that temporarily sticks out of the protoplasm (= the liquid inside a cell) of some organisms that have only one cell, used for movement and to get food
T. Ribosome - a structure composed of proteins and rRNA that provides the site where polypeptide synthesis occurs.
U. Vacuole - a space that contains air or liquid inside a living cell, often storing an important chemical or food substance.

Community – a group or association of populations of two or more different species occupying the same area at the same time.

Cyanobacteria – phylum of autotrophic gram-negative bacteria that can obtain biological energy via oxygenic photosynthesis.

Eukaryote – one of the three categories into which all forms of life can be placed. The distinguishing feature of eukaryotes is cell compartmentalization, including a cell nucleus; includes protists, fungi, plants, and animals.

Fungi – a eukaryotic kingdom of the domain eukarya that is composed of heterotrophic unicellular, multicellular, or syncytial spore-producing organisms, including molds, yeast, mushrooms, and toadstool.

Habitat – place where an organism lives.

Heterotroph – organism that cannot produce their own organic molecules and thus must obtain organic food from other organisms.

Life – a monophyletic group (refers to a group that consists of an ancestor and all of its descendants) that includes all known organisms. Characterized by a nucleic acid based genetic system (DNA or RNA), metabolism, and cellular structure. Some parasitic forms have secondarily lost some of these features and rely on the cellular environment of their host.

Microscope – a magnification tool that enables researchers to study very small structures and cells:

A. Depth of field - is determined by the distance from the nearest specimen plane in focus to that of the farthest plane also simultaneously in focus.
B. Magnification - the ratio between the size of an image produced by a microscope and its actual size.
C. Field of View - the visible area seen through the microscope when the specimen is in focus. The greater the magnification the smaller the view.
D. Focus - a specimen is in focus at the desired magnification when the image seen through the ocular lens is sharp and clear.
E. Objective lens - the primary optical system which produces a magnified image of the specimen. There are typically four objective lenses attached to the nosepiece with the magnification of each objective engraved on its side.
F. Ocular lens - the secondary optical system that you look through. The ocular lens further magnifies (10x) the image and brings the light rays to a focal point.
G. Resolution - point-to-point resolving power in the plane perpendicular and parallel to the optical axis. The ability to observe two adjacent objects as distinct from one another; a measure of clarity of an image

Multicellular – the condition of being composed of many coordinated cells.

Organism – a living thing that maintains an internal order that is separated from the environment; descended from a single-celled ancestor that appeared almost 4 billion years ago: Consist of one or more cells, Contain genetic information, Use genetic information to reproduce themselves, Are genetically related, Covert molecules obtained from their environment into new biological molecules, Extract energy from the environment and use it to do biological work, Can regulate and internal environment.

Plant – a taxonomic group that includes land plants and green algae. The group is defined as being eukaryotic with cell walls made of cellulose and the ability to make food via photosynthesis using double-membrane-bound chloroplasts and both chlorophyll a and b. The glucose product of photosynthesis is stored as starch.

Protist – any eukaryotic organism that is not an animal, land plant, or fungus. Protists do not form a natural group, or clade, but are a polyphyletic grouping of several independent clades that evolved from the last eukaryotic common ancestor.

Species – a group of related organisms that share a distinctive form in nature and (for sexually reproducing species) are capable of interbreeding

Species name – a formal system of naming species of living things by giving each a name composed of two parts (binomial nomenclature) and are italicized. The two words are as follows:

A. generic name – identifies the genus to which the species belongs and is capitalized.
B. specific name or specific epithet – distinguishes the species within the genus and is not capitalized.

Specimen – something shown or examined as an example

Taxon(a) – a group of species that are evolutionarily related to each other. In taxonomy, each species is placed into several taxons that from a hierarchy from large (domain) to small (genus):

1. Domain - one of the three major categories of life; Bacteria, Archaea, and Eukarya.
2. Kingdom
3. Phylum/division
4. Class
5. Order
6. Family
7. Genus

Taxonomy – the field of biology that is concerned with the theory, practice, and rules of classifying extinct and extant organisms and viruses.

Turbid – cloudy, opaque, or thick with suspended matter.

Turbidity – the measure of relative clarity of a liquid. It is an optical characteristic of water and is a measurement of the amount of light that is scattered by material in the water when a light is shined through the water sample. The higher the intensity of scattered light, the higher the turbidity. Material that causes water to be turbid include clay, silt, very tiny inorganic and organic matter, algae, dissolved colored organic compounds, and plankton and other microscopic organisms.

Unicellular – an organism that is composed of only one cell.

Name:_____ Team #:_____ Section #:_____

Part 1. Microscopy

A. Microscope Components

☐ Go to Biolab > Training > Technology > Microscopes (https://www.isu.edu/biology/biolab/instructor-training/technology/microscopes/) and study the parts of the microscope.

Table Intro-1. Compound microscope magnification.

Objective Lens Magnification		Ocular Lens Magnification	Total Magnification
Scanning			
Low power			
High power			
Oil immersion			

1. Look at a compound microscope and determine the magnification of each objective lens and the ocular lens then calculate the total magnification of an image viewed (Table Intro-1).
2. Describe how to make a wet mount:

B. Viewing Specimens

☐ Obtain a thread slide and place it carefully into the stage clip. In order to view each thread in focus at greater magnification, you must focus downward to view the ones underneath and upward to view the ones that are above.

☐ What is the slide's ID number / letter?_____

3. Which thread is on top, which is on bottom, and which is in the middle at each objective?

Top 4X___10X ____40X ____ Middle 4X___10X ____40X ____ Bottom 4X___10X ____40X ____

4. When you adjust the fine focus knob, are all of the threads in focus at the same time? _____
5. What happens to the depth of field when you increase to a higher magnification? Does it increase, decrease, or remain the same? _____

☐ Obtain an **e** slide and view it under the scanning (4X) objective. Make sure the **e** slide is on the stage with the e in its normal orientation.

6. Sketch the orientation of the **e** image you view unaided on the slide stage _____ compared to the view looking through the ocular lens of the microscope_____.
7. Move the slide to the left. Draw an arrow to depict the direction the **e** moved?_____

Part 2. Cells

A. Domain Bacteria

Bacteria are the most abundant of all organisms and they occur everywhere; in air, soil, water and other organisms. They include some organisms that are pathogenic (disease-causing) and others that are essential to the lives of different species with which they interact. Bacteria can be classified using morphology such as size, shape (rod, cocci, helical) and the presence of filaments, pili, endospores, flagella, or capsules. They can also be classified using staining (Gram-negative or positive), biochemical tests, and genetics. Gram staining is a method of differentiating bacteria into two large groups based on the composition of their cell walls. If the bacteria are positive (stained purple) they have a thick cell wall of peptidoglycan whereas if they are negative (stained pink) the wall is much thinner.

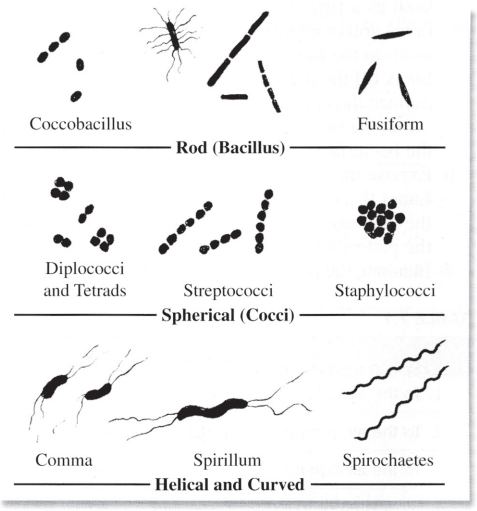

Figure Intro-1. Bacterial morphology.

B. Domain Eukaryota or Eukarya

Unicellular eukaryotes are often called protists. But the group Protista is polyphyletic which means they probably do not share a single common ancestor. These organisms have distinct nuclei and membrane-bound organelles, however, they never have an embryonic stage even if they sexually reproduce. They are extremely diverse microscopic organisms with varying structures, means of locomotion, and mechanisms for obtaining nutrients using specific organelles. Many of these organisms have acquired chloroplasts through ancient symbiotic relationships but the chloroplasts are not the same as those found in plants. You will find a variety life histories within these organisms: heterotrophs (actively hunt bacteria and other unicellular eukaryotes), parasites, detritivores, autotrophs, and a combination of the other four. .Besides the multicellular plants, animals, and fungi that we are most familiar there are other multicellular eukaryotes

8. In Figure Intro-2, label and color the listed subcellular structures and organelles:

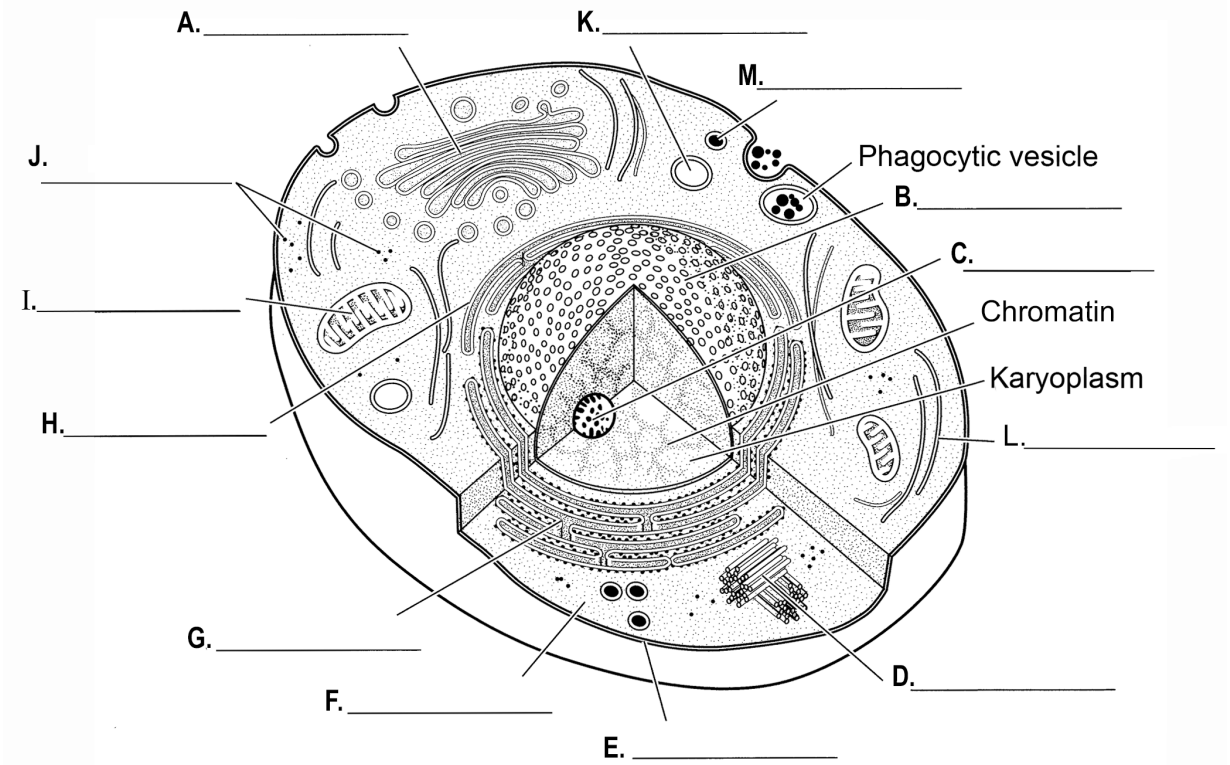

Figure Intro-2. Cell diagram, subcellular structures and organelles; A. Golgi apparatus (green), B. Nucleus (purple), C. Nucleolus (dark blue), D. Centrioles (brown), E. Plasma membrane, F. Cytoplasm (yellow), G. Rough endoplasmic reticulum (red), H Smooth endoplasmic reticulum (pink), I. Mitochondrion (orange), J. Free ribosomes. K. Lysosome, L. Cytoskeleton, and M. Peroxisome.

Part 3. Observation and Classification of Pond Life

You will take samples from the pond water communities and then identify the organisms to genus using the 'Survey of Organisms' information sheets. At least two genera as well as one drawing of an unidentified are required per table except for the following conditions:

- Bacteria - do not use genus names but instead use morphological names found on Figure Intro-1.
- Fungi - it may be difficult to find the fungi but there may be some on the decaying plant tissue.

If you find something that is interesting, bring it to the instructor microscope where your instructor will project it and take images/video using the Leica Camera.

☐ Using the available spoon, mix the substrate on the bottom of the container until the pond water is turbid. Make a wet mount from one quadrant of the turbid pond water.

9. Record your observations (genus, location) in the Tables Intro-2-7. If you are unable to identify the organism sketch it in the space provided on the table.
10. Make another wet mount from a different quadrant and record your observations (genus, location) in the Tables Intro-2-7. If you are unable to identify the organism sketch it in the space provided on the table.
11. Continue until you have make five wet mounts in total each from a different quadrant. Do not make all the wet mounts at the same time or they will dry out before you observe them. Record your observations (genus, location) in the Tables Intro-2-7. If you are unable to identify the organism sketch it in the space provided on the table.

☐ Clean the wet mounts by;

a) removing the coverslip from the slide,
b) dunk the slide in the pond to return the microbes back to the pond,
c) wipe the slide off with a paper towel,
d) place the slide back in the weigh boat, and
e) place the coverslip in the garbage.

Table Intro-2. Bacteria found in the pond water.

Bacterial morphology	Luadrant	Sketches of unidentified

Table Intro-3. Cyanobacteria found in the pond water.

Genus	Quadrant	Sketches of unidentified

Table Intro-4. Unicellular eukaryotic autotrophs (algae) found in the pond water.

Genus	Location in container	Sketches of unidentified

Table Intro-5. Unicellular eukaryotic heterotrophs (amoeboid, ciliated) found in the pond water.

Genus	Quadrant	Sketches of unidentified

Table Intro-6 Fungi (mold, mildew) found in the pond water.

Genus	Quadrant	Sketches of unidentified

Table Intro-7. Small or microscopic animals (multicellular heterotrophs) found in the pond water.

Genus	Quadrant	Sketches of unidentified

Unit I. Urinalysis

Objectives

- Study the potential of hydrogen (pH) and electrical conductivity in urine using Vernier computer interfaces (Go!Link) and software (Logger Pro).
- Study macromolecules by determining the concentration of two types of macromolecules in urine using a spectrophotometer.
- Become familiar with the scientific method and experimental design.
- Demonstrate the movement of water and ions using two NaCl solutions, water, and change in potato mass.
- Learn the dependent nature of **hypotonic (hypo- beneath or below), hypertonic (hyper- over, above, beyond), and isotonic (iso- as equal, uniform) solutions.**
- Calculate the rate of movement using Vernier electrical conductivity sensors and software.
- Learn about and calculate descriptive statistics; mean and standard deviation.
- Create tables and figures with corresponding captions using Microsoft® 365 Excel and Word.

Terms & Definitions

Aliquot - a portion of a larger whole, especially a sample taken for chemical analysis or other treatment.

Atom - the smallest unit of ordinary matter that forms a chemical element. Every atom is composed of a nucleus and one or more electrons bound to the nucleus. The nucleus is made of one or more protons and a number of neutrons:

A. Proton - a positively charged subatomic particle. The number of protons in an atom is called the atomic number which defines each type of element.
B. Electron - a negatively charged subatomic particle with the least amount of mass.
C. Neutron - an uncharged subatomic particle with the greatest amount of mass.

Active transport - the transport of a solute across a membrane against its gradient (from a region of low concentration to a region of higher concentration). Requires an input of energy. Primary active transport that uses adenosine triphosphate (ATP), and secondary active transport that uses an electrochemical gradient.

Buffer - a compound that acts to minimize pH fluctuations in the fluids of living organism . Buffer systems can raise of lower pH as needed.

Compound - a substance consisting of two or more elements.

Concentration - the amount of solute dissolved in a unit volume of solution.

Chemical - a unique form of matter with constant chemical composition and characteristic properties. Chemical substances may take the form of a single element or chemical compounds.

Chemical bonds - hold molecules together and create temporary connections that are essential to life:

A. Covalent bond - the sharing of electrons between atoms. This type of bonding occurs between two atoms of the same element or of elements close to each other in the periodic table. This bonding occurs primarily between nonmetals; however, it can also be observed between nonmetals and metals.
B. Glycosidic bond - a bond formed between two sugar molecules.
C. Hydrogen bonds - a weak chemical attraction between a partially positive hydrogen atom of a polar molecule and a partially negative atom of another polar molecule.
D. Ionic bonding - is the complete transfer of valence electron(s) between atoms. It is a type of chemical bond that generates two oppositely charged ions. In ionic bonds, the metal loses electrons to become a positively charged cation, whereas the nonmetal accepts those electrons to become a negatively charged anion. Ionic bonds require an electron donor, often a metal, and an electron acceptor, a nonmetal.
E. Peptide -a covalent bond joining the α-amino group of one amino acid to the carboxyl group of another with the loss of a water molecule.
F. Phosphodiester - two of the hydroxyl groups in phosphoric acid react with hydroxyl groups on other molecules to form two ester bonds.

Chemical reactions - the formation and breaking of chemical bonds, resulting in a change in the composition of substances:

A. Anabolic - a metabolic pathway that involves the synthesis of larger molecules from smaller precursor molecules. Such reactions usually require an input of energy
B. Catabolic - a metabolic pathway in which a molecule is broken down into smaller components, usually releasing energy (exergonic).
C. Condensation - a type of chemical reaction in which two molecules are combined to form a single molecule, usually with the loss of a small molecule such as water.
D. Dehydration - a type of condensation reaction in which a molecule of water is lost.
E. Endergonic - chemical reactions that require an addition of free energy and do no proceed spontaneously.
F. Exergonic - refers to chemical reactions that release free energy and occur spontaneously.
G. Hydrolysis - any chemical reaction in which a molecule of water breaks one or more chemical bonds.

Dialysis - the separation of particles in a liquid on the basis of differences in their ability to pass through a membrane.

Electronegative - the tendency to attract electrons to form a chemical bond.

Equilibrium (chemistry) - occurs when the rate of the forward reaction is balanced by the rate of the reverse reaction; in ecology, the situation in which the population size stays the same.

Equilibrium potential - the membrane potential at which the flow of an ion is at equilibrium, with no net movement in either direction.

Homeostasis - the tendency toward a relatively stable equilibrium between interdependent elements, especially as maintained by physiological processes.

Interstitial cells - any cell that lies in the spaces between the functional cells of a tissue.

Interstitial fluid - a fluid found in the spaces around cells. It comes from substances that leak out of blood capillaries (the smallest type of blood vessel). It helps bring oxygen and nutrients to cells and to remove waste products from them. As new interstitial fluid is made, it replaces older fluid, which drains towards lymph vessels. When it enters the lymph vessels, it is called lymph. Also called tissue fluid.

Ions - atoms or molecules that gain or lose one or more electrons and acquires a net electric charge:

A. Anions - an ion that has a net negative charge.
B. Cations - ions that have a positive net charge.

Electrolytes (ionic) - substances in water that dissociate into cations and anions. The resulting solution can then conduct an electrical current:
A. Acid - releases hydrogen ions (h+; protons) in solution; give up protons during chemical reactions.
B. Base - lowers the h+ concentration; accepts protons during chemical reactions.
C. Hydrogen ion concentration - the concentration of hydrogen ions (protons) in a solution expressed usually in moles per liter or in pH units.
D. pH - the mathematical expression of a solution's hydrogen ion (H+) concentration, defined as the negative logarithm to the base 10 of the H+ concentration.
E. pH scale - a logarithmic scale that indicates the concentration of hydrogen ions. The scale goes form 0-14, with zero representing an extremely high concentration of free H+ ions and 14 representing the lowest concentration.
F. Salt - a chemical compound consisting of an ionic assembly of positively charged cations and negatively charged anions, which results in a compound with no net electric charge. A common example is table salt, with positively charged sodium ions and negatively charged chloride ions.

Macromolecule - molecules bonded together to form a polymer:
A. Carbohydrate - organic molecules often with the general formula c(H_2O); a carbon-containing compound that includes starches, sugars, and cellulose.
B. Lipid - a molecule composed predominately of hydrogen and carbon atoms; nonpolar and insoluble in water.
C. Nucleic acids - an organic molecule composed of nucleotides. The two types of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)
D. Protein - a functional unit composed of one or more polypeptides. Each polypeptide is composed of a linear sequence of amino acids.

Membrane potential - the difference between the electric charges outside and inside a cells; also called potential difference.

Membrane transport - the movement of ions or molecules across a cell membrane.

Metabolism - the sum total of all chemical reactions that occur within an organism:
A. Anabolism - a metabolic pathway that results in the synthesis of cellular molecules and macromolecules; requires an input of energy.
B. Catabolism - a metabolic pathway in which a molecule is broken down into smaller components, usually releasing energy (exergonic).Membrane transport

Monomer - an organic molecule that can be used to form larger molecules (polymers) consisting of many repeating units of the monomer.

Nucleotides - organic molecules having three components: one or more phosphate groups, a five-carbon sugar (either deoxyribose or ribose), and a singe or double ring of carbon and nitrogen atoms known as a base.
A. Pyrimidine - single ring structured base; cytosine, thymine, and uracil (RNA only).
B. Purines - two-ring structured base; adenine and guanine.

Passive transport - a type of membrane transport that does not require energy to move substances across cell membranes:
A. Diffusion - in a solution, the process that occurs when a solute moves from a region of high concentration to a region of lower concentration.
B. Facilitated diffusion - the process of spontaneous passive transport (as opposed to active transport) of molecules or ions across a biological membrane via specific transmembrane integral proteins.
C. Filtration - movement of water and solute molecules across the cell membrane due to hydrostatic pressure generated by the cardiovascular system.
D. Osmosis - the movement of water across membranes to balance solute concentrations. Water diffuses from a solution that is hypotonic (lower solute concentration) into a solution that is hypertonic (higher solute concentration).

Peptide - short chains of amino acids linked by covalent peptide bonds.

Pressure - force per unit area:
A. Hydrostatic pressure - the physical force exerted by a fluid on a structure (HB). Blood pressure is the force exerted per unit area by the blood as it presses against the internal surface of the vessel wall. Interstitial fluid hydrostatic pressure is the force of interstitial fluid on the external surface of the blood vessel.
B. Pressure gradient - a physical quantity that describes in which direction and at what rate the pressure increases the most rapidly around a particular location.
C. Osmotic pressure - the minimum pressure which needs to be applied to a solution to prevent the inward flow of its pure solvent across a semipermeable membrane.

Phospholipid - a class of lipids that are similar in structure to triglycerides, but the third hydroxyl group of glycerol is linked to a phosphate group instead of a fatty acid; a key component of biological membranes.

Phospholipid bilayer - the basic framework of the cellular membrane, consisting of two layers of lipids.

Polymer - a large molecule formed by linking many smaller molecules called monomers.

Secretion - 1) the export of a substance from a cell; 2) the process in which some solutes are actively transported into the tubules of the excretory organ; this supplements the amount of solute that would normally be removed by filtration alone.

Selectively Permeable - the property of membranes that allows the passage of certain ions or molecules but not others.

Solute - a substance dissolved in a liquid.

Solution - a liquid that contains one or more dissolved solutes.

Stock solutions - concentrated solutions of known, accurate concentrations that will be diluted for future laboratory use.

Spectrum - used to classify something, or suggest that it can be classified, in terms of its position on a scale between two extreme or opposite points:
A. Absorption spectrum - a diagram that depicts the wavelengths of electromagnetic radiation that are absorbed by a pigment.
B. Action spectrum - the rate of photosynthesis plotted as a function of different wavelengths of lights.
C. Electromagnetic spectrum - all possible wavelengths of electromagnetic radiation, from relatively short wavelengths to much longer wavelengths.

Standard curve (calibration curve) - a method to determine the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration.

Tonicity - a measure of the effective osmotic pressure gradient; the water potential of two solutions separated by a partially-permeable cell membrane:
A. Hyper- a greater concentration of non-permeating solutes than another solution.
B. Hypo- a lower concentration of solutes than another solution.
C. Iso- the concentration is the same as that of another solution.

Wavelength - the distance from the peak of one wave to the next.

Background

There are four homeostatic functions of the human urinary/renal system; 1) regulate blood volume and blood pressure by adjusting volume of water lost in urine and releasing hormones that control red blood cell production and blood pressure, 2) regulate plasma concentrations of sodium, potassium, and chloride ions by controlling quantities lost in urine and calcium ion concentration through synthesis of the hormonally active metabolite of vitamin D, 3) help stabilize blood pH by controlling loss of hydrogen ions and bicarbonate ions in urine, and 4) conserve valuable nutrients while excreting organic waste products. Human urine is a liquid that is secreted by the urinary system. The kidneys filter blood to remove waste and extra fluid which is collected within the bladder as urine and excreted through the urethra. Urine is composed of 91–96% water and the remainder can be broadly characterized into inorganic salts, urea, organic compounds, organic ammonium salts, and small amount of epithelial cells from the bladder and external urethra. **Epithelial cells in the urine can increase from a urinary tract infection or some other cause of inflammation. Diseases can also cause urine to contain white blood cells (infection), red blood cells (kidney disease, a blood disorder or another underlying medical condition, such as bladder cancer), bacteria or yeasts (infection), tube-shaped proteins called casts (kidney disorders), and crystals (sign of kidney stones).**

A. Urinalysis

Urinalysis evaluates samples of urine to detect and assess a wide range of disorders, such as urinary tract infections, kidney disease, and diabetes. It involves examining the appearance, concentration and content of urine. Abnormal urinalysis results may point to a disease or illness. Most routine clinical tests are completed by using a commercial dipstick. A dipstick has absorbent paper impregnated with specific chemicals and several different chemical tests can be performed with one dipstick. Dipsticks can screen for the presence of excessive glucose or protein in urine. Other tests that can be performed using a dipstick include detecting the presence of bilirubin, blood, leukocytes, ketones, nitrite, urobilinogen, and pH. Urine can also be used for pregnancy testing and drug screenings. For example, pregnancy testing measures a hormone called human chorionic gonadotropin (HCG). Drug screenings detect specific drugs or their metabolic products, depending on the purpose of the testing. Quantitative tests for pH, conductivity, and macromolecules in urine are not routinely done. During the Unit I - Urinalysis & Dialysis we will study a variety of urine variables and measure them quantitatively.

pH & Conductivity

The pH level of urine indicates the amount of acid in urine. Abnormal pH levels may indicate a kidney or urinary tract disorder and possibly the formation of stones. Electrical conductivity (EC) can show how concentrated the urine is and can indicate dehydration. Also, red blood cells, pus cells, calcium oxalate monohydrate, calcium oxalate dihydrate, uric acid, sodium, and phosphates that can be found in urine and are correlated with high EC.

- pH - 4.6 to pH 8.0
- Conductivity (EC) - 2.1- 8.1 dS/m (decisiemens per meter)

Macromolecules

The major classes of macromolecules can be detected in urine. Analysis of urine for the major classes of macromolecules is clinically important in assisting diagnosis metabolism disorders and understanding the pathologic significance of the disorders.

Carbohydrate - There are eight carbohydrates that are often tested for: maltose, lactose, D-mannose, D-glucose, D-ribose, D-xylose, L-arabinose and D-galactose. Detection of glucose in a urine test usually calls for follow-up testing for diabetes. **Glucose - not usually found in urine. If it is, further testing is needed. Normal glucose range in urine: 0 - 0.15 mg/ml**

Protein - Low levels of protein in urine are normal but large increases of protein may indicate a kidney problem such as diabetic kidney damage. More than 150 mg/24 hours is considered excessive and can be caused by a variety of conditions, from benign to serious. Transient increases can occur with a fever, exposure to cold, emotional stress or severe exercise. However, persistent increases are most commonly associated with kidney diseases such as polycystic kidney disease, and nephrotic syndrome. **Protein - 0 to 0.2 mg/ml in a random urine sample. The normal value is less than 0.8 mg/ml in a 24-hour urine collection.**

Nucleic Acids - Urine is not considered an ideal source of nucleic acids because there are few nucleated cells found in urine but those that are found are typically white blood cells and epithelial cells. The need for the use of urine as an identification tool may arise from a crime scene, or in a toxicology laboratory. At a crime scene, urine may be used to identify the perpetrator of a crime, or to place a victim at a particular site. In a laboratory, DNA analysis may be needed to positively identify an individual as the submitter of a particular urine sample.

Lipid - Human urine usually contains only very small amounts of lipids. However, under certain nephrotic syndromes the urinary excretion of cholesterol, cholesterol esters, triglycerides, free fatty acids and phospholipids is considerably increased. Many of the lipids found in urine come from the phospholipids of cell membranes.

B. Filtration

In order to survive, all organisms need to move molecules in and out of their cells. Molecules such as gases (e.g., O_2, CO_2), water, food, and wastes pass across the cell membrane. There are two ways that the molecules move through the membrane: **passive transport** and **active transport.** While active transport requires that the cell uses chemical energy to move substances through the cell membrane, passive transport does not require such energy expenditures. Passive transport occurs spontaneously, using heat energy from the cell's environment. **Diffusion is the movement of molecules by passive transport from a region in which they are highly concentrated to a region in which they are less concentrated.** Diffusion continues until the molecules are randomly distributed throughout the system. **Osmosis, the movement of water across a membrane, is a special case of diffusion. Water always flows down a pressure gradient or up a solute concentration gradient**. Water molecules are small and can easily pass through the membrane. Other molecules, such as proteins, DNA, RNA, and sugars are too large to diffuse through the cell membrane. The membrane is said to be semipermeable, since it allows some molecules to diffuse through but not others.

Kidneys filter blood in a three-step process to produce urine. Nephrons, the functional unit of the kidney, are composed of a Glomerular capsule (Bowman's capsule, renal corpuscle) and a long convoluted renal tubule (proximal convoluted tube + loop of Henle + distal convoluted tubule). Nephrons are involved in the formation of filtrate by 1) renal ultrafiltration of the blood plasma, 2) selective reabsorption of most of the filtered water and other small molecules (amino acids, glucose, and sodium ions), and the 3) secretion of some excretory products (Potassium ions K+, Hydrogen ions H+, Ammonium ions NH4+, Creatinine, Urea, some hormones, some drugs). Renin (blood pressure regulation), erythropoietin (stimulate production of erythrocytes), and calcitriol (activated vitamin D promoting intestinal absorption of calcium and the renal reabsorption of phosphate) are hormones produced by the nephrons.

Glomerular filtration filters out almost all solutes, except for proteins, due to high blood pressure and specialized membranes in the **afferent arteriole**. A process called **tubular reabsorption** allows almost all nutrients to be reabsorbed in the **proximal convoluted tubule** (PCT). Reabsorption of water and some key electrolytes are regulated and can be influenced by hormones. Sodium (Na+) is the most abundant ion and most of it is reabsorbed by active transport and then transported to the **peritubular capillaries**. Because Na+ is actively transported out of the tubule, water follows it to even out the osmotic pressure. Water is also independently reabsorbed into the peritubular capillaries due to the presence of aquaporins, or water channels, in the PCT. This occurs due to the low blood pressure and high osmotic pressure in the peritubular capillaries. However, every solute has a transport maximum and the excess is not reabsorbed. In the loop of Henle, the filtrate continues to exchange solutes and water with the renal medulla and the **peritubular capillary network**. Water is also reabsorbed during this step. Then, additional solutes and wastes are secreted into the kidney tubules during **tubular secretion**.

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Part 1. pH & Conductivity

An electrolyte is any solute that produces ions in solution such as sodium, potassium, chloride calcium, and hydrogen ions controlled by the urinary system. The resulting solution can then conduct an electrical current. Most biological organisms require electrolytes to maintain the pumps and channels across their plasma membranes. If the balance of electrolytes between the inside and outside of a membrane is disrupted due to dehydration or salt reduction, cardiac and neurological complications can occur in most multicellular organisms.

The electrolyte that we most commonly recognize is salt but most acids and bases are also electrolytes. All of these solutes produce ions in solution:

NaCl (table salt) when dissolved in water produces Na^+ and Cl^- ions.

HCl (gastric acid) when dissolved in water produces H^+ and Cl^- ions.

The potential of a solution to pass an electric current is called electrical conductivity (EC) and it is usually measured in microSiemens per centimetre (µS/cm). This is often expressed simply as an 'EC Unit'. Strong acids, such as sulfuric acid or hydrochloric acid, and strong bases, such as sodium hydroxide or potassium hydroxide, are strong electrolytes because when they dissolve in water, almost every molecule dissociates to produce ions. On the other hand, weak electrolytes, such as weak acids and weak bases, produce relatively few ions when dissolved in water. Citric acid and acetic acid (in vinegar) are weak acids. Baking soda and ammonia are weak bases. When weak electrolytes dissolve in water, the solution is a poor conductor. As the concentration of ions in a solution increases, so does the EC reading. In this lab we **will use deciSiemens per metre (dS/m)** to read conductivity.

☐ Write you Team number on the top of the first page next to your name.

☐ Check that the; a) Go!Links (Fig. UI-1) are connected to the computer, b) pH and conductivity sensors are connected to the Go!Links, and c) sensors are connected to the ring stand clamp (they can fit in the clamp together).

☐ Download and then open the 'Urinalysis.cmbl' file that is linked to Canvas (it will be opened by the Logger Pro software).

☐ You may need to set the conductivity sensor range to 20,000 µS using the switch on the sensor.

☐ A window will open. Click on the 'Use Sensor Settings' button.

Put on goggles and gloves

IMPORTANT: Before, between, and after each use - rinse the tips of the pH and conductivity sensors thoroughly using the wash bottle and plastic beaker, then dry with a KimWipe.Do not let the pH electrode dry out. Keep the tip covered in the buffer of the storage vial between tests and after use.

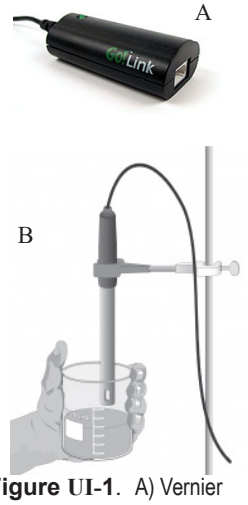


Figure UI-1. A) Vernier computer interface; Go!Link. B) Conductivity sensor attached to ring stand. You will attach both sensors to the ring stand using one clamp.

A. Controls

☐ Obtain specimen vials for the controls:
- positive - 0.85 NaCl mg/ml (37 mEq/L)
- negative - distilled (deionized) water

☐ Test the controls:

- Uncap a control sample and set it on the ring stand base. Lower the sensors into the sample.
- Briefly swirl the solution and then let the sensors sit in the solution for 3 minutes before taking a recording.
- Click the Collect button. The pH and EC will be tested in a 20 second run by the Logger Pro software.
- Once collection is complete, click the ½ button.

☐ **Record the mean pH and EC of the 20 second run in the appropriate column of Table UI-1.**

- Recap the sample, wash and dry the sensors.

- Repeat for the other control.

B. Urine Samples

☐ Obtain a specimen vial for one of the three different urine samples.

☐ Repeat the steps used to test the pH and EC of the controls; then repeat for the other two samples.

☐ **Record the mean pH and EC of the 20 second run in the appropriate column of Table UI-1.**

☐ Recap the sample, wash and dry the sensors.

C. Interpretation

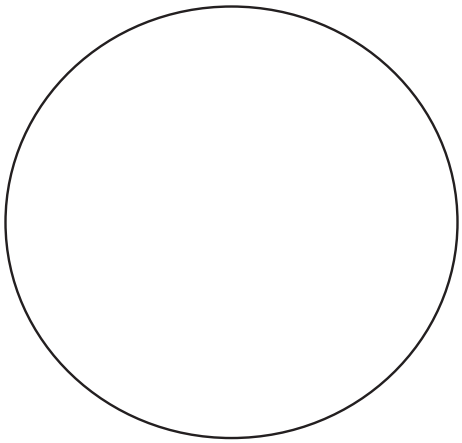
1. View and sketch the urine specimen slide using the **10X** objective lens. Label the following:
   A. Epithelial cells and casts
   B. Specimen, Species, Domain, and Kingdom names.
   C. Total magnification.

☐ Download and open the Urinalysis workbook found on Canvas. Add your group's data to the Part 1 spreadsheet table. The graph will automatically populate.

2. Open a Word document and at the top right of the document type:
   - Biol 1101L
   - Unit I - Urinalysis; Part 1 - pH & Conductivity
   - Your section #
   - Your team #
   - Names of everyone on your team
3. Insert the table and figure into the document.
4. Create a table caption ABOVE the table.

Table UI-1. pH and EC of control and urine samples tested.

	pH	EC
Negative control		
Positive Control		
Urine Sample 1		
Urine Sample 2		
Urine Sample 3		



Specimen:_____

Species:_____

Domain:_____

Kingdom: _____

Total Magnification: _____

5. Create a figure caption BELOW the figure.
6. Type the answers to the following questions into the document:
   A. Why were controls used?
   B. How did the control measurements compare to the sample measurements?
   C. Which sample(s) had the greatest pH and conductivity?
   D. Which sample(s) were out of range for a normal (healthy) urine sample for each type of measurement?
   E. What type of disorders do you think could be causing the abnormal measurements?
   F. What components of urine result in pH and conductivity; Why?
   G. What type of investigator error could have caused the controls to show a response similar to the urine samples?

☐ **Send the document to your lab instructor using your ISU Google Account Gmail**

Name:_____ Team #:_____ Section #:_____

Part 2. Macromolecules

1. Before attending the lab, use the suggested reading on Canvas to complete Table UI-2 by filling in the characteristics of each of the four classes of biologically important large molecules (proteins, nucleic acids, carbohydrates, and lipids).

Table UI-2. The characteristics of A) PROTEINS, B) CARBOHYDRATE, C) NUCLEIC ACID, and D) LIPIDS.

A) PROTEIN

Sketch the general structure of a **protein monomer** and name all characteristic chemical groups:
Sketch the general structure of a **protein polyme**r and name all characteristic chemical group:
Name of bond between monomers:

B) CARBOHYDRATE

Sketch the general structure of a **carbohydrate monomer** and name all characteristic chemical groups:
Sketch the general structure of a **carbohydrate polymer** and name all characteristic chemical group:
Name of bond between monomers:

C) NUCLEIC ACID

Sketch the general structure of a **nucleic acid monomer** and name all characteristic chemical groups:
Sketch the general structure of a **nucleic acid polyme**r and name all characteristic chemical group:
Name of bond between monomers:

D) LIPID

Sketch the general structure of a **triglyceride molecule**, name the characteristic chemical groups that form the molecule, and label the chemical linkages between the groups:
Sketch the general structure of a **phospholipid molecule**, name the characteristic chemical groups that form the molecule, and show how phospholipids align to create cell membranes.
Name the characteristic that distinguishes all **lipids** from other large molecules:

☐ Go to Biolab > Training > Technology > Spectrophotometers (https://www.isu.edu/biology/biolab/instructor-training/technology/spectrophotometers/#d.en.90794) and study the content at that web page.

A standard curve is a quantitative research technique where multiple samples with known concentrations are measured and graphed. Concentrations can be determined for other samples by following the line from the y-axis measurement of the graph to where it intersects the standard curve. The corresponding value on the x-axis is the concentration of substance in the unknown samples. Today we will use the spectrophotometers and a standard curve to determine the concentration of two types of macromolecules found in urine; protein and carbohydrate (glucose).

☐ Write you Team number on the top of the first page next to your name.

☐ Remove the plastic protective cover of the Genesys 20 Spectrophotometer and turn on the spectrophotometer using the power switch located on the back of the machine, lower left side. Once it has warmed up (it may take a few minutes), it should automatically be set to absorbance (A) mode. If it is not, press the A/T/C button to select absorbance (A).

A: Protein - Albumin

The Bradford assay is mediated by Coomassie Brilliant Blue dye and generates a protein-dependent color change response that will vary with regard to protein type and concentration. The assay is accurate but non-linear and is used in many research and clinical applications. Binding of the dye to protein stabilizes the blue anionic dye form, detected at 595 nm.

☐ Using a permanent marker, label six cuvette tubes **near the top** of the tubes with the sample information: B, N, P, 4, 5, and 6.

Put on goggles and gloves

☐ Aliquot 3 ml of the Blank solution into the B cuvette tube. Stretch Parafilm over the top of the tube. You will use the Blank (B) cuvette in Parts 2A and 2B.

☐ Prepare samples (swirl solutions before aliquoting):

- Aliquot 1 ml of each sample into its corresponding cuvette tube (N, P and 4, 5, and 6).
- Aliquot 2 ml of Bradford reagent into each cuvette tube N, P and 4, 5, and 6.
- Cover the cuvette tubes by stretching Parafilm over the top of each tube and then mix by inverting the tube three times.
- Let the tubes sit for ten minutes.
- Mix by inverting the tubes three times.

☐ Set the wavelength to **595 nm** by pressing either the buttons.

Important: Do not cross-contaminate; use a different transfer pipette for each sample.

Table UI-3. Urine sample with Bradford reagent absorbance at 595 nm and determined protein concentration.

	Absorbance	Absorbance Adjusted	Protein Concentration (mg/ml)
Negative **-** Control (N)			
Positive **+** Control (P)			
Urine Sample **4**			
Urine Sample **5**			
Urine Sample **6**			

☐ Insert the B cuvette tube into the cell holder of the sample chamber. The tube should be sitting on the bottom of the cell.

☐ Close the sample chamber.

☐ Press the 0 ABS 100% T button to set the blank to 0 concentration.

☐ Measure the Negative - (N) control sample by inserting the cuvette into the cell holder and closing the sample chamber.

☐ **Record the absorbance in Table UI-3**.

☐ Repeat for the Positive + (P) albumin control and the urine samples 4, 5, and 6.

☐ Reserve the B cuvette tube for Part 2B.

☐ Cleaning the cuvette tubes:
- Empty tubes N, P, 4, 5, and 6 into the correct waste container.
- Scrub the tubes with water and dish detergent.
- Rub off the marker label.
- Invert the tubes on the tube rack.

Calculations

☐ Determine the adjusted absorbance:
- Subtract the absorbance of the negative control from positive control and the sample (4, 5, and 6).
- Record the adjusted absorbance for all samples in Table UI-3.

☐ Determine the protein concentration of the urine samples:
- Using the standard curve (Fig. UI-3A) of albumin dilutions, find the adjusted absorbance of each sample on the y-axis and determine the concentration from the x-axis.
- Use a ruler to draw a line from the adjusted absorbance (from y-axis) to the standard curve down to the protein concentration (x-axis).

☐ **Record the protein mg/ml for all controls and samples in Table UI-3.**

B: Carbohydrate - Glucose

Glucose is oxidized by Copper (Cu^{2+}) and copper is reduced by glucose. Gluconic acid and a reddish precipitate of copper oxide are formed when glucose is oxidized by the copper ions in the Benedict's reagent. Color change is measured spectroscopically. The amount of the red precipitate is proportional to the amount of glucose in the sample. Glucose concentration in a sample can be measured by measuring the absorption at 520 nm.

Table UI-4. Urine sample with Benedict reagent absorbance at 520 nm and determined glucose concentration.

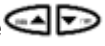
	Absorbance	Absorbance Adjusted	Glucose Concentration (mg/ml)
Negative **-** Control (N)			
Positive **+** Control (P)			
Urine Sample **7**			
Urine Sample **8**			
Urine Sample **9**			

☐ Using a permanent marker, label five cuvette tubes **near the top** of the tubes with the sample information: N, P, 7, 8, and 9.

☐ Prepare samples (swirl solutions before aliquoting)
- Aliquot 0.5 ml of each sample into its corresponding cuvette tube (N, P and 7, 8, and 9).
- Aliquot 2 ml of Benedict reagent into each tube N, P and 7, 8, and 9 BUT NOT the B tube.

☐ Water bath 70°C:
- Place the cuvette tubes in the bath for 20 minutes.
- Remove the tubes and let them cool for 5 minutes.

☐ Cover the cuvette tubes:
- Stretch Parafilm over the top of each tube.
- Mix by inverting or flicking the bottom of the tube three times.

☐ Set the wavelength to **520 nm** by pressing either the  buttons.

☐ Insert the B cuvette tube into the cell holder of the sample chamber. The tube should be sitting on the bottom of the cell.

☐ Close the sample chamber.

☐ Press the (0 ABS 100% T) button to set the blank to 0 concentration.

☐ Measure the Negative - (N) control sample by inserting the cuvette into the cell holder and closing the sample chamber.

☐ **Record the absorbance in Table UI-3.**

☐ Repeat for the Positive + (P) glucose control and urine samples 7, 8, and 9.

☐ Cleaning the cuvette tubes:
- Empty tubes B, N, P, 7, 8, and 9 into the correct waste container.
- Scrub the tubes with water and dish detergent.
- Rub off the marker label.
- Invert the tubes on the tube rack.

Calculations

☐ Determine the adjusted absorbance:
- Subtract the absorbance of the negative control from positive control and the sample (7, 8, and 9).
- Record the adjusted absorbance for all samples in Table UI-4.

☐ Determine the glucose concentration of the urine samples:
- Using the standard curve (Fig. UI-3B) of glucose dilutions, find the adjusted absorbance of each sample on the y-axis and determine the concentration from the x-axis
- Use a ruler to draw a line from the adjusted absorbance (from y-axis) to the standard curve down to the glucose concentration (x-axis).

☐ **Record the glucose mg/ml for all controls and samples in Table UI-4.**

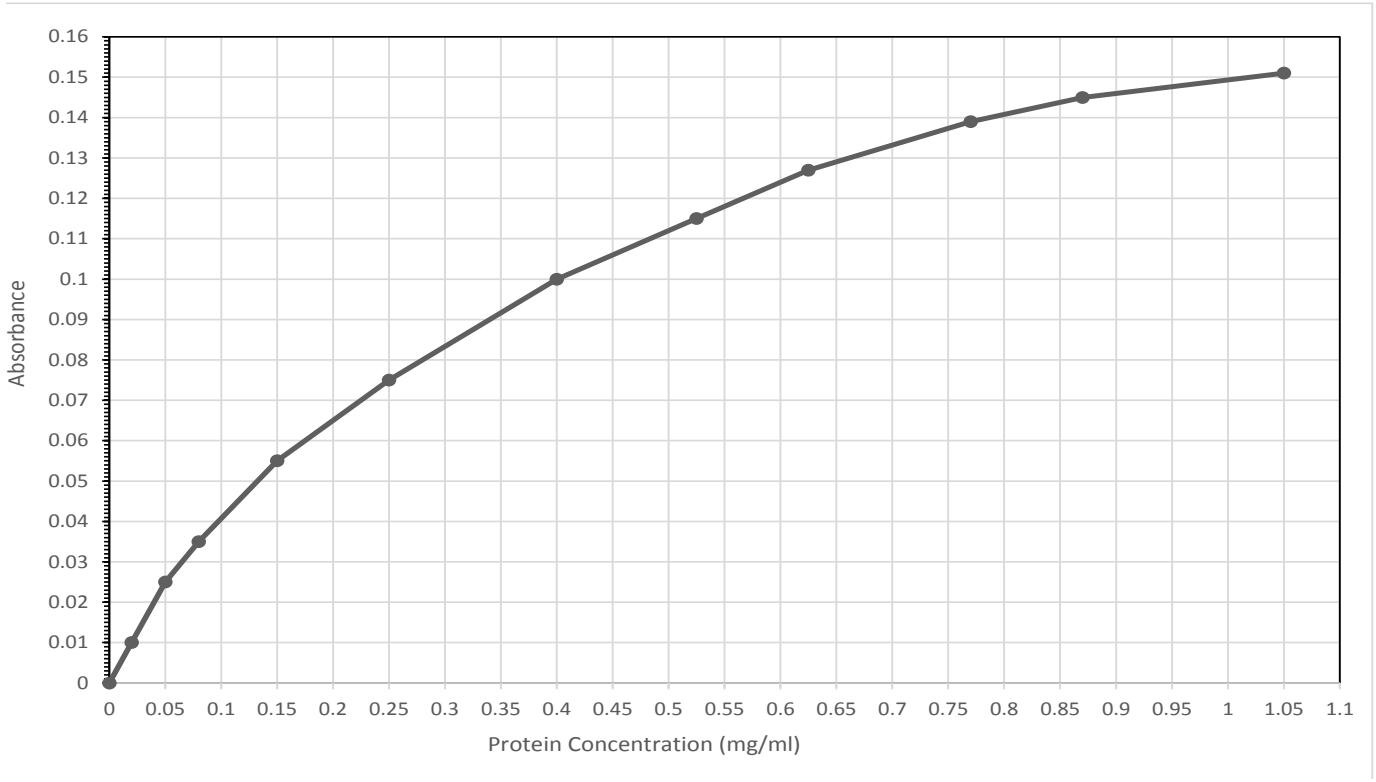
C: Interpretation

☐ Download and open the Urinalysis workbook found on Canvas. Add your group's data to the Part 2 spreadsheet table. The graph will automatically populate.

2. Open a Word document and at the top right of the document type:
   - Biol 1101
   - Unit I- Urinalysis; Part 2 - Macromolecules
   - Your section #
   - Your team #
   - Names of everyone on your team
3. Insert the table and graph into the document.
4. Create a table caption ABOVE the table.
5. Create a figure caption BELOW the figure.
6. Type the answers to the following questions into the document:
   A. What are the major classes of biological macromolecules and which of those that can be found in urine indicate disease?
   B. Which sample(s) had the greatest concentration of protein and glucose?
   C. Which sample(s) were out of range for a normal (healthy) urine sample for each type of macromolecule?
   D. What type of disorders do you think could be causing the abnormal measurements? Why?
   E. Explain how the concentrations were determined using the spectrophotometer and standard curves?

☐ **Send the document to your lab instructor using your ISU Google Account Gmail**

A



B

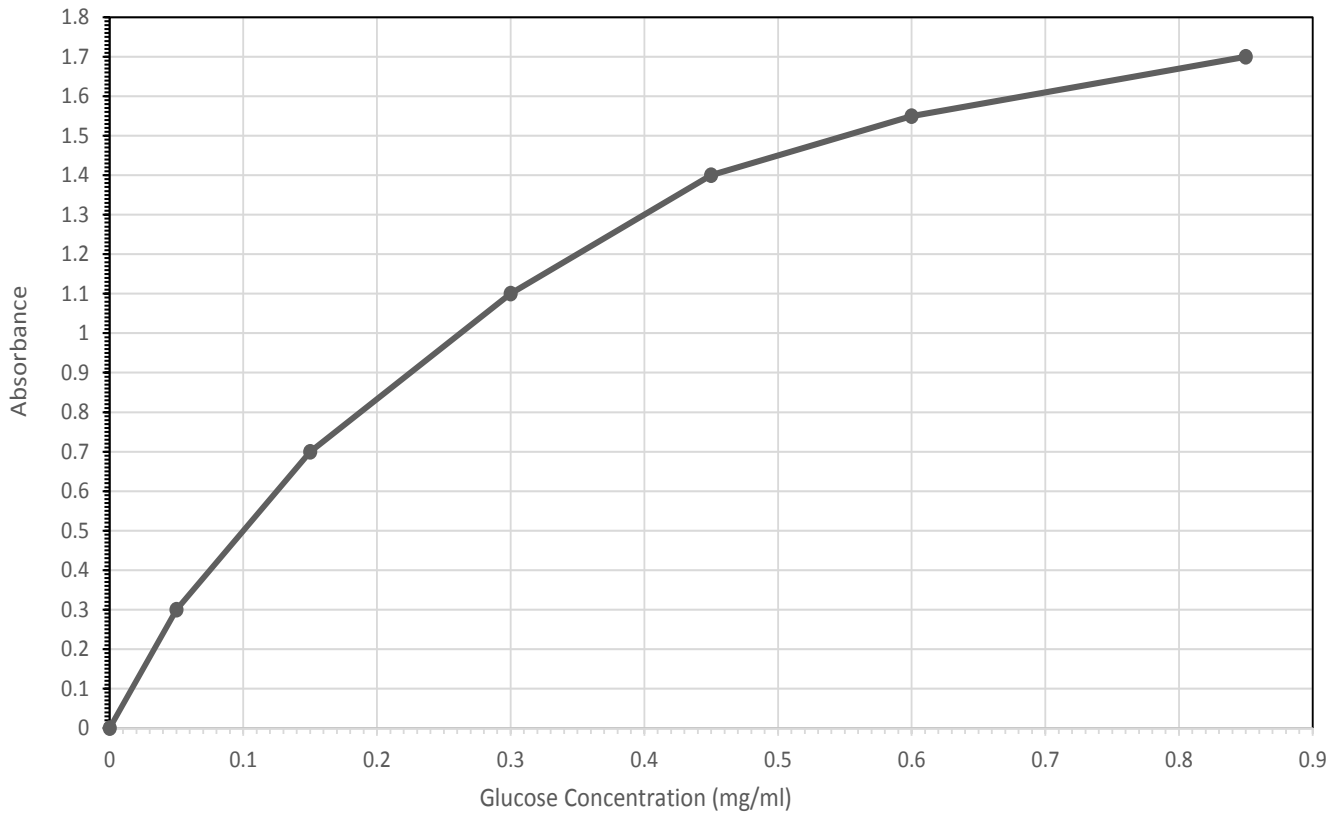


Figure UI-3. Standard curve created from; **A)** albumin standard showing protein concentration (mg/ml) and absorbance at wavelength 595 nm but adjusted for the absorbance of the Bradford Reagent, and **B)** glucose standard showing glucose concentration (mg/ml) and absorbance at wavelength 520 nm but adjusted for the absorbance of the Benedict Reagent. Concentrations were determined for controls, samples 4-6 (protein), and 7-9 (glucose).

Name:_____ Team #:_____ Section #:_____

Part 3. Osmosis & Diffusion

Water that contains ions is able to conduct electricity. The more ions in solution, the easier it is for an electric current to flow. The potential of a solution to pass an electric current is called electrical conductivity (EC) and it is usually measured in microSiemens per centimetre (µS/cm) or deciSiemens per metre (dS/m). This is often expressed simply as an 'EC Unit'. As ion concentration of a solution increases, so to does the EC reading. In this lab will determine the change in solution conductivity and potato mass as well as the rate of osmosis (dS/m/minute) to study diffusion/osmosis in a potato tuber and to learn the dependent nature of **hypotonic (hypo- beneath or below), hypertonic (hyper- over, above, beyond), and isotonic (iso- as equal, uniform) solutions**.

The part of the potato plant (*Solanum tuberosum,* Domain Eukarya, Kingdom Plantae) we eat is called the tuber, which is actually an enlarged underground stem. Potato tubers are mostly made of parenchyma. Parenchyma is a plant ground tissue that forms, among other things, the cortex and pith of stems, the cortex of roots, the mesophyll of leaves, the pulp of fruits, and the endosperm of seeds. Parenchyma cells are living cells and may remain meristematic at maturity—meaning that they are capable of mitosis if stimulated. Each tuber consists of individual parenchyma cells with cellulose **cell walls** cemented together with pectins and weak cell membranes. The cell membrane is a thin bilayer of phospholipids with protein molecules that separates the interior of the cell from its environment. Cell membranes are selectively permeable, controlling what moves into and out of the cell. Inside each cell is a **nucleus** and **cytoplasm** where respiration and starch synthesis occurs. **Starch grains** can be observed in the cytoplasm of most tuber cells.

A. Experimental Design

☐ Observe the Pre-Lab video to find the question, hypothesis, and prediction.

☐ Open Word document, answer question 1 and 2, and then save the document.

1. At the top right of the document type:
   - Biol 1101L
   - Unit I- Osmosis & Diffusion
   - Your section #
   - Your team #
   - Names of everyone on your team

2. Type the answers to the following questions into the Word document.
   A. Background: What is the species name of your model organism? To which domain and kingdom does it belong? Plants store energy as starch, what type of macromolecule is starch? Plant cell walls are made of what type of organic compound? What type of macromolecule is the organic compound?
   B. Question?
   C. Determine your variables: Which is your response (dependent) variable? Which is your predictor (independent) variable? Are the variables categorical and/or quantitative?
   D. Describe the control and experimental groups for your experiment:
   E. Describe the type of relationship between the variables (positive, negative, or neutral).
   F. Develop a hypothesis.
   G. What is your prediction as to the outcome of your experiment.

B. Data Collection

☐ Download and then open the 'Osmosis.cmbl' file (it will be opened by the Logger Pro software).
- You may need to set the conductivity sensor range to 20,000 µS using the switch on the sensor.
- A window will open. Click on the 'Use Sensor Settings' button.

☐ Check that the; a) Go!Link is connected to the computer, b) conductivity sensor is connected to the Go!Link, and c) sensor is connected to the ring stand clamp.

☐ Pour 100 mL of dechlorinated water into a 250-mL glass beaker.

☐ Lower the conductivity sensor into the solution until the top of the oblong opening containing the metal rod is covered.

☐ Potato (initial):
- The instructor will have six fresh half slices on a paper towel for you.
- Blot the potato slices dry using the paper towel.
- On the scale, push the blue lid back and place the washed and dry weigh boat on the scale.
- Zero the scale.
- Place all the potato slices in the weigh boat.
- Weigh all the slices together in grams (g).

☐ **Record the initial weight in Table UI-5.**

☐ The slices need to be vertically positioned around the sensor tip that is submerged in the solution. Let the sensor/potatoes sit in the solution for 30 seconds.

☐ Run:
- Set a timer for 18 minutes.
- Click the 'Collect' button.
- Swirl the solution every two minutes of the run. Keep the sensor in the solution but gently slide the beaker around on the ring stand or tray.

Table UII-5. The initial and final potato mass (g) and percent change (%Δ) when raw potato is placed in three solutions (water, 0.9% NaCl, and 10% NaCl) for 18 minutes. %Δ is calculated by subtracting the initial mass FROM the final mass, dividing by the initial mass, and then multiplying by 100.

Solution	Potato Mass (g)		%Δ Mass
	Initial	Final	
Dechlorinated water			
0.9% NaCl			
10% NaCl			

☐ **Record the initial and final dS/m at time 18 minutes in Table UII-6.**

☐ Move your data to a stored run. To do this, choose Store Latest Run from the Experiment menu. DO NOT close Logger Pro.

☐ Potato (final):
- Pour out the water from the beaker and dump the potato slices onto a paper towel.
- Blot the slices dry.
- On the scale, push the blue lid back and place the washed and dry weigh boat on the scale.
- Zero the scale.
- Place all the potato slices in the weigh boat.
- Weigh all the slices together in grams (g).

Important: Rinse and dry the beaker and sensor before and after use.

☐ **Record the final mass of the potato slices in Table UI-5.**

☐ Repeat the above steps using the 0.9% NaCl solution and fresh potato slices and then again with the 10% NaCl solution and another set of fresh potato slices.

☐ DO NOT close Logger Pro.

☐ Rate of osmosis (dS/m/minute):
- Go back to the open Logger Pro with all your stored runs.
- Select a run by clicking on the corresponding graph. Roll the mouse over the graph and place the cursor at the lower left portion of the graph.
- Left click the mouse and, while keeping the left button depressed, drag the shaded area up and right over the first 10 minutes of the run.
- Release the left mouse button.
- Click on the Linear Fit  button.

☐ **Record the value of the slope, m, in Table UI-6**

☐ Percent change (%Δ): %Δ=(f- i)/i X 100:
- Potato mass - subtract the initial mass FROM the final mass, divide by the initial mass, and then multiply by 100. **Record the value in Table UI-5.**
- Electrical conductivity (dS/m) - subtract the initial dS/m FROM the final dS/m, divide by the initial dS/m, and then multiply by 100**. Record the value in Table UI-6.**

☐ **Record in rate of osmosis, %Δ potato mass, and %Δ conductivity in the Osmosis workbook on the instructor computer.**

Table UII-6. The initial and final electrical conductivity (dS/m), percent change (%Δ) of electrical conductivity, and the rate of osmosis (dS/m/min) when raw potato is placed in three solutions (water, 0.9% NaCl, and 10% NaCl) for 18 minutes. %Δ is calculated by subtracting the initial dS/m FROM the final dS/m, dividing by the initial dS/m, and then multiplying by 100.

Solution	dS/m			Rate (dS/m/minute)
	Initial	Final	%Δ	
Dechlorinated water				
0.9% NaCl				
10% NaCl				

C. Interpretation

3. In Figure UI-4, label and color the listed subcellular structures:

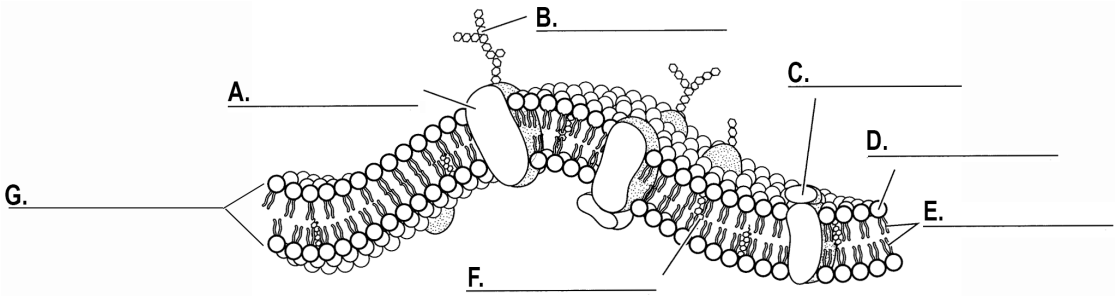
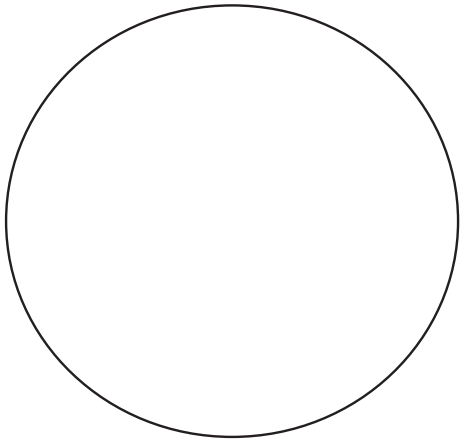


Figure UI-4. Plasma membrane diagram, subcellular structures; A. Integral protein (green), B. Carbohydrate chain (purple), C. Peripheral (dark blue), D. Phosphate molecule (brown), E. Lipid Layer, F. Cholesterol molecule (yellow), and G phospholipid bilayer.

4. View and sketch the potato tuber slide using the 40X objective lens. Label the following:
   A. Cell wall and starch grains.
   B. Specimen, Species, Domain, and Kingdom names.
   C. Total magnification.



☐ Your instructor will send your lab section's completed workbook to each student's ISU Google Account Gmail.

☐ Open the Word document from Section A.

5. Insert the table and graph into the document.
6. Create a table caption ABOVE the table.
7. Create a figure caption BELOW the figure.
8. Type the answers to the following questions into the Word document you created and saved in section A (Questions 1 & 2):
   H. Look at the 2-D column chart that you created showing mean %Δ potato mass:
   - Which potato/solution had the greatest positive change in mass? What caused the change?
   - Which potato/solution had the greatest negative change in mass? What caused the change?
   - Which solution showed the least change in mass.? Why?

Specimen:_____

Species::_____

Domain:_____

Kingdom: _____

Total Magnification _____

   I. Look at the 2-D column chart that you created showing mean %Δ solution conductivity:
   - Which potato/solution had the greatest positive change in conductivity? What caused the change?
   - Which potato/solution had the greatest negative change in conductivity? What caused the change?
   - Which solution showed the least change in conductivity? Why?

J. We calculated rate of osmosis (dS/m/minute) by taking the slope of the lines in the graph generated by LoggerPro:
- What does rate of osmosis mean if slope is rate of change, what was changing?
- Which solution had a fairly flat slope. Was equilibrium achieved? What does that mean?
- Did one of the solutions have a negative slope? What does that mean?

K. After looking at the slide of a potato tuber section; describe how water and ions were moving with respect to the potato tuber cells and each solution.

L. Did you reject or support your hypothesis**?** WHY?

M. Compare the raw data to the means and standard deviations that were calculated. Which data points could have caused the standard deviation to widen. What type of investigator error could have caused the standard deviation that you see?

☐ **Send the document to your lab instructor using your ISU Google**

9. Fill in Figure UI-5 (represents a beaker with solution and the circle is potato) using the class %Δ potato mass data to:
   A. Determine which diagram (A-C) represents the movement of water in each of your potato/solution experiments and fill in the figure and figure caption.
   B. Determine the tonicity of the potato and solution relative to each other in each diagram.
10. Figure UI-6, label the arrows; 1) filtration, 2) reabsorption, 3) secretion, and 4) excretion.

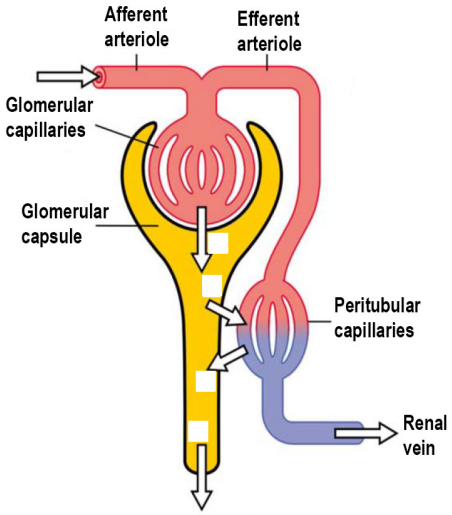
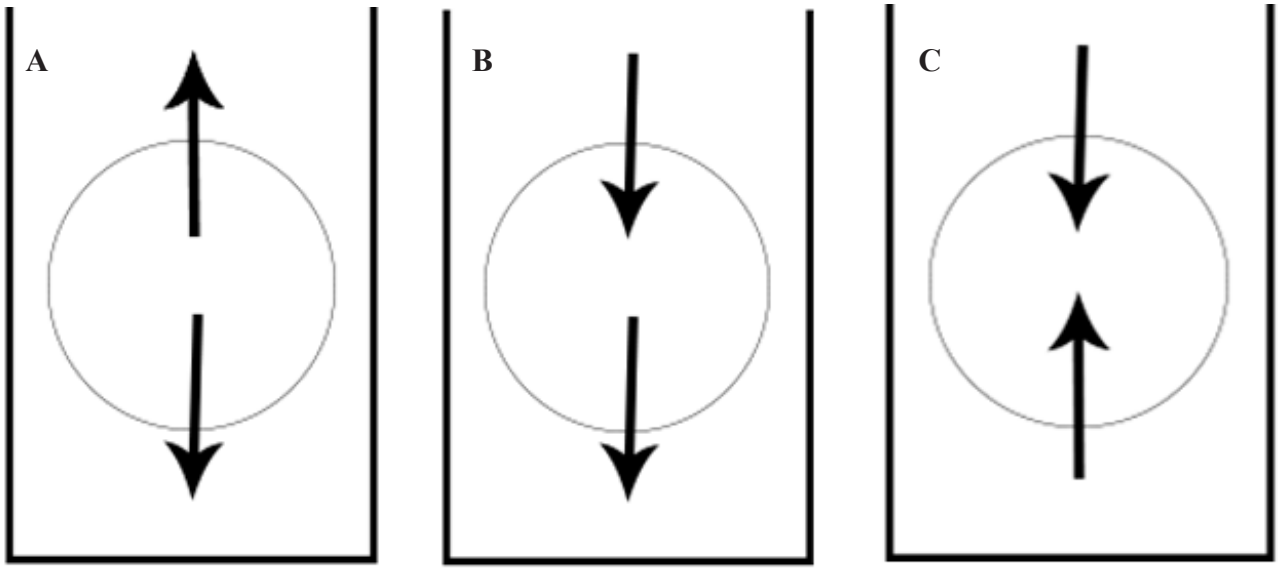


Figure UI-6. Diagram showing the basic physiologic mechanisms of the kidney and the three steps involved in urine formation. Urinary Excretion = Filtration - Reabsorption + Secretion.



Solution type_____ Solution type_____ Solution type_____

Potato tonicity:_____ Potato tonicity:_____ Potato tonicity:_____

Solution tonicity:_____ Solution tonicity:_____ Solution tonicity:_____

Figure UII-5. Tonicity and water movement in a beaker containing a solution and potato slices. Three solutions types: ___) water, ___)0.9% NaCl, and ___)10% NaCl. Arrows depict the direction of water movement into and out of potato cells.

Unit II - Energy & Metabolism

Objectives

- Continue to practice the scientific method through experimental design, hypothesis construction, and descriptive statistics (mean and standard deviation).
- Learn about enzymes and describe their activity in cells.
- Observe and measure changes in CO_2 concentration during respiration of lactose with and without lactase by yeast and determine the rate of respiration.
- Observe and measure changes in gas pressure during fermentation of sugars by yeast and determine the rate of fermentation.
- Observe the changes in CO_2 and O_2 concentrations caused by *Spinacia oleracea* (spinach) during photosynthesis and respiration.
- Determine the absorption spectrum of *S. oleracea* chloroplasts.
- Create tables and figures with corresponding APA captions using Microsoft® Office Excel and Word.

Terms & Definitions

Abaxial – facing away from the stem of a plant and denoting the lower surface of a leaf.

Adaxial – facing toward the stem of a plant and denoting the upper surface of a leaf.

Aerobic processes - refers to a process that occurs in the presence of oxygen; a form of metabolism that does requires oxygen.

Anaerobic processes - refers to a process that occurs in the absence of oxygen; a form of metabolism that does not require oxygen.

Adenosine triphosphate (ATP) - a common energy source for all cells; it converts either to adenosine diphosphate (ADP) or to adenosine monophosphate (AMP).

Catalyst - a substance that lowers the activation energy of a chemical reaction, thereby speeding up the reaction:
A. Active site - the location in an enzyme where a chemical reaction takes place
B. Amylase - a digestive enzyme in saliva and the pancreas involved in the digestion of starch.
C. Coenzyme - substances such as vitamins that help enzymes catalyze chemical reactions.
D. Enzymes - macromolecular biological catalysts that are usually proteins that accelerate chemical reactions. Enzymes are biological catalysts and catalyze the rate of a chemical reaction.
E. Induced fit - a change in shape of the active site of an enzyme so that it binds tightly to a substrate.
F. Isomerase - a general class of enzymes that convert a molecule from one isomer to another such as fructose to glucose.
G. Lactase - a family of enzymes involved in the hydrolysis of the disaccharide lactose into constituent galactose and glucose monomers. It is located in the brush border of the small intestine of humans and other mammals.
H. Maltase - an enzyme that catalyses the hydrolysis of the disaccharide maltose to glucose monomers.
I. Sucrase/invertase - an enzyme that catalyzes the hydrolysis (breakdown) of sucrose (table sugar) into fructose and glucose monomers.
J. Substrate - the substance upon which an enzyme reacts.
K. Specificity - phenomenon of enzyme shape determining the reaction the enzyme catalyzes.

Cellular respiration - a process by which living cells obtain energy from organic molecules and release waste products.

Cellulose - a structural polysaccharide found in cell walls and composed of glucose molecules.

Chitin - a tough, nitrogen-containing polysaccharide that forms the external skeleton of many insects and the cell walls of fungi.

Chloroplast - a plastid organelle found in plant and algal cells that carries out photosynthesis:
A. Intermembrane space - a thin region about 10–20 nanometers between the outer and inner chloroplast membranes.
B. Inner membrane - a membrane borders the stroma and regulates passage of materials in and out of the chloroplast.
C. Outer chloroplast membrane - a semi-porous membrane that small molecules and ions can easily diffuse across.
D. Stroma - the fluid-filled region of the chloroplast between the thylakoid membrane and the inner membrane.
E. Thylakoid - a flattened, platelike membranous region found in cyanobacteria and the chloroplasts of plants and algae.

Citric acid cycle (KREBS) - a cycle that results in the breakdown of carbohydrate to CO_2; also known as the krebs cycle.

Digestion - the process of breaking down food by mechanical and enzymatic action in the gastrointestinal tract into substances that can be used by the body.

Electron Transport Chain - a group of protein complexes and small organic molecules within the inner membranes of mitochondria and chloroplasts and the plasma membrane of prokaryotes. the components accept and donate electrons to each other in a linear manner and produce a H+ electrochemical gradient.

Energy - the capacity of a body to do work.

Disaccharide - a carbohydrate composed of two monosaccharides:
A. Lactose - β-D galactose + β-D glucose “milk sugar” (lactase)
B. Maltose - α-D-glucose + α-D-glucose “malt sugar. (maltase)
C. Sucrose - glucose + fructose; “table sugar” (sucrase/invertase)

Fermentation - the breakdown of organic molecules to produce energy without any net oxidation.

Fermentation (Lactic Acid) - a metabolic process by which glucose or other six-carbon sugars (also, disaccharides of six-carbon sugars, e.g. sucrose or lactose) are converted into cellular energy and the metabolite lactate, which is lactic acid in solution.

Flavin adenine dinucleotide (FAD) - a redox-active coenzyme associated with various proteins, which is involved with several important enzymatic reactions in metabolism. Fad can exist in four different redox states, which are the flavin-n(5)-oxide, quinone, semiquinone, and hydroquinone. Fad is converted between these states by accepting or donating electrons. FAD, in its fully oxidized form, or quinone form, accepts two electrons and two protons to become $FADH_2$ (hydroquinone form). The semiquinone ($FADH\cdot$) can be formed by either reduction of fad or oxidation of $FADH_2$ by accepting or donating one electron and one proton, respectively.

Fungi - a eukaryotic kingdom of the domain eukarya that is composed of heterotrophic unicellular, multicellular, or syncytial spore-producing organisms, including molds, yeast, mushrooms, and toadstools.

Glycogen - a polysaccharide found in animals cells (animals starch) and fungus.

Glycolysis - a metabolic pathway that breaks down glucose to pyruvate.

Leaf - the principal appendages of a vascular plant stem, usually borne laterally above ground and specialized for photosynthesis.

Mitochondrial Matrix - the space within the inner membrane; contains the mitochondria's DNA, ribosomes, soluble enzymes, small organic molecules, nucleotide cofactors, and inorganic ions. The enzymes in the matrix facilitate reactions responsible for the production of ATP, such as the citric acid cycle, oxidative phosphorylation, oxidation of pyruvate, and the beta oxidation of fatty acids.

Monosaccharide - a simple sugar:
A. Fructose - hexose monosaccharide "fruit sugar" (isomerase to convert to glucose)
B. Galactose - hexose monosaccharide (five different types of enzymes)
C. Glucose - hexose monosaccharide is found in all living cells and is often referred to as "blood sugar"Mucosa

Nutrients - substances that provide nourishment.

Nutrient cycling - the process by which decomposers break down dead organisms or waste products, release the chemical elements locked in the biological material, and return them to the environment.

Nicotinamide adenine dinucleotide phosphate (NADP+_) - a cofactor used in anabolic reactions, such as the calvin cycle and lipid and nucleic acid syntheses, which require NADPH as a reducing agent. It is used by all forms of cellular life. NADPH is the reduced form of NADP+. NADP+ differs from NAD+ in the presence of an additional phosphate group on the 2' position of the ribose ring that carries the adenine moiety.

Oxidation - a process that involves the removal of electrons; occurs during the breakdown of small organic molecules.

Oxidative phosphorylation - a process during which NADH and $FADH_2$ are oxidized to make more ATP via the phosphorylation of ADP.

Photorespiration - a series of reactions triggered by the closing of stomatal openings to prevent water loss.

Photosynthesis - the process whereby light energy is captured by plan, algal, or bacterial cells and is used to synthesize organic molecules from CO2 and H_2O.

Photosystem I & II - distinct complexes of proteins and pigment molecules in chloroplasts that absorbs light (PSI) or generates oxygen from water (PSII) during the light reaction of photosynthesis.

Plant photosynthetic pigments - a molecule present in chloroplasts or other photosynthetic organisms that can capture and absorb light energy which can then be converted to chemical energy by the organism:
A. Carotenoid - accessory pigment in photosynthetic organism that extend the range of absorbed wavelengths (470 nm and 500 nm) and protect the chlorophyll from oxidation.
B. Chlorophyll - the primary pigment in photosynthetic organisms and the primary electron donor in the electron transport chain of PSII (P680) and PSI (P700). Absorbs light most strongly in the blue portion of the electromagnetic spectrum as well as the red portion while reflecting a majority of green. It is found in the mesosomes of cyanobacteria, as well as in the chloroplasts of algae and plants. Chlorophyll a (430 nm and 662nm) and chlorophyll b (453 nm and 642 nm) absorb in slightly different ranges of the general chlorophyll spectrum.

Plastid - a membrane-bound organelle found in some eukaryotic organisms. They are considered to be intracellular endosymbiotic cyanobacteria.

Pyruvate - the simplest of the alpha-keto acids, with a carboxylic acid and a ketone functional group; an intermediate in several metabolic pathways throughout the cell.

Ribose - the five-carbon sugar in RNA.

Starch - a polysaccharide composed of repeating glucose units that is produces by the cells of plants and some algal protists.

Spectrum - used to classify something, or suggest that it can be classified, in terms of its position on a scale between two extreme or opposite points:
A. Absorption spectrum - a diagram that depicts the wavelengths of electromagnetic radiation that are absorbed by a pigment.
B. Action spectrum - the rate of photosynthesis plotted as a function of different wavelengths of lights.
C. Electromagnetic spectrum - all possible wavelengths of electromagnetic radiation, from relatively short wavelengths to much longer wavelengths.

Wavelength - the distance from the peak of one wave to the next.

Yeast - a fungus that can occur as a single cell and that reproduces by budding.

Name:_____ Group #:_____ Section #:_____

Part 1. Enzymes

Living organisms perform a multitude of chemical reactions very rapidly because of the participation of enzymes. Enzymes are biological catalysts, compounds that speed up chemical reactions without being used up or altered in the reaction. The material with which the catalyst reacts, the substrate, is modified during the reaction to form a new product. Because the enzyme itself emerges from the reaction unchanged, a small amount of enzyme can alter a relatively large amount of substrate. The active site of an enzyme will bind with the substrate, forming an enzyme-substrate complex. It is here that the reaction takes place, and when it is complete, the complex dissociates into the enzyme and a product or products. Enzymes are, in part or in whole, proteins that are highly specific in function. Because enzymes lower the energy of activation needed for reactions to take place, they accelerate the rates of reactions. They do not, however, determine the direction in which a reaction will go or its final equilibrium. Enzyme activity is affected by many factors. Varying environmental conditions such as temperature and pH may alter the three-dimensional shape of an enzyme, thereby affecting its rate of activity. Similarly, the amount of enzyme relative to the amount of substrate can have an affect on the rate of enzyme activity. Sugars are a vital source of food for all living organisms but much be broken down in order to be used. Glycoside hydrolase is a class of enzyme involved in the hydrolysis of the glycosidic linkage/bond of a disaccharide. A hydrolysis reaction is a chemical reaction that utilizes water to break apart molecules. This class of enzyme includes sucrase, maltase, and lactase. The disaccharides sucrose, maltose, and lactose have glycosidic bonds that under go a hydrolysis reaction with the aid of their respective hydrolase enzyme and water. *Saccharomyces cerevisiae* (Domain Eukarya, Kingdom Fungi) is the species name of the yeast used as a leavening agent in baking bread where it converts the fermentable sugars present in the dough into carbon dioxide and ethanol. Brewer's yeast is another strain of *S. cerevisiae* commonly used in alcoholic fermentation (beer and wine). It is a single-cell microorganism found on and around the human body and was domesticated by humans long ago. Yeast cells are unable utilize all of the sugars equally well but they do synthesize a range of enzymes with some more effective than others. Yeast metabolizes sugars aerobically (in the presence of oxygen) the process is known as respiration; when yeast metabolizes sugars anaerobically (in the absence of oxygen) the process is known as fermentation. *Saccharomyces cerevisiae* is glucophilic, meaning glucose will be used at a faster rate than fructose. For example, sucrose is a disaccharide and yeast cells use the **enzyme sucrase/invertase** to break it into glucose and fructose. Finally, it uses the glucose in respiration or fermentation. Depending on the strain of *S. cerevisiae* or the conditions of yeast incubation, the yeast may not produce enough maltase enzyme to split maltose in the time frame we are running the experiments. We generally see (but not always), (**1 being the sugar that yeast utilize at the greatest rate**):

1. Sucrose - disaccharide = glucose + fructose; "table sugar" (sucrase/invertase)
2. Glucose - hhexose monosaccharide is found in all living cells and is often referred to as "blood sugar"
3. Maltose - disaccharide = α-D-glucose + α-D-glucose "malt sugar" (maltase)
4. Fructose - hexose monosaccharide "fruit sugar" (isomerase to convert to glucose)
5. Galactose - hexose monosaccharide (five different types of enzymes)
6. Lactose - disaccharide = β-D galactose + β-D glucose "milk sugar" (lactase)

Part 2. Respiration

The following equation shows that in the presence of oxygen, glucose is converted by yeast through the process of cellular respiration to water, carbon dioxide and energy:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ H_2O + 6\ CO_2 + \text{energy}$$

During this process, oxygen gas (O_2) is consumed at the same rate that carbon dioxide (CO_2) is produced. The rate of cellular respiration in yeast can be determined by measuring either the amount of O_2 consumed or the amount of CO_2 produced in a specified period of time.

A. Experimental Design

☐ Observe the Pre-Lab video to find the question, hypothesis, and prediction.
☐ Open Word document, answer question 1 and 2, and then save the document.

1. At the top right of the document type:
   - Biol 1101L
   - Unit II - Respiration
   - Your section #
   - Your team #
   - Names of everyone on your team
2. Type the answers to the following questions into the Word document.
   A. Background: What is the species name of your model organism? To which domain and kingdom does it belong? Yeast cell walls are made of what type of organic compound? What type of macromolecule is the organic compound?
   B. Question?
   C. Determine your variables: Which is your response (dependent) variable? Which is your predictor (independent) variable? Are the variables categorical and/or quantitative?
   D. Describe the control and experimental groups for your experiment:
   E. Describe the type of relationship between the variables (positive, negative, or neutral).
   F. Develop a hypothesis.
   G. What is your prediction as to the outcome of your experiment.

B. Data Collection

You will work in a team of two or three students, depending on the number of people in your lab section. Part of your team should prepare your computer for collecting data while others on your team prepare the glassware and reagents for conducting your experiment. Everyone should read the entire procedure before beginning.

☐ Connect the CO_2 sensor (Figure UII-1) to the Go!Link.

☐ Download and then open the **Respiration.cmbl** file (it will be opened by the Logger Pro software).

☐ Remove the CO_2 sensor from the reaction chamber and calibrate it following your instructor's direction; it should fluctuate between 300 and 400 ppm. Set to 0-100,000 ppm.

☐ Label four separate tubes with the control solution names and the test solution names, then fill each tube with 4 mL of the corresponding solution:
- Control solutions - Water (tube 1) & Sucrose (tube 2)
- Test solutions - Lactose (tube 3) & Lactose with enzyme lactase (tube 4)

Figure UII-1. CO_2 sensor. Keep the sensor upright at all times.

☐ Water bath (38-40°C):
- Place the large beaker at the base of the ring stand.
- Fill ½ up with water from the electric hot water bath.
- Attach the 250-mL reaction chamber to the ring-stand clamp.
- Lower the chamber into the water with the bottom half submerged in the beaker water.
- Continuously monitor and maintain the bath temperature at 38-40°C; using the Styrofoam cups and basters, move the water between the beaker and the electric hot water bath. Cooler water from the beaker can be added back to the electric hot water bath.

☐ Pre-incubation (See Figure UII-2):
- Gently swirl the yeast suspension flask to resuspend the cells.
- Transfer 4 mL of the yeast suspension into the labeled tube.
- Gently mix the yeast into the control- water.
- Transfer the solution from the tube into the reaction chamber and incubate for 10 minutes.
- Continuously monitor and maintain the bath temperature at 38-40°C.

Important: Start the incubation for the second tube AFTER the first seven (7) minutes of the first tube's pre-incubation. See Figure UIII-2.

☐ Run(See Figure UII-2):
- At the 10 minute pre-incubation time, place the CO_2 gas sensor shaft into the opening of the reaction chamber.
- Begin measuring CO_2 concentration for four (4) minutes by clicking the collect button.
- Continuously monitor and maintain the bath temperature at 38-40°C.
- DO NOT click on the Stop button. The software will stop collection on its own.
- **Store Latest Run from the Experiment menu. DO NOT close Logger Pro.**

☐ Repeat the pre-incubations and runs for the other three tubes.

Incubation= I Before each run you need to maintain a 37°C water bath to incubate your tubes for 10 minutes.
Run= R During each run, as you collect data, you need to maintain a 37°C water bath to incubate your tubes.
NA= no incubation or run at this time.

	Time in minutes																																
Solution	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Water	I	I	I	I	I	I	I	I	I	I	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sucrose	NA	NA	NA	NA	NA	NA	NA	I	I	I	I	I	I	I	I	I	I	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Lactose	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	I	I	I	I	I	I	I	I	I	I	R	R	R	R	NA	NA	NA	NA	NA	NA	NA
Lactose w/ lactase	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	I	I	I	I	I	I	I	I	I	I	R	R	R	R

Figure UII-2. Respiration incubation and run schedule.

☐ Disconnect the CO_2 sensor from the Go!Link and set it upright in a dry beaker.

☐ Rinse and shake dry the respiration chamber.

☐ Click on the Linear Fit button. Note: you can use the buttons to view the slope region of interest.

☐ **Record the value of the slope, m, for each of the equations that describe the straight-line relationships, y = mx + b, in Table UII-1 AND to the Respiration workbook on the instructor computer.**

☐ Cleaning the tubes:
- Scrub the tubes with water and dish detergent.
- Rub off the marker label.
- Invert the tubes on the wooden tube rack.

Table UII-1. Rate of respiration (ppm/min) by yeast, as determined from the slope of the best-fit linear regression line of the plot of CO_2 gas versus time.

Solution (tube)	°C	Respiration rate
Water (1)		
Sucrose (2)		
Lactose (3)		
Lactose w/ enzyme (4)		

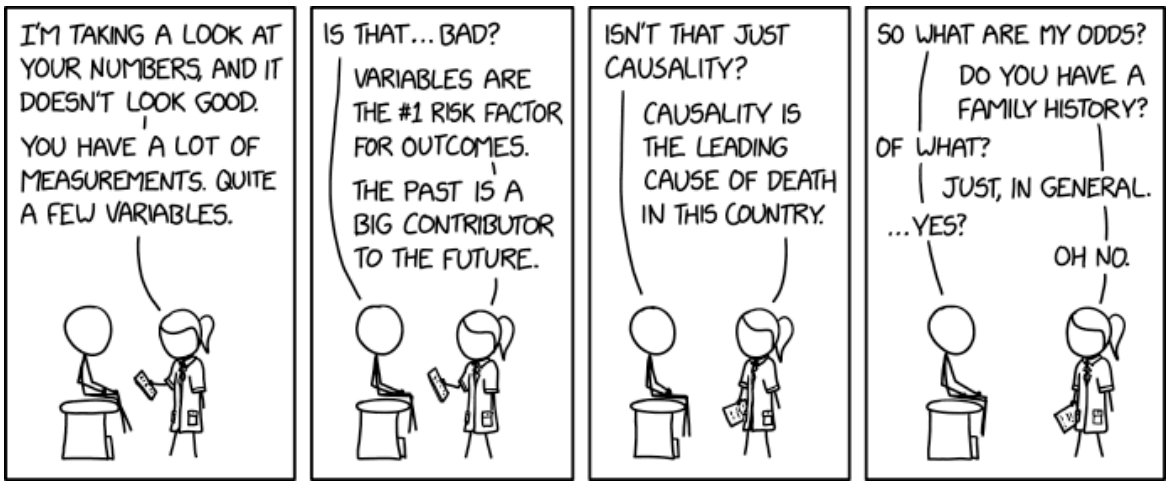
C. Interpretation

☐ Your instructor will send your lab section's completed workbook to each student's ISU Google Account Gmail.

☐ Open the Word document from Section A.

3. Insert the table and graph into the document.
4. Create a table caption ABOVE the table.
5. Create a figure caption BELOW the figure.
6. Type the answers to the following questions into the document:
   H. Which solution was the positive control and which was the negative control?
   I. Describe the patterns shown by your graphs and discuss what the patterns are indicating.
   J. Do yeast utilize all lactose and sucrose for respiration equally? Why does this difference between the sugars exist?
   K. Did you reject or support your hypothesis? Explain in DETAIL why it was supported or rejected?
   L. Using the following terms (active site, substrate, activation energy, enzyme, disaccharide, monosaccharide) describe how lactose is modified by yeast with and without lactase.
   M. Humans have lactose intolerance. Do you think the intolerance is based on a similar mechanism? Why?
   N. Compare the raw data to the means and standard deviations that were calculated. Which data points could have caused the standard deviation to widen. What type of investigator error could have caused the standard deviation that you see?

☐ **Send the document to your lab instructor using your ISU Google Account Gmail**



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Name:_____ Team #:_____ Section #:_____

Part 3. Fermentation

During the process of fermentation, which occurs when oxygen (O_2) is not available, yeast converts glucose to ethanol, carbon dioxide (CO_2) and energy:

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2 + \text{energy}$$

CO_2 is still produced, but ethanol is produced instead of water. Ethanol is an undesirable waste product from the perspective of yeast because it can eventually reach toxic concentrations. By measuring the change in pressure in a vessel in which it is possible to determine whether yeast is using a particular sugar as a source of food.

A. Experimental Design

☐ Observe the Pre-Lab video to find the question, hypothesis, and prediction.
☐ Open Word document, answer question 1 and 2, and then save the document.

1. At the top right of the document type:
   - Biol 1101L
   - Unit II - Fermentation
   - Your section #
   - Your team #
   - Names of everyone on your team
2. Type the answers to the following questions into the Word document.

A. Background: What is the species name of your model organism? To which domain and kingdom does it belong? Yeast cell walls are made of what type of organic compound? What type of macromolecule is the organic compound?

B. Question: What is one question about fermentation and sugar type?

C. Determine your variables: Which is your response (dependent) variable? Which is your predictor (independent) variable? Are the variables categorical and/or quantitative?

D. Describe the control and experimental groups for your experiment.

E. Describe the type of relationship between the variables (positive, negative, or neutral).

F. Develop a hypothesis.

G. What is your prediction as to the outcome of your experiment.

B. Data Collection

☐ Connect the gas pressure sensor (Figure UII-3A) to the Go!Link.

☐ Download and then open the 'Fermentation.cmbl' file (it will be opened by the Logger Pro software).

☐ Label four separate tubes with the control solution names and the test solution names, then fill each tube with 4 mL of the corresponding solution:

- Control solutions - Water (tube 1) & Sucrose (tube 2)
- Test solutions - Maltose (tube 3) & Galactose (tube 4)

OR Glucose (tube 3) & Fructose (tube 4)

☐ Set up your water bath (38-40°C) without the reaction tube (Figure UII-3B).

☐ Pre-incubation (See Figure UII-5):

- Gently swirl the yeast suspension flask to resuspend the cells.
- Transfer 4 mL of the yeast suspension into the tube and gently mix.
- Attach the tube to the ring-stand clamp.
- Lower the tube into the water with the bottom half submerged.
- 👁 Add a drop of vegetable oil to the surface of the yeast-sugar mixture to create an anaerobic seal. Do not drip oil on the inside wall of the test tube, as it will keep a seal from forming.
- Insert the black rubber stopper into the test tube. DO NOT connect the pressure sensor to the stopper. Gently twist the stopper into the tube to create an airtight seal (but do not twist so hard that you break the glass tube!).
- Incubate the test tube for 10 minutes.
- Continuously monitor and maintain the bath temperature at 38-40°C.

☐ Run (See Figure UII-4):

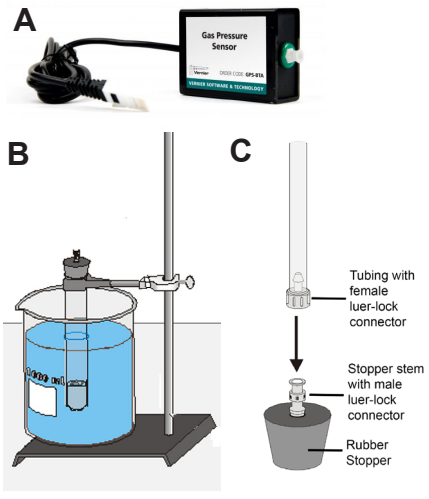

Figure UII-3. A) Gas pressure sensor. **B)** Reaction tube suspended in a water bath using a ring stand and clamp. Do not connect to the pressure sensor until the end of incubation, once you begin to collect data, and **C)** Connector.

Incubation= I Before each run you need to maintain a 37°C water bath to incubate your tubes for 10 minutes.
Run= R During each run, as you collect data, you need to maintain a 37°C water bath to incubate your tubes.
NA= no incubation or run at this time.

Solution	Time in minutes: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Control Solutions																																								
Water (tube 1)	I	I	I	I	I	I	I	I	I	I	R	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sucrose (tube 2)	NA	NA	NA	NA	NA	NA	NA	NA	I	I	I	I	I	I	I	I	I	I	R	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Test solutions																																							
_____ (tube 3)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	I	I	I	I	I	I	I	I	I	R	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA
_____ (tube 4)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	I	I	I	I	I	I	I	I	I	I	R	R	R	R	R

Figure UII-4. Fermentation incubation and run schedule.

- After 10 minutes of pre-incubation, connect the free end of the plastic tubing to the connector in the rubber stopper (Figure UII-3C).
- Begin measuring gas pressure for five (5) minutes by clicking the collect button.
- Continuously monitor and maintain the bath temperature at 38-40°C.
- DO NOT click on the Stop button. The software will stop collection on its own.
- **Store Latest Run from the Experiment menu. DO NOT close Logger Pro.**

☐ Repeat the pre-incubations and runs for the other three tubes.

☐ Click on the Linear Fit button. Note: you can use the buttons to view the slope region of interest.

☐ **Record the value of the slope, m, for each of the equations that describe the straight-line relationships, y = mx + b, in Table UII-2 AND to the Fermentation workbook on the instructor computer.**

Important: Start the incubation for the second tube AFTER the first eight (8) minutes of the first tube's pre-incubation. See Figure UII-4.

Table UII-2. Rate of fermentation (kPA/min) by yeast, as determined from the slope of the best-fit linear regression line of the plot of gas pressure versus time.

Solution (tube)	°C	Fermentation rate
Controls		
Water (1)		
Sucrose (2)		
Assigned test solutions		
_____(3)		
_____(4)		

☐ Cleaning the tubes:
- Scrub the tubes with water and dish detergent.
- Rub off the marker label.
- Invert the tubes on the wooden tube rack.

C. Interpretation

☐ Your instructor will send your lab section's completed workbook to each student's ISU Google Account Gmail.

☐ Open the Word document from Section A.

3. Insert the table and graph into the document.
4. Create a table caption ABOVE the table.
5. Create a figure caption BELOW the figure.
6. Type the answers to the following questions into the document:

H. Describe the patterns shown by your graphs and discuss what the patterns are indicating.

I. Do yeast equally utilize all sugars for fermentation?

J. Why does this difference between sugars exist?

K. Did you reject or support your hypothesis? Explain in DETAIL why it was supported or rejected?

L. Using the following terms (active site, substrate, activation energy, enzyme, disaccharide, monosaccharide) describe how sucrose is modified by yeast.

M. Do you think similar enzymes are used in humans? Why?

N. Compare the raw data to the means and standard deviations that were calculated. Which data points could have caused the standard deviation to widen. What type of investigator error could have caused the standard deviation that you see?

☐ **Send the document to your lab instructor using your ISU Google Account Gmail.**

Name:_____ Team #:_____ Section #:_____

Part 4: Photosynthesis

Although there are over 100 elements, only 12 or so are used to make biological materials. Living organisms are built predominantly from non-metal elements. However, trace amounts of many metal elements are essential for healthy growth. The most abundant elements in living organisms are Carbon (C), Hydrogen (H), Oxygen (O), Nitrogen (N) , Sulfur (S), and Phosphorus (P).

Table UII-3. Elements in the human and plant body (% mass dry weight) are as follows.

Human		Plant	
Oxygen*	65	Carbon	44
Carbon	18	Hydrogen*	44
Hydrogen*	10	Oxygen*	6
Nitrogen	3	Nitrogen	1–4
Calcium	1.5	Potassium	0.5–6
Phosphorus	1	Calcium	0.2–3.5
Potassium	0.35	Magnesium	0.1–0.8
Sulfur	0.25	Phosphorus	0.1–0.8
Sodium	0.15	Sulfur	0.05–1
*these elements make up water; 55-60% of a human and >60% of a plant body are water.			

Plants are essential components of the food webs of all ecosystems because they are autotrophs. An autotroph converts energy from an inorganic source into organic molecules that can be used for biological functions such as cell growth and reproduction. Because plants utilize sunlight as their primary source of inorganic energy, they are more specifically known as phototrophs or autotrophs. Through the process of photosynthesis, occurring in the chloroplast of a plant cell, solar energy is used by plants to convert carbon dioxide and water into glucose, water and oxygen, as shown in the following equation:

$$6\ CO_{2(g)} + 12\ H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6\ H_2O + 6\ O_{2(g)}$$

Through this reaction, light energy is stored as potential energy in the electrons that make up the bonds of the glucose molecule. This enables plants to provide themselves with the organic molecules necessary for their own survival and growth. Heterotrophic organisms, which include almost all bacteria, fungi and animals, rely on autotrophs as an important source of organic molecules for both energy and the raw materials to make macromolecules.

Both autotrophs and heterotrophs release the chemical energy stored in glucose through the process of cellular respiration. The potential energy contained in the electrons that form bonds within glucose is released as the electrons are transferred to other molecules. Some of the energy that is released is captured as adenosine triphosphate (ATP) and some is lost as heat. Eventually, the energy-depleted electrons are transferred to a final electron acceptor. When the electron acceptor is oxygen, cellular respiration is known as aerobic respiration; when the electron acceptor is an inorganic molecule other than oxygen, cellular respiration is known as anaerobic respiration. You will recall from your observations of cellular respiration by yeast, that this process involves the consumption of oxygen and the release of carbon dioxide, as shown in the following equation:

$$C_6H_{12}O_6 + 6\ O_{2(g)} \rightarrow 6\ H_2O + 6\ CO_{2(g)} + \text{heat energy}$$

Spinach (*Spinacia oleracea*, Domain Eukarya, Kingdom Plantae) is an edible flowering plant native to central and western Asia. It is an annual herb whose leaves are commonly used as a vegetable or salad green. They are an excellent source of vitamins A, B and C, iron, phosphorus and chlorophyll. Spinach leaves typically grow in a basal clump to 12” tall. Greenish-yellow flowers appear when the plants bolt. Flowers have no ornamental value.

Spinach and all other plants have organelles called chloroplasts. Chloroplasts are small, reaching a maximum size of 5µm in diameter. Inside chloroplasts there are stacked thylakoids that require a variety of pigments to power photosynthesis. Plant pigments selectively absorb certain wavelengths of light and reflect others. The reflected wavelengths of light determine the color the pigment will appear to the eye. Chlorophyll a (430 nm & 662 nm) and Chlorophyll b (453 nm & 642 nm) reflect green. Chlorophyll is usually at a higher concentration giving plants their typical green color. Carotenoids (470 nm & 500 nm) are accessory pigments and reflect red, orange, or yellow colors. They assist in photosynthesis by gathering wavelengths of light not readily absorbed by chlorophyll.

A. Experimental Design

☐ Observe the Pre-Lab video to find the question, hypothesis, and prediction and to observe spinach chloroplasts.

☐ Open Word document, answer question 1 and 2, and then save the document.

1. At the top right of the document type:
   - Biol 1101L
   - Unit II - Photosynthesis
   - Your section #
   - Your team #
   - Names of everyone on your team

2. Type the answers to the following questions into the Word document.

   A. Background: What is the species name of your model organism? To which domain and kingdom does it belong? Does it have chloroplasts? What are the names of the pigments in the chloroplasts that allow a plant to capture light energy? How do plants benefit from using a variety of pigments?
   B. Question?
   C. Determine your variables: Which is your response (dependent) variable? Which is your predictor (independent) variable? Are the variables categorical and/or quantitative?
   D. Describe the control and experimental groups for your experiment:
   E. Describe the type of relationship between the variables (positive, negative, or neutral).
   F. Develop a hypothesis.
   G. What is your prediction as to the outcome of your experiment.

B. Chloroplast Absorption Spectrum

☐ Obtain a cuvette of chloroplast suspension diluted with buffer and another cuvette blank (filled with buffer only.)

Important: Wipe the bottom half of the cuvette tubes with a Kim-Wipe and isopropanol. Mix by inversion to keep the chloroplasts as evenly suspended as possible.

☐ Start with the spectrophotometer set at 375 nm, blank the instrument with the cuvette filled with only buffer.

- Evenly suspend the chloroplasts by inverting the suspension.
- Place the chloroplast solution into the sample holder and determine the absorbance.
- Record the absorbance reading in Table UII-4.
- Remove the sample and reset the wavelength to 400 nm.
- Reblank the instrument and then determine the absorbance of the sample at that wavelength.
- Repeat at 25 nm intervals up to 700 nm.

☐ **Record the absorbance readings in Table UII-6.**

☐ Download, open, and fill in the Spectrum.xlxs table with your data.

☐ Add your data to spreadsheet in the Photosynthesis workbook labelled Spectrum.

☐ Open the Word document from Section A, answer questions 1-5, and the save the file.

3. Insert the table and graph into the document.
4. Create a table caption ABOVE the table.
5. Create a figure caption BELOW the figure.
6. On your figure label the chlorophyll a peaks, the chlorophyll b peaks, and the carotenoid peaks.

Table UII-4. Absorption spectrum of chloroplast suspension.

Wavelength	Absorbance
375	
400	
425	
450	
475	
500	
525	
550	
575	
600	
625	
650	
675	
700	

C. Data Collection

Important: Keep the sensors upright at all times.

☐ Hardware and software set up:
a. Restart the computer at your station.
b. Login into the ISU network and your Canvas account.
c. Download, save, and open the Photosynthesis.cmbl file.
d. Check that the GoLinks are connected to the computer and the CO_2 and O_2 sensors.

☐ Remove the sensors from the BioChamber and calibrate them following your instructor's direction; CO_2 should fluctuate between 0.03-0.04% and O_2 should read 20.9%

Dark (shielded)

☐ Light Incubation:
- Obtain 20 grams of leaves.
- Remove the BioChamber lid (keep the lid upright), place the leaves adaxial side up in the BioChamber.
- Place the BioChamber under the light, turn on the light if it is not already on, and set the timer for 3 minutes.

☐ Dark Incubation:
- Put on the chamber lid.
- Wrap the reaction chamber in foil to prevent any light from reaching the spinach.
- Set the timer for 10 minutes.

☐ Run 1:
- After the 10-minute dark incubation, set the timer for 25 minutes.
- Click 'Collect' Collect to begin data collection.
- The program is set for 40 minutes but the run only needs to be 25 minutes.
- Concentration will be measured in two separate graphs (CO_2 and O_2)
- After 25 minutes, select Experiment menu > Store Latest Run.

☐ Determine the rate of change for CO_2 and O_2 concentrations using the linear fit button .
- CO_2 - highlight the entire line.
- O_2 - highlight the last 5 minutes only.

Table UII-5. Rates of change in the concentrations of carbon dioxide (CO_2) and oxygen gas (O_2) when spinach was shielded from or exposed to light. Rate was determined by calculating the slope of the linear regression line obtained when concentration was plotted against time

	Rate of Change (%)	
	O_2	CO_2
Dark (run 1)		
Light (run 2)		

☐ **Record the slope, m, for both CO_2 and O_2 in Table UII-5 AND to the Photosynthesis workbook on the instructor computer.**

Light (exposed)

☐ Set up:
- Place the BioChamber under the light.
- Create a foil reflector under the BioChamber.
- Make sure the light is ~1½ ft above counter top and turn on the light.

☐ Run 2 (latest run):
- Set the timer for 40 minutes.
- Click 'Collect' to begin data collection.
- Concentration will be measured in two separate graphs (CO_2 and O_2).
- The program is set for 40 minutes.
- After 40 minutes, select Experiment menu > Store Latest Run.

☐ Determine the rate of change for CO_2 and O_2 concentrations using the linear fit button .
- CO_2 - highlight the entire line.
- O_2 - highlight the entire line.

☐ **Record the slope, m, for both CO_2 and O_2 in Table UII-5 AND to the Photosynthesis workbook on the instructor computer.**

☐ Place the spinach back in the storage bag. Keeping the lid upright, wipe out the BioChamber and then set the lid back on the chamber.

D. Interpretation

☐ Your instructor will send your lab section's completed workbook to each student's ISU Google Account Gmail.

☐ Open the Word document from Section A

7. Insert the table and graph into the document.
8. Create a table caption ABOVE the table.
9. Create a figure caption BELOW the figure.
10. Type the answers to the following questions into the Word document:

H. A positive value for m indicates the regression line has a positive slope; a negative value for m indicates the regression line has a negative slope:
- Which slope indicates whether carbon dioxide was consumed?
- Which slope indicates whether carbon dioxide was produced?
- Which slope indicates whether oxygen gas was consumed?
- Which slope indicates whether oxygen gas was produced?

I. Did you reject or support your hypothesis? Explain in DETAIL why it was supported or rejected? Describe the evidence you collected indicating that respiration and photosynthesis were occurring in the leaves?

J. Your instructor showed you an absorption spectra of 3 plant pigments. How did it differ from the chloroplast absorption spectrum you created?

K. Compare the raw data to the means and standard deviations that were calculated. Which data points could have caused the standard deviation to widen. What type of investigator error could have caused the standard deviation that you see?

L. Given your understanding of photosynthesis and respiration in spinach leaves and the structure of macromolecules (Unit I); what makes it possible for a plant seed (such as the bean in Figure UII-4) to sprout and grow into a seedling? Contrast this with a human newborn growing into a toddler. Make reference to starch, glycogen (multibranched polysaccharide of glucose), cellulose, autotroph, and heterotroph. Please use information from this lab and previous labs to help answer the question.

☐ **Send the document to your lab instructor using your ISU Google Account Gmail**

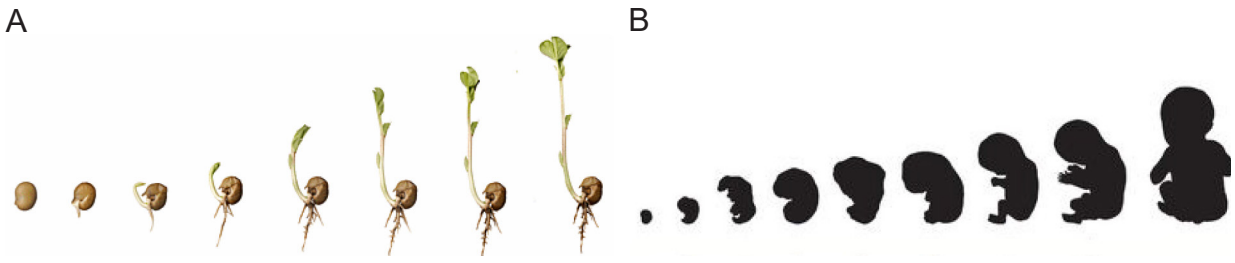

Figure UII-4. Growth and development; A) a bean seed sprouting into a seedling and B) a human fetus growing into a infant.

Unit III - Cellular Reproduction

Objectives

- Gain a better understanding of the molecular structure of cell membranes, nuclear membranes and deoxyribonucleic acid (DNA) by extracting DNA from cheek cells.
- Gain a better understanding of the molecular structure of DNA and the process of DNA replication and transcription by constructing simple models.
- Translate a gene to synthesize a protein.
- Use electrophoresis to observe variations in cleavage patterns produced by the action of restriction enzymes on samples of DNA.
- Identify the perpetrator of a crime using the logic of DNA "fingerprinting."
- Name, identify, describe, and compare cell cycle events and the division and maturation of blood cells.
- Observe the cell cycle and blood tissue using prepared animal and plant tissue slides.

Terms & Definitions

Agarose gel - a jelly-like slab used to separate molecules on the basis of molecular weight.

Amino acid - monomer subunit of a protein. Contains an amino, a carboxyl, and a unique side group.

Bivalent/tetrad - the association of a pair of homologous chromosomes (4 sister chromatids) physically held together by at least one synaptic DNA cross-over. This physical attachment allows for alignment and segregation of the homologous chromosomes in the meiosis I.

Base - a component of nucleotides that is a single or double ring of carbon and nitrogen.
Blood - a fluid connective tissue in animals consisting of cells and (in mammals) cell fragments suspended in a solution of water containing dissolved nutrients, proteins, gases, and other molecules.

A. Basophils - a type of white blood cell. Basophils are the least common type of granulocyte, representing about 0.5% to 1% of circulating white blood cells
B. Clotting - coagulation, is an important process that prevents excessive bleeding when a blood vessel is injured. Platelets and proteins in plasma work together to stop the bleeding by forming a clot over the injury.
C. Erythrocyte (red blood cell, RBC) - the most common type of blood cell and the vertebrate's principal means of delivering oxygen (O2) to the body tissues through the circulatory system. RBCs take up oxygen in the lungs, or in fish the gills, and release it into tissues while squeezing through the body's capillaries.
D. Hemoglobin - an iron-containing protein that binds oxygen and is found within the cytosol of red blood cells.
E. Leukocyte (White blood cell, WBC) - A type of blood cell that is made in the bone marrow and found in the blood and lymph tissue and are part of the body's immune system (B & T Lymphocyte, Monocyte,Neutrophil,Eosinophil,Basophil)
F. Thrombocytes (Platelet) - cell fragments in the blood of mammals that play a crucial role in the formation of blood clots.
G. Plasma - fluid, composed of about 92% water, 7% vital proteins, clotting factors, and 1% mineral salts, sugars, fats, hormones and vitamins.

Blood group systems - blood type that is determined by the presence or absence of certain antigens on the cell membranes of erythrocytes.

Bone marrow - a semi-solid tissue found within the spongy or cancellous portions of bones. In birds and mammals, bone marrow is the primary site of new blood cell production or hematopoiesis. It is composed of hematopoietic cells, marrow adipose tissue, and supportive stromal cells.

A. Hematopoiesis - the production of blood cells and platelets, which occurs in the bone marrow.
B. Erythropoiesis - the production of red blood cells.

Blood Vessels - a vessel (arteries, veins, and capillaries) in the human or animal body in which blood circulates.

Calcitonin - a hormone secreted by the thyroid that has the effect of lowering blood calcium.

Cell cycle - the series of phases a eukaryotic cell progresses through from its origin until it divides by mitosis:

A. Interphase - it is the portion of the cell cycle during which the chromosomes are decondensed and found in the nucleus. G1 (first gap cycle), S (DNA synthesis phase), and G2 (second gap cycle) are stages of interphase.
B. Prophase - phase of mitosis during which the chromosomes condense and the nuclear membrane begins to vesiculate.
C. Prometaphase - phase of mitosis during which the mitotic spindle is completely formed.
D. Metaphase - the phase of mitosis during which the chromosomes are aligned along the metaphase plate -
E. Anaphase - the phase of mitosis during which the sister chromatids separate from each other and move to opacity poles; poles themselves also move farther apart.
F. Telophase - the phase of mitosis during which the chromosomes decondense and the nuclear membrane re-forms.
C. Cytokinesis - the division of the cytoplasm to produce two distinct daughter cells.

Cell cycle structures:

A. Centriole - a cylindrical organelle composed mainly of tubulin protein that helps anchor microtubules during cell division.
B. Centromere - a region (not a true structure) of a chromosome where sister chromatids are attached and to which microtubules bind.
C. Centrosome - a structure near the cell nucleus that forms the main microtubule organizing center during division. Each centrosome is composed of two centrioles at right angles to each other. Duplication occurs during the G1 phase and S Phase.
D. Kinetochore - a protein structure that can be found in the centromere region of a chromosome where the microtubules attach during cell division to pull sister chromatids apart.
e. Microtubule - protein structure that moves chromosomes around during mitosis and meiosis.

Cellular division - in eucaryotic cells, the process by which one cell divides into two cells:

A. Mitosis - the process in which nuclear division results in two nuclei, each of which receives the same complement of chromosomes.
B. Meiosis - the process by which haploid cells are produced from a cell that was originally diploid.

Codon - a sequence of three nucleotides bases that specifies a particular amino acid or a stop codon; codons function during translation:

A. Anticodon - a three nucleotide sequence in tRNA that is complementary to a codon in mRNA.
B. Start - the three-base sequences (start usually aug) that specify the first animo acid in a polypeptide in translation
C. Stop - those that signal the end of translation (stop UAA, UAG, UGA)

Denature - the process where proteins unravel and change their native shape thus losing their biological activity.

Deoxyribonucleic acid (DNA) - the genetic material that serves as the code for building each unique organism.

Deoxyribonuclease (DNAse) - an enzyme that catalyzes the hydrolytic cleavage of phosphodiester linkages in the DNA backbone, thus degrading DNA.

Chromosome - a discrete unit of genetic material composed of DNA and associated proteins. Eukaryotes have chromosomes in their cell nuclei and in their plastids and mitochondria:

A. Autosome - non-sex chromosome, of which there are 22 pairs in humans.
B. Chromatin - highly organized chromosome complex composed of DNA and histone proteins.
C. Histone - a group of proteins involved in the formation of nucleosomes that aid in the compaction of eukaryotic DNA.
D. Homologous (Homologs) - a pair of chromosomes consisting of one chromosome received from the father and one from the mother.
E. Nucleosome - the structural subunit of chromatin and is composed of eight histones wrapped with DNA.
F. Sex chromosome - a distinctive set of chromosomes that are different in males and females.
G. Sister chromatid - either of the two duplicated, identical copies of a chromosome formed after DNA synthesis. Acidophilic cells

DNA fingerprinting - a technology that identifies particular individuals using properties of their DNA.

DNA replication - the copying of DNA strand.

A. Directionality - the end-to-end chemical orientation of a single strand of nucleic acid. In a single strand of DNA or RNA. The chemical convention of naming carbon atoms in the nucleotide pentose-sugar-ring means that there will be a 5′ end (typically contains a phosphate group attached to the 5′ carbon of the ribose ring) and a 3′ end that is unmodified from the ribose -OH substituent. In a DNA double helix, the strands run in opposite directions to permit base pairing between them, which is essential for replication or transcription of the encoded information.
B. Helicase - separates double-stranded DNA into single strands.
C. Lagging strand - a single DNA strand that is replicated in the 5′ – 3′ direction (opposite direction to the replication fork).
D. Leading strand - a single DNA strand that is replicated in the 3’ – 5’ direction (same direction as the replication fork). DNA is added to the leading strand continuously, one complementary base at a time.
E. Ligase - covalently attaches adjacent Okazaki fragments in the lagging strand.
F. Okazaki fragments - DNA added to the lagging strand in discontinuous sections.
G. DNA Polymerase - an enzyme involved in making new DNA molecules from the four nucleotide bases, using the existing DNA as a template. They can add nucleotides to the 3’-end of an existing nucleic acid, requiring a primer be bound to the template before DNA polymerase can begin a complementary strand.
H. DNA Primase - are enzymes that must be continuously active and catalyze the creation of small RNA molecules that are employed as DNA polymerase primers.
I. RNA primer - four to fifteen nucleotides long single-stranded nucleic acid used by all living organisms in the initiation of DNA synthesis.
J. Topoisomerase - enzymes that play essential roles in DNA replication, transcription, chromosome segregation, and recombination: type I, which makes single-stranded cuts in DNA, and type II enzymes, which cut and pass double-stranded DNA. They remove the tightened coils ahead of the replication fork.

Essential Amino Acid - any of the amino acids that humans cannot synthesize and thus must be obtained from the diet. Gametogenesis - the process in which cells undergo meiosis to form gametes.

Gel Electrophoresis - a technique used to separate macromolecules by using an electric field that causes them to pass trough a gel matrix.

Genome - the complete genetic composition of a cell or a species..

Locus - the physical location of a gene on a chromosome.

Nucelotide - organic molecules composed of a nitrogenous base (nucleobase), a pentose sugar (ribose or deoxyribose) and a phosphate containing one to three phosphates. They serve as monomeric units of the nucleic acid polymers – deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

A. Nucleotide bases (nucleobases, nitrogenous bases) - nitrogen-containing biological compounds; adenine (A), cytosine (C), guanine (G), thymine (T), and uracil (U)
B. Purine bases - guanine (G) and adenine (A) have a fused-ring skeletal structure derived of purine; characterized by their single amino group (−NH2), at the C6 carbon in adenine and C2 in guanine.
C. Pyrimidine bases - cytosine (C), thymine (T), and uracil (U) have a simple-ring structure derived of pyrimidine
D. Nucleosides - glycosylamines that can be thought of as nucleotides without a phosphate group.
D. Base pair - the structure in which two bases in opposite strands of DNA hydrogen-bond with each other.
F. Complementary - bases pair with each other by hydrogen bonding across the DNA helix; adenine with thymine and cytosine to guanine.

Ploidy - the number of sets of chromosomes in a cell, or in the cells of an organism.

A. Diploid - carrying two complete sets of chromosome; denotes by 2n.
B. Haploid - carrying one set of chromosomes; designated as 1n.

Pole - opposite ends of a sphere, such as a cell or of a planet.

Polymerase Chain Reaction - a technique used for replication DNA that can produce millions of copies of a DNA sequence in just a few hours from a small initial amount of DNA; primers are used that flank the region of DNA to be amplified.

Primers - short segments of RNA, typically 10-12 nucleotides in length, that are needed to begin DNA replication

Progenitor cell - a cell that can differentiate into specialized cell types but cannot maintain an undifferentiated status as does a stem cell.

Promoter - the site in DNA where transcription begins.

Protease - an enzyme that cuts proteins into smaller polypeptides.

Protein synthesis - the process whereby biological cells generate new proteins.

Proteolysis - a processing event within a cell in which enzymes called proteases cut proteins into smaller polypeptides.

Restriction enzyme - a protein isolated from bacteria that cleaves DNA sequences at sequence-specific sites along the phosphate backbone producing DNA fragments with a known sequence at each end.

Secondary Messenger - intracellular signaling molecules released by the cell in response to exposure to extracellular signaling molecules—the first messengers.

Somatic cell - the type of cell the constitutes all cells of an animal or plant body except those that give rise to gametes.

Stem cell - a cell that can go through mitotic cell division numerous times without differentiating into a specific cell type but they can also differentiate into specialized cell types.

Ribonucleic acid (RNA) - one of two classes of nucleic acids; the other is deoxyribonucleic acid (DNA). RNA consists of a single strand of nucleotides.

A. Messenger RNA (mRNA) - RNA that contains the information to specify a polypeptide with a particular amino acid sequence.
B. RNA Primers - short segments of RNA, typically 10 - 12 nucleotides in length, that are needed to begin DNA replication
C. Ribosomal RNA (rRNA) - an RNA that forms part of ribosomes, which provide the site where translation occurs.
D. Transfer RNA (tRNA) - is used to translate mRNA into polypeptides and carries an amino acid to the protein synthesizing ribosome.

TAQ Polymerase - is a thermostable DNA polymerase that is frequently used in the polymerase chain reaction (PCR).

Telomerase - an enzyme that helps prevent the degradation of the tips of chromosomes, active during development and sometimes reactivated in cancer cells.

Transcription - the use of a gene sequence to make a copy of RNA.

Translation - the process of synthesizing a specific polypeptide on a ribosome.

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Part 1: Deoxyribonucleic Acid

Deoxyribonucleic acid (DNA) contains the genetic instructions specifying the biological development of all cellular forms of life, and most viruses. DNA is a long polymer of nucleotides (a polynucleotide) and encodes the sequence of the amino acid residues in proteins using the genetic code, which consists of a triplet code of nucleotides. In organisms that belong to the domains Archaea and Bacteria, DNA is not separated from the cytoplasm by a nuclear envelope. This is one of the characteristics that distinguish these organisms as prokaryotes. In complex eukaryotic organisms comprised of plants, animals, fungi and protists, most of the DNA is located in the cell nucleus. Plant chloroplasts and the mitochondria of eukaryotic organisms also carry DNA. DNA is often referred to as the molecule of heredity because it is responsible for the genetic propagation of most inherited traits. In humans, these traits can range from hair color to disease susceptibility. Every person's DNA, their genome, is inherited from both parents. The mother's mitochondrial DNA, together with twenty-three chromosomes from each parent, combine to form the genome of a zygote, the fertilized egg. As a result, with certain exceptions such as red blood cells, most human cells contain 23 pairs of chromosomes, together with mitochondrial DNA inherited from the mother. Studies of relatedness in humans are based on the fact that a) mitochondrial DNA is inherited only from one's mother, and b) the male Y chromosome is inherited only from one's father. Ribonucleic acid (RNA) is a polynucleotide that "reads" the information encoded in DNA and directs the synthesis of proteins, which have a variety of functions. Both DNA and RNA consist of nucleotides comprised of a phosphate molecule, a nitrogenous base and a five-carbon sugar, ribose. The nitrogenous bases that form nucleotides in DNA include the purines - adenine (A) and guanine (G), and the pyrimidines - cytosine (C) and thymine (T). In RNA, the pyrimidine uracil (U) replaces thymine.

A. Replication

DNA replication is the process by which a double-stranded DNA molecule is copied to produce two identical DNA molecules. Replication is an essential process because, whenever a cell divides, the two new daughter cells must contain the same genetic information, or DNA, as the parent cell.

The replication process relies on the fact that each strand of DNA can serve as a template for duplication. DNA replication initiates at specific points, called origins, where the DNA double helix is unwound. A short segment of RNA, called a primer, is then synthesized and acts as a starting point for new DNA synthesis. An enzyme called DNA polymerase next begins replicating the DNA by matching bases to the original strand. Once synthesis is complete, the RNA primers are replaced with DNA, and any gaps between newly synthesized DNA segments are sealed together with enzymes.

DNA replication is a crucial process; therefore, to ensure that mistakes, or mutations, are not introduced, the cell proofreads the newly synthesized DNA. Once the DNA in a cell is replicated, the cell can divide into two cells, each of which has an identical copy of the original DNA.

☐ Construct a DNA segment model with five base-pairs (which equals 10 nucleotides) and their corresponding bonds following the instructions on sheets A-B of the DNA modeling manual.

☐ Replicate four base-pairs of the DNA model you constructed by following the instructions on sheets D-E.

1. In Figure UIII-1 label the following:
   A. 3' and 5' ends (boxes)
   B. Helicase
   C. Lagging strand (template & synthesized)
   D. Leading strand (template & synthesized)
   E. Ligase
   F. Okazaki fragments (OF)
   G. DNA Polymerase
   H. DNA Primase
   I. RNA primer
   J. Topoisomeras
2. Once you have completed your model and Q1 you must demonstrate the replication process to your TA. **TA initials** _____

☐ Reverse your replication and save the resulting DNA molecule model for Part 1B.

B. Transcription & Translation

Transcription is the process by which the information in a strand of DNA is copied into a new molecule of messenger RNA (mRNA). DNA safely and stably stores genetic material in the nuclei of cells as a reference, or template. Meanwhile, mRNA is comparable to a copy from a reference book because it carries the same information as DNA but is not used for long-term

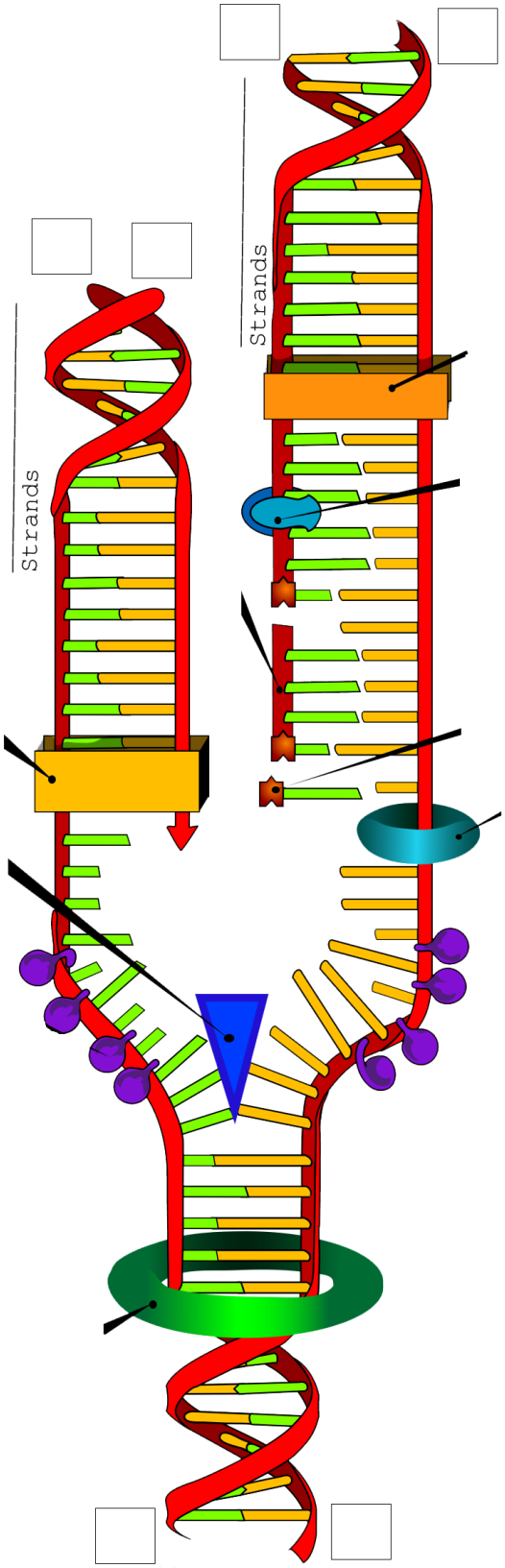

Figure UIII-1. DNA structure and replication.

storage and can freely exit the nucleus. Although the mRNA contains the same information, it is not an identical copy of the DNA segment, because its sequence is complementary to the DNA template.

Transcription is carried out by an enzyme called RNA polymerase and a number of accessory proteins called transcription factors. **Transcription factors** can bind to specific DNA sequences called enhancer and promoter sequences in order to recruit RNA polymerase to an appropriate transcription site. Together, the transcription factors and RNA polymerase form a complex called the transcription initiation complex. This complex initiates transcription, and the RNA polymerase begins mRNA synthesis by matching complementary bases to the original DNA strand. The mRNA molecule is elongated and, once the strand is completely synthesized, transcription is terminated. The newly formed mRNA copies of the gene then serve as blueprints for protein synthesis during the process of translation.

Translation is the process by which a protein is synthesized from the information contained in a molecule of messenger RNA (mRNA). During translation, an mRNA sequence is read using the genetic code, which is a set of rules that defines how an mRNA sequence is to be translated into the 20-letter code of amino acids, which are the building blocks of proteins. The genetic code is a set of three-letter combinations of nucleotides called codons, each of which corresponds with a specific amino acid or stop signal. Translation occurs in a structure called the ribosome, which is a factory for the synthesis of proteins. The ribosome has a small and a large subunit and is a complex molecule composed of several ribosomal RNA molecules and a number of proteins. Translation of an mRNA molecule by the ribosome occurs in three stages: initiation, elongation, and termination. During initiation, the small ribosomal subunit binds to the start of the mRNA sequence. Then a transfer RNA (tRNA) molecule carrying the amino acid methionine binds to what is called the start codon of the mRNA sequence. The start codon in all mRNA molecules has the sequence AUG and codes for methionine. Next, the large ribosomal subunit binds to form the complete initiation complex. During the elongation stage, the ribosome continues to translate each codon in turn. Each corresponding amino acid is added to the growing chain and linked via a bond called a peptide bond. Elongation continues until all of the codons are read. Lastly, termination occurs when the ribosome reaches a stop codon (UAA, UAG, and UGA). Since there are no tRNA molecules that can recognize these codons, the ribosome recognizes that translation is complete. The new protein is then released, and the translation complex comes apart.

☐ Perform a transcription on four base-pairs of DNA model you built during Part 2 by following the instructions on sheets F-G.

3. Once you have completed your model you must demonstrate transcription to your TA. **TA initials** _____

☐ Deconstruct the DNA model into its individual pieces.

4. Given the coding strand 5'-ATG ATG GTT CCC CTA ACA CCG GAC CAA TAG TAG-3'.
   A. Determine the template strand.
   B. Transcribe the template strand.
   C. Translate the transcription

Part 2: RFLP Analysis of DNA

Restriction Fragment Length Polymorphism (RFLP) is widely used in genotyping, DNA fingerprinting, mapping of genes, and diagnosis of genetic disorders. Individuals, species, or organisms can be distinguished on the basis of their RFLP pattern. RFLP refers to differences (or variations) in DNA sequences at sites recognized by restriction endonucleases (restriction enzymes). Such variation results in different sized (or length) DNA fragments produced by digesting the DNA with a restriction enzyme. It is a commonly employed molecular tool to check the small but specific variations in a sequence of double-stranded DNA. Restriction endonucleases recognize a set of nucleotides at a restriction site and cleave the DNA at those sites. A specific RFLP pattern emerges during electrophoresis separation of the digested DNA, producing variable lengths of cleavage fragments which are characteristic of a sequence of DNA.

A. PCR

RFLP requires the use of a molecular laboratory technique called **polymerase chain reaction** (PCR). PCR (Fig. UII-2) rapidly produces/amplifies millions to billions of copies of a specific segment of DNA. The amplified DNA segment can then be studied in greater detail. PCR involves using short synthetic DNA fragments called **primers** to select a segment of the genome allowing multiple rounds of DNA synthesis to amplify that segment. Primers are designed by researchers to target/bind to a specific DNA sequence which then directs the ***Taq* polymerase** to build new DNA starting at the primer location and continuing along the sequence. After the initial elongation the sample is heated again to denature the newly formed DNA duplex, cooled to allow primer binding and extension to happen again. Each time the sample cycles through the different temperatures the amount of DNA doubles. This simple cycle (anneal, extend, denature) is the basis of PCR. By repeating this sequence of heating and cooling many times billions of a specific DNA sequence in a sample are produced in a matter of minutes.

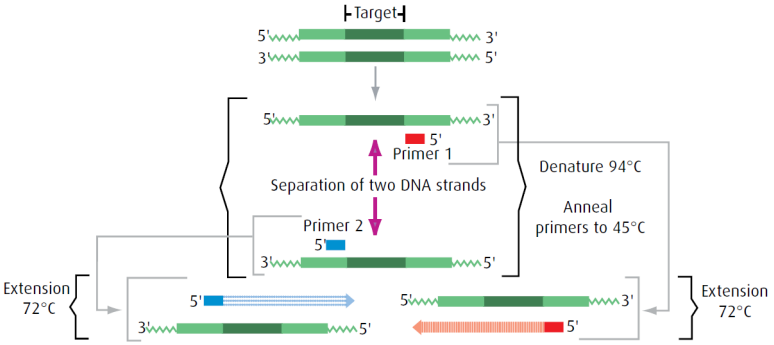

Figure UIII-2. Diagram of the polymerase chain reaction.

B. Restriction Enzymes

After amplification, the DNA is digested with restriction enzymes that cut double-stranded DNA in a sequence-specific manner. Most restriction endonucleases recognize palindromic or partially palindromic sites. For example, EcoRI (pronounced "eco R one") is a 377 amino acid restriction endonuclease enzyme isolated from the bacteria, *Escherichia coli*. It cleaves DNA double helices into fragments at specific sites. EcoRI cuts DNA after G forming sticky ends with AATT (Figure UIII-3).The triangle denotes the site where the phosphodiester bond is broken.

5′... G▼A A T T C ...3′
3′... C T T A A▲G ...5′

Figure UIII-3. A palindrome is defined as dyad symmetry around an axis. The recognition site of EcoRI with triangle indicating the cut site.

C. Electrophoresis

Electrophoresis describes the migration of charged particles under the influence of an electric field. Gel electrophoresis refers to the technique in which molecules are forced by an electric current to move through a gel. Most DNA electrophoresis requires a gel made from agarose. The majority of agar used in culinary and commercial applications is extracted from the cell wall of the red seaweed, *Gelidium amansii*. Agar consists of a mixture of two polysaccharides: agarose and agaropectin. Agarose is a linear polymer, made up of repeating units of agarobiose, a disaccharide made up of D-galactose and 3,6-anhydro-L-galactopyranose. **Because the phosphate groups in the backbone of DNA are negatively charged**, **the entire DNA molecule has a negative charge.** During gel electrophoresis, the **negatively charged DNA molecules move toward the positive pole of the electrophoresis chamber**.

Agarose gel electrophoresis separates molecules into discrete bands, each comprising molecules of the same size. Movement of the molecules is inversely proportional to the size/length of the molecule. ($\log_{10}$ of the molecules length). Larger fragments of DNA move through the gel slowly because they frequently collide with particles in the gel matrix. Smaller fragments of DNA are less likely to collide with particles in the matrix, and therefore move through the gel more quickly. Once the fragments of DNA have been separated by molecular size, their locations within the gel can be determined using a stain. The pattern of stained bands is compared to the pattern produced by other DNA samples. DNA standard markers (molecular weight standards in base pairs) can be used to determine the size of the unknowns running on the gel. It some cases, a standard curve will need to be created to extrapolate the size of each unknown.

5. Why do DNA molecules move towards the negative or positive electrode?

6. What is RFLP analysis and explain how restriction endonucleases function in RFLP analysis?

7. What is PCR, how are primers used in PCR, and explain the basic cycle of PCR? ?

8. What is *Taq* polymerase and what is its function?

9. What does recognition site mean with respect to restriction endonucleases?

10. Explain the movement of DNA fragments during gel electrophoresis?

11. What are DNA standard markers?

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Part 3: Molecular Analysis of Cancer

Most cancers result from somatic (body cell) mutation during a person's life (Figure UIII-4A). These changes occur either though exposure to mutagens that change the DNA sequence or through errors in DNA replication. Not all change will cause cancer because some changes may occur in non-coding DNA sections or they may be recessive to the function of a gene. Hereditary germline mutations are directly inherited through DNA changes that can be passed form one generation to another as they are present in the gametes (Figure UIII-4B).

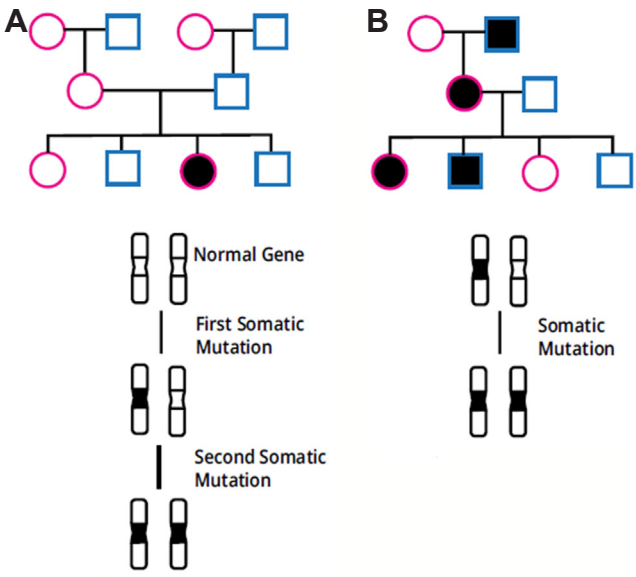


Figure UIII-4. Pedigree models of *TP53* gene inactivation: **A)** Sporadic somatic mutation that produces single tumors that are usually unilateral and occur after the age of 45, and **B)** Hereditary germline mutation that produces multiple tumors that can be bilateral and occur before the age of 45.

Cancer genomes or the whole DNA sequence from cancer cells can be analyzed using techniques like RFLP analysis. The TP53 gene is a tumor suppressor gene that acts as a master regulator for the genes responsible for cell division and death by coding for the p53 protein that functions as a transcriptional regulator. Specific amino acids in the p53 protein allow it to bind to specific DNA sites activating the transcriptions of genes necessary for cell division and cell death. This gene is often called the "Guardians of the Genome" as it plays a major role in preventing changes to the genomic DNA. Just a single mutation in the gene greatly increases a person's susceptibility of cancer and TP53 is the most commonly mutated gene in cancer.

Figure UIII-5. A palindrome is defined as dyad symmetry around an axis. The recognition site of *Pvu*II with triangle indicating the cut site.

We will determine if an individual has mutations in one or both copies of the TP53 gene (both TP53 alleles). Breast cancer is the second most common form of cancer in women and most often occurs in the milk ducts although they can develop in other breast tissue. Besides breast tissue, mutations in the TP53 gene can also cause cancer in other tissues of the body. PvuII restriction endonuclease enzyme recognizes the TP53 gene mutant sequence at codon position 165 (Figure UIII-5). The normal DNA at this site can not be cut because it is not recognized by the enzyme. In this exercise; 1) a blood sample, 2) a breast tumor sample, and 3) a healthy breast tissue sample were collected from a female patient that had a precancerous breast lump biopsy. DNA was extracted from the sample, amplified using PCR, and then the DNA was digested with PvuII restriction endonuclease enzyme.

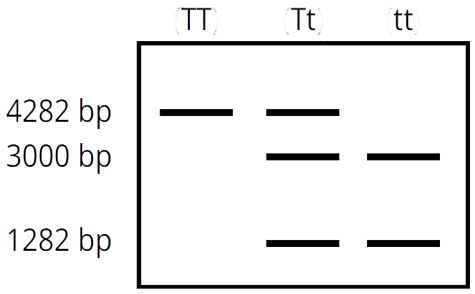


Figure UIII-6. *Pvu*II restriction enzyme. Columns; 1) normal TT p53 alleles, 2) Heterozygous Tt p53 alleles, 3) Mutated tt p53 alleles.

When a person is homozygous recessive (tt) for the TP53 gene mutation it means a mutation has occurred in both alleles (UIII-6 column 3) by either pathways (UIII-4). If the person is heterozygous (Tt) it means that the mutation is a sporadic somatic mutation in a single allele of the gene. The homozygous dominate (TT) indicates that no TP53 gene mutation has occurred in either alleles (UIII-6 column 1).

A. Gel Preparation

☐ Place the gel tray in the extra gel box so that the rubber gaskets seal against the edges of the unit.

☐ Place the six-tooth well comb in the first set of notches at the end of the tray.

☐ Add the following ingredients to an Erlenmeyer flask: 30ml **Tris Acetate EDTA buffer, Ph 7.8** (1X TAE buffer) and 0.3 g agarose.

☐ Swirl the mixture to disperse clumps of agarose powder.

☐ Place the flask in the microwave, heat for 10 seconds. Use a hot glove and gently swirl the solution.

☐ Repeat the process two more times to dissolve the agarose particles.

Caution: Flask and solution will be HOT! Be sure to use protective heat gloves or oven mitts. Use caution when handling the hot agarose solution to avoid boiling over.

☐ Once the flask has cooled for 4 minutes, pour the agarose mixture into the center of the casting tray.

☐ Allow the gel to solidify until opaque (approximately 15 minutes).

☐ As you wait for the gel to solidify, practice loading samples into the previous section's gel (found in the Petri dish) using micropipettes and tips.

☐ After the gel has solidified, gently remove the casting tray (with gel) from the center platform of the electrophoresis chamber, rotate 90 degrees, and place back on the center platform of the chamber.

☐ Pour running buffer (1X TAE buffer) into the chamber. Make sure the gel is covered with 5 mm of buffer.

☐ Carefully remove comb from the gel by pulling it straight up. Do not tear the wells created by the teeth of the comb.

B. Electrophoresis

☐ Position the gel wells farthest from the positive electrode (closest to the black).

☐ Remove the comb.

☐ Using a micropipette:

- Pipette 30 µL of sample your assigned sample into the correct well (Figure UIII-7).
- Discard the pipette tips into the plastic beaker between each sample that is loaded.
- Repeat by pipetting 30 µL of the other assigned samples into the appropriate wells.

☐ After the DNA samples are loaded:

- Insert the plug of the black wire into the black input of the power source (negative input) and insert the plug of the red wire into the red input of the power source (positive input).

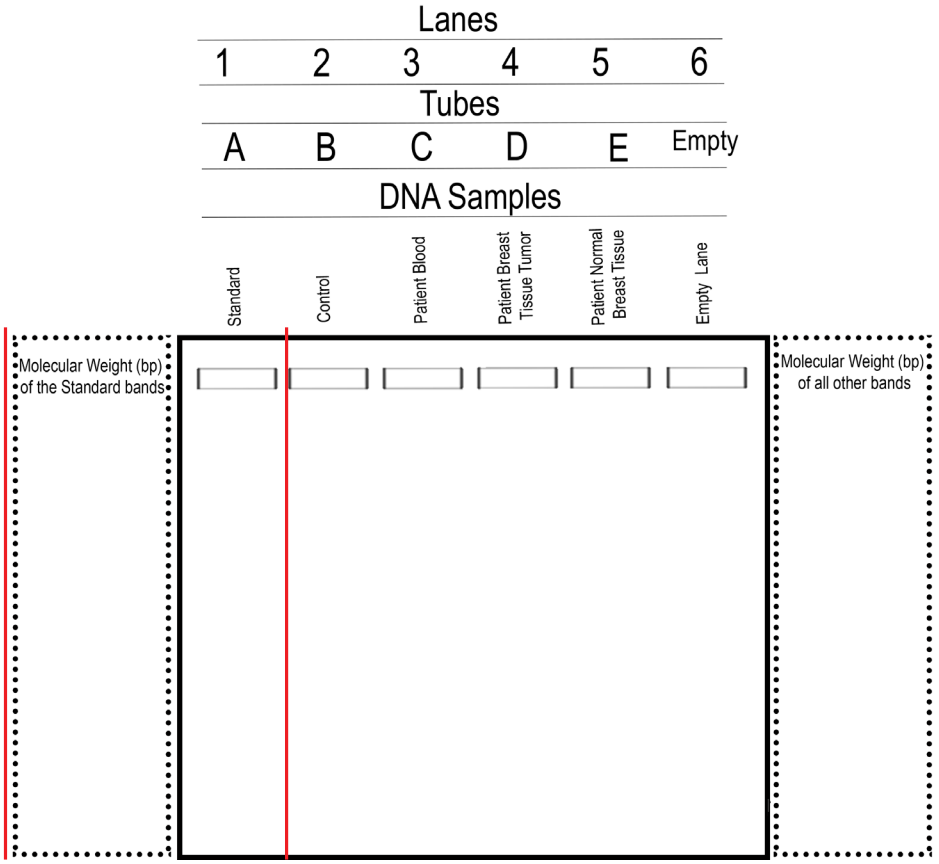


Figure UIII-7. Comparisons of bands produced from RFLP aanlysis of patient DNA samples using PCR, *Pvu*II restriction enzyme, and separated by gel electrophoresis.

- Turn on the electrophoresis power supply and check that it is set at ~134 volts (both blue and white supplies), low (blue supply only), and 25 minutes (white supply only).
- Press the run button (white supply only).

☐ Check that current is flowing by observing bubble formation at the thin wire electrodes.

C. Staining

- ☐ Remove the casting tray (with the gel) from the electrophoresis chamber and slide the gel off the tray into the Petri dish lid (upside down).
- ☐ Pour the 1X TAE buffer into the labeled 'Reuse Running Buffer' pitcher.
- ☐ Place the blue dye side of an InstaStain MetBlue card on the gel and run your fingers over the surface several times to ensure good contact between the card and the gel.
- ☐ Place the Petri dish base on the card and then place a beaker on the base.
- ☐ After one (1) minute, remove the staining card and move the gel back into the Petri dish base.
- ☐ Destain for 5 minutes by adding 37ºC water from the hot water baths to the Petri dish, submerging the gel. Replace the water in the Petri dish every minute. Discard the used blue water in the sink.
- ☐ Place the Petri dish with gel in it on the light box and observe the position of the bands.
- ☐ Measure the distance the bands traveled using a ruler.
- ☐ Add water to the Petri dish with the gel, cover with the lid, and place the dish on the original brown tray.

1. In Figure UIII-7 **sketch in the bands.** The six small boxes at the top of the figure correspond to the wells in which the DNA samples were placed.
2. In Figure UIII-7 write in the molecular weight (Table UIII-1 columns 4 & 5) of the DNA standard marker bands (lane 1) and then the other bands (lanes 2-5).
3. **Measure the distance** in millimeters (mm) of each band from the well in which the DNA sample was placed. Write the distance on Table UIII-1 (column 6).

Table UIII-1. Comparisons of bands produced from RFLP analysis of patient DNA samples using PCR, *Pvu*II restriction enzyme, and separated by gel electrophoresis.

Lane	Tube	Sample	Band	Molecular weight in base pairs (bp)	Distance band migrated (mm)
1	A	DNA standard markers	1 2 3 4 5 6 7	6751 3652 2827 1568 1118 825 630	
2	B	Control DNA is unmutated p53 allele	1	4282	
3	C	Patient Peripheral Blood DNA	1 2 3	4282 3000 1282	
4	D	Patient Breast Tumor DNA	1 2	3000 1282	
5	E	Patient Normal Breast Tissue DNA	1 2 3	4282 3000 1282	
6	Empty				

D. Interpretation

4. Open a Word document and at the top right of the document type:
   - Biol 1101L
   - Unit III - DNA
   - Your section #
   - Your team #
   - Names of everyone on your team
5. Type the answers to the following questions into the document:
   A. In detail, describe what *TP53* and p53 are and what each do?
   B. In detail, describe what *Pvu*II is and what it does?
   C. What is the correct order of steps when adding sample to a gel well after you initially draw up sample into the micropipette tip?.
   D. What information did Lane 1 give you. Explain?
   E. Why does Lane 2, sample B have only one band? Explain.
   F. Why does Lane 3, sample C have three bands? Explain.
   G. Why does Lane 4, sample C have only two bands? Explain.
   H. Why does Lane 5, sample C have three bands? Explain
   I. What does the comparison of Lanes 3 and 5 and Lanes 4 and 5 tell you in reference to UIII-6?

☐ **Send the document to your lab instructor using your ISU Google Account Gmail**

Name:_____ Team #:_____ Section #:_____

Part 4: The Cell Cycle

In **diploid** organisms each body (**somatic**) cell contains two copies of the genome. Thus, each somatic cell contains two copies of each chromosome, and two copies of each gene. The exceptions to this rule are the sex chromosomes that determine sex in a given species. For example, in the XY system that is found in most mammals males have one X chromosome and one Y chromosome (XY) and females have two X chromosomes (XX). The paired chromosomes that are not involved in sex determination are called **autosomes**, to distinguish them from the sex chromosomes. Human beings have 46 chromosomes: 22 pairs of autosomes and one pair of sex chromosomes (X and Y). A cell cycle is a series of events that takes place in a cell as it grows and divides. A cell spends most of its time in what is called interphase (G_1, S, and G_2 stages of interphase), and during this time it grows, replicates its chromosomes, and prepares for cell division. The cell then leaves interphase, undergoes mitosis, and completes its division. The resulting cells, known as daughter cells, each enter their own interphase and begin a new round of the cell cycle. Cell cycle has different stages called G_1, S, G_2, and M. G_1 is the stage where the cell is preparing to divide. To do this, it then moves into the S phase where the cell copies all the DNA. So, S stands for DNA synthesis. After the DNA is copied and there's a complete extra set of all the genetic material, the cell moves into the G_2 stage, where it organizes and condenses the genetic material, or starts to condense the genetic material, and prepares to divide. The next stage is M. M stands for mitosis. This is where the cell actually partitions the two copies of the genetic material into the two daughter cells. After M phase completes, cytokinesis occurs resulting in two identical cells.

1. Fill in Table UIII-2 with the correct information.

Table UIII-2. Distinguishing characteristics of **mitotic** cellular division.

How many chromosomes does each human cell contain?_____	
DNA replication occurs during the _____ phase of _____.	
Characteristic	**Mitosis**
Location of the dividing cells?	
How many cellular divisions?	
Homologous chromosome bivalents are formed?	
Sister chromatids pair up?	
Crossing over occurs?	
Sister chromatids line up on the metaphase plate?	
Bivalents line up on either side of the metaphase plate?	
Sister chromatids separate?	
Number of daughter cells?	
Ploidy (haploid or diploid) level of daughter cells?	

☐ Working in your assigned team, match each image to the correct description sheet.

2. Turn your task sheets into you instructor as a team before you present your matching. Each group member must participate in the following:

   A. Describe what each phase is.

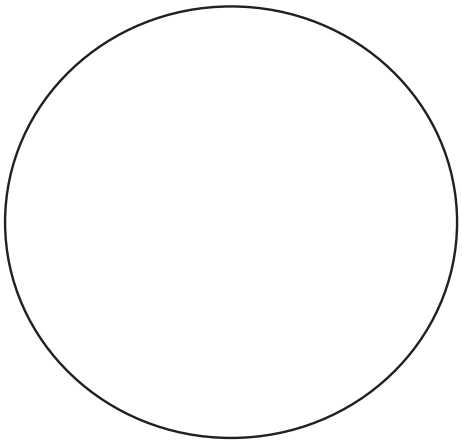
   B. Point out the structures listed on the image sheets.

   C. Instructor initials_____.

A. Onion Root Tips & Whitefish Cells

☐ View the onion and crayfish/white fish specimen slides using the 4X, 10X, then 40X objective lenses. NOTE: on the onion slide the **apical meristem** is near the tip of the root behind the root cap.

3. Sketch each at the **40X** objective and label the following:
   A. The stages of mitosis and look carefully for evidence of cytokinesis.
   B. Specimen, Species, Domain, and Kingdom names.
   C. Total magnification.



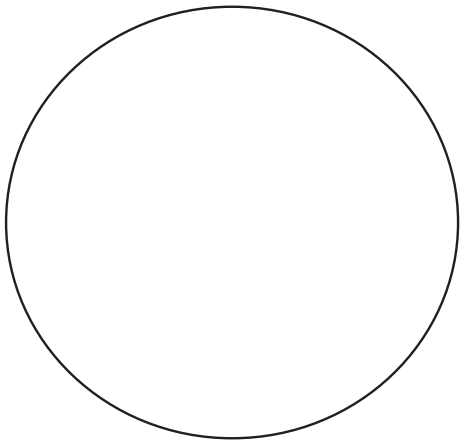
Specimen:_____

Species::_____

Domain:_____

Kingdom: _____

Total Magnification _____



Specimen:_____

Species::_____

Domain:_____

Kingdom: _____

Total Magnification _____

B. Duration of the Cell Cycle

The way in which biologists know the amount of time a cell spends in various stages of the cell cycle is by determining the frequency of cells at each stage in a cell population. You will determine the lengths of the phases of the onion root tip cell cycle, which is on average 16 hours (960 minutes) long.

☐ Obtain a prepared slide of and onion root tip.

☐ Select a field of view in the apical meristem region and focus using the 40X objective.

Table UIII-3. Number of cells from three different fields of view in each cell cycle phase.

	field 1	field 2	field 3	total
interphase				
prophase				
prometaphase				
metaphase				
anaphase				
telophase				

☐ **Count the number of cells in each phase of the cell cycle and enter these numbers into the correct column of Table UIII-3 and in Cell Cycle workbook on Canvas. Repeat for three non-overlapping fields of view. Save the workbook.**

C. Interpretation

4. Open a Word document and at the top right of the document type:
   - Biol 1101L Unit III - Cellular Reproduction (Cell Cycle)
   - Your section #
   - Your team #
   - Names of everyone on your team

5. Insert the graph into the document.
6. Create a figure caption BELOW the graph.
7. Type the answers to the following questions into the Word document:
   A. Why is mitosis important to both unicellular and multicellular eukaryotic organisms?
   B. Do chromosomes exist during interphase? Explain.
   C. Why are the activities of G1 important for a cell preparing to divide?
   D. The centrosome is the major microtubule-organizing centre in eukaryotic cells, being comprised of two centrioles surrounded by an electron-dense matrix. Why does it need to self-replicate during the cell cycle?
   E. Indicate if each statement is true of the G1, S, G2, or M phase of the cell cycle. A given statement may be true of one, several or none of the phases.
      - The amount of nuclear DNA in the cell doubles.
      - The nuclear envelope breaks into fragments.
      - Sister chromatids separate from each other.
      - Chromosomes are present as diffuse, extended chromatin.
      - This phase is part of interphase.
   F. Type this sentence with the missing words included: During the S phase a chromosome is duplicated to produce two sister _____ (identical copies formed by the DNA replication of a chromosome), each is called a _____ and they are connected at the _____. They are still in a loose less-condensed _____ form.

☐ **Send the document to your lab instructor using your ISU Google Account Gmail**

Part 5: Blood Cell Cycle

Stem have the potential to develop into many different types of cells in the body. They have asymmetric cell division that results in daughter cells with their own unique life course. One of the daughter cells has a *finite capacity* for mitosis and begins to differentiate, whereas the other daughter cell remains a stem cell with *unlimited proliferative* ability. Progenitor cells are early descendants of stem cells that can divide and differentiate to form one or more kinds of cells, but cannot divide indefinitely. Mammalian blood cells are produced in the bone marrow by multipotent stem cells called hematopoietic cells that can go through mitosis to produce more hematopoietic cells or progenitor cells that will differentiate into a variety of blood cells.

Both bone marrow and blood are considered connective tissue. The whole blood of humans (*Homo sapiens*) contain red blood cells, white blood cells, and platelets (~45% of volume) suspended in plasma (~55% of volume). **Red blood cells (RBCs, erythrocytes)** carry oxygen using a complex iron containing protein called **hemoglobin**. RBCs carry oxygen from the lungs to your body's tissue and also take carbon dioxide back to your lungs to be exhaled. Once mammalian hematopoietic cells (including those of humans) differentiate into RBCs the organelles disintegrate. This means that the RBCs do not have a nucleus or mitochondria, whereas all other vertebrates do have those organelles. Because of this, mature mammalian RBCs do not go through mitosis and cannot use the oxygen they transport. Instead, RBCs produce ATP through glycolysis and lactic acid fermentation. **White blood cells (WBCs, leukocytes)** are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. Leukocytes are found throughout the body, including the blood and lymphatic system. **Platelets** (a type of **thrombocyte**) are small, colorless cell fragments in the blood whose main function is to interact with clotting proteins to stop or prevent bleeding. **Plasma** is a fluid, composed of about 92% water, 7% vital proteins, clotting factors, and 1% mineral salts, sugars, fats, hormones and vitamins. **Serum** is the blood plasma not including the clotting factors.

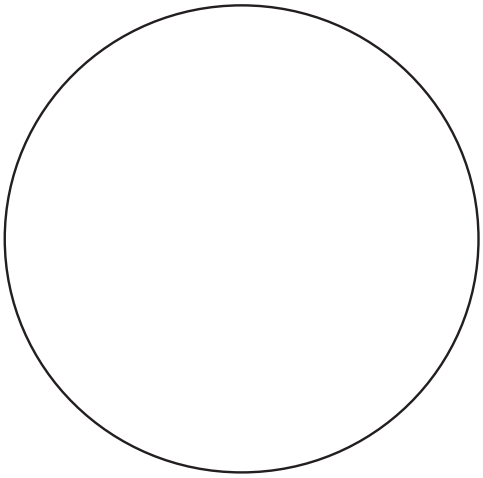
8. Observe the blood model:
   A. Sketch a representative of each of the seven types of blood cells depicted.
   B. Circle the ones that are leukocytes (white blood cells).
   C. Which ones produce B cells.

Erythrocyte (red blood cell) Lymphocyte Monocyte Neutrophil Eosinophil Basophil Thrombocyte

9. View each blood slide using the **40X** objective lens. Sketch each and label the following:
   A. RBC, WBC, RBC nucleus where possible.
   B. Specimen, Species, Class, Kingdom, and Domain names.
   C. Total magnification.

10. What class of tissue do both bone marrow and blood belong? _____

☐ Each group will be given a set of hematopoiesis cards to learn the order of blood cell division and maturation.



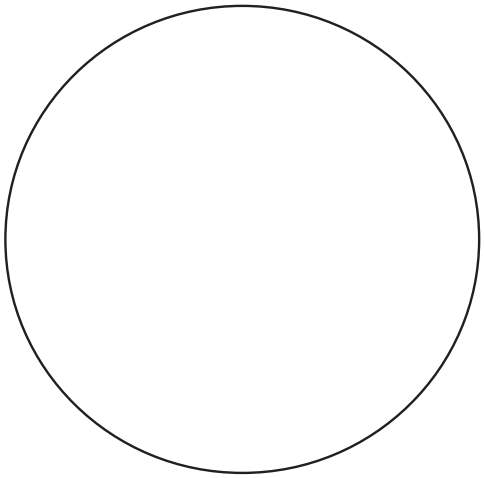
Specimen: _____

Species: _____

Class: _____

Kingdom:_____

Domain:_____ Total Magnification _____



Specimen: _____

Species: _____

Class: _____

Kingdom:_____

Domain:_____ Total Magnification _____

☐ Arrange your cards left to right with all the cards branching from the stem and progenitor cells.

11. Turn your task sheets into you instructor as a group before your arrangement. Each group member must participate in the following:
   A. Describe what each step is.
   B. Point out the structures listed on the image sheets.
   C. Instructor initials_____.

Unit IV - Genetics

Objectives

- Learn about meiosis and its connection to genetics.
- Learn about the Rh and ABO blood systems.
- Explain simple genetic dominance using Punnett squares.
- Use statistical null hypothesis testing for monohybrid and dihybrid crosses.
- Use the Hardy-Weinberg equations to calculate the Rh and ABO allele and genotype frequencies.

Terms & Definitions

Agglutinate - the process that occurs if an antigen is mixed with its corresponding antibody.

Cross:
A. Monohybrid - A cross that studies one pair of alleles at one genetic locus that is used to determine the dominance relationship between two alleles.
B. Dihybrid - A cross that studies two pairs of alleles at two genetic loci on two separate chromosomes that is used to determine the dominance relationship between the four alleles.

Chi-square test - a statistical hypothesis test where the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true.

Dendritic Cells - a type of antigen-presenting cell (APC) that form an important role in the adaptive immune system.

Frequency - the number, proportion, or percentage of items in a particular category in a set of data:
A. Allele - the number of copies of a particular allele in a population divided by the total number of alleles in that population; the relative proportion of all alleles that are of a designated type.
B. Genotype - the number of copies of a particular genotype in a population divided by the total number of individuals in that population; the proportion of individuals within a population that are of a specific genotype.

Gene - a unit of heredity that contributes to the characteristics or traits of an organism. At the molecular level, a gene is composed of organized sequences of DNA. Every person has two copies of each gene, one inherited from each parent:
A. Equilibrium - is a condition where a gene pool is not changing in frequency across generations.
B. Expression - gene function both at the level of traits and at the molecular level.
C. Flow - occurs when individuals migrate between different populations and results in changes in the genetic composition of the resulting populations.
D. Pool - all of the genes found in a population.
E. Sex-linked - refers to genes that are found on one sex chromosome but not the other.
F. X-linked - a gene found on the x chromosome but not on the y.
G. Y-linked - a gene found on the y chromosome but not on the x.

Genotype - the alleles or variants an individual carries for a particular gene:
A. Allele - one of two or more versions of DNA sequence (a single base or a segment of bases) at a given gene locus. An individual inherits two alleles, one from each parent, for any given gene where such variation exists. If the two alleles are the same, the individual is homozygous for that allele. If the alleles are different, the individual is heterozygous.
B. Heterozygous - two different alleles at the same gene.
C. Homozygous genotype - two identical alleles at the same gene.

Genetic drift - the random changes in a population's allele frequencies from one generation to the next that is attributed to chance. It occurs more quickly in small populations:
A. Bottleneck - an effect caused by adverse environmental conditions.
B. Founder - an effect caused by geographic separation of a subset of the population.

Dominant - a term that describes the displayed trait in a heterozygote:
A. Co - an allele from each parent is expressed in the offspring and the phenotypes of both parents are simultaneously expressed in the same offspring.
B. Complete - the effect of one allele in a heterozygous genotype completely masks the effect of the other. The allele that masks the other is said to be dominant to the latter, and the allele that is masked is said to be recessive to the former.

Meiosis - the process by which haploid (1n) cells are produced from a cell that was originally diploid (2n):
A. I - the separation of homologous chromosomes but the sister chromatids remain together resulting in two haploid cells (1n); occurs only in germ cells.
B. II - similar to a mitotic division but the connected sister chromatids remaining from meiosis I will separate to form four haploid cells.

Heritability - the amount of variation for a trait in the population that can be explained by differences in genes among individuals.

Phenotype - physical and physiological traits of an individual.

Population - a group of individuals of the same species that occupy the same environment and can interbreed with one another:
A. Biological - individuals of the same species that live and breed in the same geographic area.
B. Ecological - the study of how populations grow and what factors promote or limit growth.
C. Genetics - study of the factors in a population that determine allele frequencies and their change over time.

Polymorphism - the phenomenon that many traits or genes may display variation within a population.
A. Balanced - the phenomenon in which two or more alleles are kept in balance and maintained in a population over the course of many generations. The gene pools of most populations contain a number of deleterious alleles that reduce the overall fitness of a population.
B. Deleterious - a mutation that reduces the fitness of a variant.
C. Fitness - relative survival and reproduction of one variant compared to another in a population.

Principles of:
A. Independent assortment - states that the alleles of different genes assort independently of each other during gamete formation.
B. Segregation - separation of pairs of alleles during the production of gametes. Results in a 50% probability that a given gamete contains one allele rather that the other.
C. Uniformity - heterozygotes share a common phenotype due to dominance
D. Hardy-Weinberg - predicts an equilibrium if no new mutations are formed, no natural selection occurs, the population size is very large, the population does not migrate, and mating is random.

Punnett square - a common method for predicting the outcome of simple genetic crosses.

Recessive - applies to an allele with an effect that is not visible in a heterozygote.

Recombination - recombination occurs randomly in nature as a normal event of meiosis and is enhanced by the phenomenon of **crossing over**, in which gene sequences called linkage groups are disrupted, resulting in an exchange of segments between paired chromosomes that are undergoing separation.

Reproductive terms - the tissues and cells used in reproduction.
A. Egg cell - gamete produced by a female organism.
B. Embryo - the developmental stage commencing after the first mitotic divisions of the zygote and ending when body structures begin to appear.
C. Fertilization - the union of two gametes, such as an egg cell with a sperm cell, to form a zygote.
D. Gamete - a haploid cell that is involved with sexual reproduction, such as a sperm or egg cell.
E. Germ cells - any biological cell that gives rise to the gametes of an organism that reproduces sexually.
F. Gonad - a reproductive, sex, mixed gland that produces the gametes and sex hormones of an organism.
G. Oocytes - an immature ovum, or egg cell. An oocyte is produced in the ovary during female gametogenesis. The female germ cells produce a primordial germ cell (PGC), which then undergoes mitosis, forming oogonia. During oogenesis, the oogonia become primary oocytes.
H. Ovarian Follicles - a roughly spheroid cellular aggregation set found in the ovaries. It secretes hormones that influence stages of the menstrual cycle.
HI. Ovaries - an organ found in the female reproductive system that produces an ovum and secrete hormones that play a role in the menstrual cycle and fertility.
J. Ovum - the female reproductive cell, or gamete, egg
K.Sexual reproduction - a process that requires a fertilization event in which two gametes unite to produce a cell called a zygote.
L. I. Sperm cell - gamete produced by a male organism.
M. Seminiferous Tubules - located within the testes, and are the specific location of meiosis, and the subsequent creation of male gametes, namely spermatozoa.
N. Zygote - a diploid cell formed by the fusion of two haploid gametes.

Synapsis - the pairing of two chromosomes that occurs during meiosis. It allows matching-up of homologous pairs prior to their segregation, and possible chromosomal crossover between them. Synapsis takes place during prophase I of meiosis.

Variation - refers to the differences or deviations from the recognized norm or standard.
A. Continuous - a range of slightly different values for a trait in a population.
B. Genetic - differences in alleles that exist among individuals in a population.

White Blood Cells - also called leukocytes or leucocytes, are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All white blood cells are produced and derived from multipotent cells in the bone marrow known as hematopoietic stem cells. Leukocytes are found throughout the body, including the blood and lymphatic system.

Natural selection - the process that eliminates those individuals that are less likely to survive and reproduce in a particular environment, while allowing other individuals with traits that confer greater reproductive success to increase in numbers.
A. Balancing - a type of natural selection that maintains genetic diversity in a population.
B. Diversifying - natural selection for individuals at both ends of a range of phenotype but against the “average” phenotype.
C. Sexual - a type of natural selection that is directed at certain traits of sexually reproducing species that make it more likely for individuals to find or choose a mate and/or engage in successful mating.
D. Stabilizing selection - a pattern of natural selection that favors survival of individuals with intermediate phenotypes.

Spatial isolation - a mechanism for reproductive isolation that depends on the geographic separation of populations

Name:_____ Team #:_____ Section #:_____

Part 1: Patterns of Inheritance

The process of sexual reproduction involves two parents, each contributing one gamete. Gametes are produced by a process called meiosis, which starts by the duplication of the chromosomes, followed by two rounds of cell divisions and halving of the chromosome number. Gametes have half the chromosome number of other adult cells of an organism. Sexual reproduction has two processes that maximize diversity in a species. The different forms of a **gene** that are found at a specific point (or locus) along a given chromosome are known as alleles. Diploid organisms have two alleles for each autosomal gene - one inherited from the mother, one inherited from the father. One crucial process is that diploid cells give rise to unique haploid cells through genetic recombination between homologous chromosomes during meiosis. Exchange of genetic material between maternally and paternally derived chromosomes markedly increases the genetic diversity of the resultant haploid cells. One theoretical advantage of sexual reproduction is that the process of meiosis permits the random recombination of genetic material, thereby increasing the range of traits displayed by members of the species. This diversity increases the chances of success of the species in adapting to an ever-changing environment. A second process of sexual reproduction is that the haploid cells fuse during fertilization to form a new and unique diploid cell. The single-cell diploid zygote has all the genetic information necessary to grow and develop into an adult organism.

Organisms that reproduce sexually are thought to have an advantage over organisms that reproduce asexually, because novel combinations of genes are possible in each generation. Furthermore, with few exceptions, each individual in a population of sexually reproducing organisms has a distinct genetic composition. We have meiosis to thank for this variety. **Recombination** is the formation of new gene combinations in a gamete. It results from two events in meiosis, independent assortment and crossing over. **Independent assortment** occurs in meiosis I when each pair of homologous chromosomes lines up on the metaphase plate. The pairs of **homologous chromosomes** line up independently of other pairs and the paternal chromosome may be on the left or right. The number of possible combinations of maternal and paternal chromosomes in the nuclei produced by meiosis equals 2 raised to the power of *n*, where *n* is the number of pairs of chromosomes. For the 23 pairs of human chromosomes, this amounts to over 8 million combinations. **Crossing over** occurs when homologous chromosomes undergo **synapsis** during prophase I, equivalent sections may be exchanged between non-sister chromatids. This adds further variability among the **gametes** produced during meiosis.

A. Meiosis

There are two types of nuclear division: mitosis and meiosis. Meiosis reduces the chromosome number in daughter nuclei to half that of the parent nucleus. Gametes (germ cells) in animals and spores in plants are produced by meiotic division. The mother's mitochondrial DNA, together with twenty-three chromosomes from each parent, combine to form the genome of a zygote, the fertilized egg. As a result, with certain exceptions such as red blood cells, most human cells contain **23 pairs of chromosomes,** together with mitochondrial DNA inherited from the mother. Studies of relatedness in humans are based on the fact that a) mitochondrial DNA is inherited only from one's mother, and b) the male Y chromosome is inherited only from one's father. Although the names given to various phases of meiosis are similar to those of mitosis, there are obviously important differences in what occurs during the phases.

Stage I

In early prophase I, the sister chromatids of homologous chromosomes undergo synapsis, organizing themselves in a formation known as a **bivalent/tetrad**. This allows crossing over and the exchange of genetic material between segments of **homologous chromosomes** during late prophase I. In metaphase I, the tetrads migrate to the metaphase plate. In anaphase I, each homologous pair of sister chromatids is pulled to one **pole**. In telophase I, new nuclear membranes form around the daughter nuclei that consist of sister chromatids that are still attached by centromeres. Telophase I may or may not be followed by cytokinesis.

Stage II

Meiosis II begins with the formation of a spindle in prophase II. The sister chromatids, still attached with centromeres, move toward the metaphase plate. At metaphase II, the chromatids are lined up and attached to spindle fibers. Anaphase II begins when the centromeres separate and the sister chromatids, now considered chromosomes, be-

gin moving in opposite directions. During telophase II the nuclear membrane re-forms, the spindle disappears and cytokinesis divides the cytoplasm. The result is four haploid cells, none of which are alike because of the **genetic recombination.**

1. Fill in Table UIV-1 with the correct information.

Table UIV-1. Distinguishing characteristics of **meiotic** cellular division.

How many chromosomes does each human cell contain?_____		
DNA replication occurs during the _____ phase of _____.		
Characteristic	**Meiosis**	
	Stage I	**Stage II**
Location of the dividing cells?		
How many cellular divisions?		
Homologous chromosome bivalents are formed?		
Sister chromatids pair up?		
Crossing over occurs?		
Sister chromatids line up on the metaphase plate?		
Bivalents line up on either side of the metaphase plate?		
Sister chromatids separate?		
Number of daughter cells?		
Ploidy (haploid or diploid) level of daughter cells?		

B. Simple Inheritance

There may be a number of alleles for a given gene. Individuals that have two copies of the same allele are referred to as **homozygous** for that allele; individuals that have copies of different alleles are known as **heterozygous** for that allele. The inheritance patterns observed will depend on whether the allele is found on an autosomal chromosome or a sex chromosome, and on whether the allele is **dominant** or **recessive**. **Complete dominance** occurs when the phenotypes of heterozygous and dominant homozygous are indistinguishable. **Codominance** occurs when the phenotypes of both parents are simultaneously expressed in the same offspring. **Codominance** is the specific term for a system in which an allele from each homozygote parent combines in the offspring, and the offspring simultaneously demonstrates both phenotypes. An example of codominance occurs in the human ABO blood group system.

Observations of the way traits, or characteristics, are passed from one generation to the next in the form of identifiable phenotypic traits probably represents the oldest form of genetics. However, the scientific study of patterns of inheritance is conventionally said to have started with the work of the Austrian monk, Gregor Mendel, in the second half of the nineteenth century. Mendel described three principles of inheritance; **1) Uniformity**: heterozygotes share a common phenotype due to dominance, **2) Segregation**: pairs of gene variants (alleles) are separated into reproductive cells during meiosis producing gametes, and **3) Independent Assortment**: genes independently separate from one another when reproductive cells develop which means that the pairs of homologous chromosome are divided in half to form haploid cells and this assortment of homologous chromosomes is random.

Part 2: Human Blood Immunity & Genetics

Although all blood is made of the same basic elements, not all blood is alike. The International Society of Blood Transfusion recognizes 33 blood group systems that are described by the **presence or absence of certain antigens on the cell membranes of red blood cells (RBCs)**. The antigens that determine blood type can either be proteins, such as the D antigen, or carbohydrates; A and B antigens are polysaccharides. An **antibody** is a protein secreted by **plasma B cells (differentiated from B lymphocytes)** and is part of the immune response. The antibodies travel through the body to reach antigens identical to those that stimulated their production. Natural antibodies are antibodies produced in the absence of overt antigenic stimulation as in the ABO system. D antigen will illicit an immune response in people who are Rh- .

There are 33 blood group systems but we will focus on only two, ABO and Rh. In these two systems there are two independent genes that provide instructions for making specific blood antigens (Table UIV-2). The *ABO* gene is found on chromosome 9 and the *RHD* gene is found on chromosome 1 (Figure UIV-1). Both genes are inherited from your parents through a **Mendelian pattern of inheritance**. Rh and ABO alleles **segregate independently** of each other when gametes are formed during meiosis.

A. Blood Group Systems

The **Rh blood group system** is the second most clinically significant of the blood groups, second only to ABO. It has one genetic locus that exhibits two (2) alleles: D and d and two phenotypic traits: Rh+ and Rh-. The Rh blood groups are determined by the presence (+) or absence (-) of the D antigen, also known as the Rhesus (Rh) factor, on surface of red blood cells. There are two possible alleles for the Rh factor: a dominant allele (D) which encodes for the **D antigen protein** on red blood cells, and a recessive allele (d) which does not encode for the D antigen.

The **ABO blood group system** is the most clinically significant of the blood groups. It has one genetic locus that exhibits three (3) alleles: I^A, I^B, and i and four phenotypes (also known as blood groups or types): A, B, AB, and O. The four major blood groups are determined by the presence or absence of **two carbohydrate antigens (A and/or B)** on the surface of red blood cells. Not only does the ABO system have three alleles at one locus but I^A and I^B are dominant over i and **codominant** to each other.

Table UIV-2. The genotypes and phenotypes of the ABO and Rh blood group systems and the antigens that determine the systems.

Genotypes	Alleles	Phenotypic Trait (Blood Groups or Types)	Antigen on Red Blood Cell		Antibodies in Plasma	
			A	B	A	B
I^AI^A, I^Ai	I^A, i	A	yes	no	no	yes
I^BI^B, I^Bi	I^B, i	B	no	yes	yes	no
I^AI^B	I^A, I^B	AB	yes	yes	no	no
ii	i	O	no	no	yes	yes
Genotypes	**Alleles**	**Phenotypic Trait**	**D Antigen on Red Blood Cell**		No Natural Antibodies Present in Plasma	
DD, Dd	D, d	Rh +	yes			
dd	d	Rh -	no			

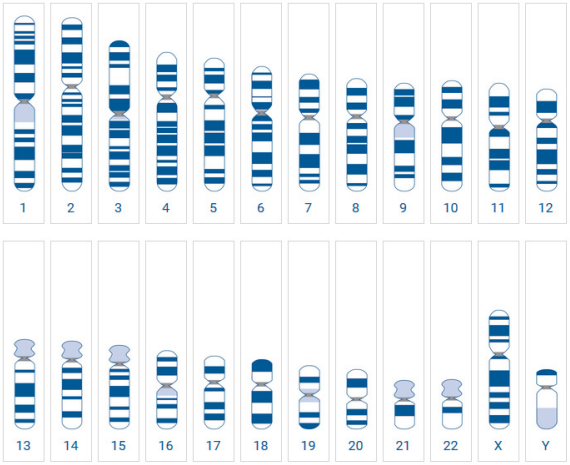


Figure UIV-1. Humans normally have 23 pairs of chromosomes, for a total of 46. Twenty-two of these pairs, called autosomes, and look the same in both males and females. The 23rd pair, the sex chromosomes, differ between males and females. Females have two copies of the X chromosome, while males have one X and one Y chromosome. *RHD* gene is found on Chromosome 1 and the *ABO* gene on Chromosome 9.

2. Go to: https://ghr.nlm.nih.gov/chromosome/1 and fill in the blanks of the following sentence: Chromosome ____ is the largest human chromosome, spanning about _____ million DNA building blocks (base pairs) and representing approximately_____% of the total DNA in cells.

3. Go to: https://ghr.nlm.nih.gov/chromosome/9 and fill in the blanks of the following sentence. Chromosome _____ is made up of about _____ million DNA building blocks (base pairs) and represents approximately _____% of the total DNA in cells.

4. Red blood cells have surface antigens on their plasma membranes. Which macromolecule type is the main component of surface antigens for the:

   A. ABO blood system?

   _____

   B. Rh (D antigen) blood system?

   _____

5. What is the main macromolecule component of an antibody?

   _____

6. Which kind of blood cell makes antibodies? _____

B. Your Blood Groups

☐ Obtain four sections of paper towel, one blood typing card, one safety medium-flow lancet, four blood mixing sticks, and a bandage.

☐ Set everything on three layers of paper towel. Reserve the other for your fingertip.

☐ Fill in the date and your name on the card.

☐ Place one stick above each circle on the card.

☐ Add a drop of water to each of the circles.

IMPORTANT: Do not cross-contaminate samples. When mixing; 1) use the respective blood mixing sticks, 2) keep the blood and water drop inside its circle, and 3) mix until the colored antisera is fully dissolved in the water/blood.

Table UIV-3. Reaction to antisera.

Dried antisera	Reaction Occurred? (Y/N)
Anti-A	
Anti-B	
Anti-D (Rh)	
Control	

☐ Use alcohol with a cotton ball to gently clean the fingertip you will use for testing.

☐ Remove the cap from the lancet. Place the lancet on the fingertip, and using firm pressure, press down until the lancet pierces the fingertip.

☐ Anti-A (antisera with anti-A anitbodies):
- Collect a droplet of blood on the concave surface of the Anti-A blood mixing stick.
- Swirl it on the circle labeled Anti-A(blue antisera).
- Place the stick above the Anti-A circle.

☐ Anti-B (antisera with anti-B anitbodies):
- Collect a droplet of blood on the concave surface of the Anti-B blood mixing stick.
- Swirl it on the circle labeled Anti-B (yellow antisera).
- Place the stick above the Anti-B circle.

☐ Anti-D (antisera with anti-D anitbodies):
- Collect a droplet of blood on the concave surface of the Anti-D blood mixing stick.
- Swirl it on the circle labeled Anti-D (pink antisera).
- Place the stick above the Anti-D circle.

☐ Control (no antibodies):
- Collect a droplet of blood on the concave surface of the Control blood mixing stick.
- Swirl it on the circle labeled Control (green antisera).
- Place the stick above the Control circle.

☐ Dispose of the lancet in the sharps container.

☐ Dispose of the paper towels, blood mixing sticks, and cotton ball in the biohazard container.

☐ Let your card air dry (you can skip ahead to Part 3 while you wait for it to dry) and then look at the agglutination under a stereo microscope.

☐ Carefully examine the thin film left behind and enter the reaction in Table UV-4:
- Film remains uniform in appearance, there is no agglutination.
- Film appears granular, agglutination has occurred. A positive agglutination reaction indicates the blood type

7. What was your phenotype for the ABO and Rh blood group systems? ABO _____ Rh _____

8. What are your possible genotypes? _____ _____ _____

9. Add your phenotype to the BloodType workbook on the instructor computer. Your ABO and Rh types need to be added to separate columns (D & E) but in the same subject row. What is your subject #_____.

☐ Place your card in the plastic bag, seal the bag, and take it home with you.

Part 3. Statistical Null Hypothesis Testing

For statisticians, a population contains all possible outcomes from a variable. Ideally we would measure the entire population. Usually, however, this is not possible. As a result, we sample some subset of the population, and draw inferences concerning the population from these data. One approach is to test whether or not the pattern indicated by the sample data coincides with a population that represents “no effect” or a “default effect”. This population is

often called a null distribution, and tests concerning this distribution are called null hypothesis tests. In a null hypothesis test we make one of two possible decisions; we either reject the null hypothesis (H_0) or we fail to reject the null hypothesis. There are four steps to null hypothesis testing:

Table UIV-4. Critical values for the chi-square test at three levels of alpha, 0.05, 0.01, and 0.001.

Degrees of Freedom (df)	Critical Values at alpha 0.05, 0.01, and 0.001		
	0.05	0.01	0.001
1	3.84	6.64	10.83
2	5.99	9.21	13.82
3	7.82	11.34	16.27
4	9.49	13.28	18.47
5	11.07	15.09	20.52
6	12.59	16.81	22.46
7	14.07	18.48	24.32
8	15.51	20.09	26.12
9	16.92	21.67	27.88
10	18.31	23.21	29.59

1) Define the null (H_0) and alternative (H_A) hypotheses as well as the **alpha (α).** H_A is the mathematical opposite of the H_0 For instance, if we defined the null hypothesis as: H_0: X = 0, then we would use the alternative hypothesis H_A: X ≠ 0. Alpha is a probabilistic standard for limiting a particular kind of mistake: rejecting H_0 when H_0 is true. Reflecting conventional practice, we will use a probability of 0.05 for alpha. This means that, given an infinite number of tests, we would only reject the null hypothesis 5% of the time when the null is actually true. We can assess the validity of H_0 by comparing a test statistic outcome (see below) to an entity called a critical value (see below). If the test statistic is greater than the critical value then we reject H_0, and conclude in favor of H_A. It should be emphasized that rejecting H_0 does not mean the null is false. It only means that the process we are making inference to is very poorly described by null . Conversely, failing to reject H_0 does not mean that the null is true, but we have insufficient data to reject it. In our work today, we will consider the following null and alternative hypotheses:

H_0 = 3:1 is the TRUE dominant to recessive phenotypic ratio for a monohybrid cross, and 9:3:3:1 is the TRUE ratio for a dihybrid cross.

H_A = 3:1 is NOT the TRUE dominant to recessive phenotypic ratio for a monohybrid cross, and 9:3:3:1 is NOT the TRUE ratio for a dihybrid cross.

2) Calculate a test statistic. We will use a chi-square test. This test is appropriate when data are counts with respect to categories. The chi-square test statistic is a relatively simple mathematical calculation that measures how well the observed experimental results (the observed count) correspond to the expected results under null. The formula for chi-square test statistic is:

$$X^2 = \sum_{i=1}^{c} \frac{(o_i - e_i)^2}{e_i}$$

where o_i and e_i are the observed and expected counts, respectively.

If the null hypothesis is true, then the test statistic, x^2, will be a random outcome from a chi-square distribution (Table UIV-4) with *c* - 1 degrees of freedom where *c* = the number of categories being considered.

3) Determine degrees of freedom and the critical value. Degrees of freedom (*df*) are the number of categories under consideration minus one. For the monohybrid example, there are two categories (Rh+ and Rh-). Thus, in this case, the degrees of freedom are 2 - 1 = 1. For the dihybrid example, there are four categories (A+ or B+, A- or B-, O+, O-). In this case, the degrees of freedom are 4 - 1 = 3. A critical value is the minimum value the test statistic must be in order to reject H_0 at a particular alpha.

4) Draw a conclusion. If the test statistic is greater than or equal to the critical value, then H_0 is rejected. If the test statistic is less than the critical value we fail to reject H_0. See Table UV-3.

A. Monohybrid Cross

In a monohybrid cross, **one pair of alleles is studied**. If both parents (P) are homozygous, with one parent homozygous dominant and the other parent homozygous recessive, the first generation (F_1) will be heterozygous exhibiting the dominant phenotype. As Mendel demonstrated, 75% of the second generation (F_2) offspring

will have the dominant phenotype and 25% the recessive phenotype (a 3:1 ratio). This prediction can be validated using the chi-square statistical test. In this exercise you will perform a chi-square test on the second-generation offspring of a monohybrid cross with 3:1 phenotypic ratio.

☐ Divide into twelve separate groups with **one-to-two people per group**.

☐ You will be given a deck of cards for a Rh F_1 monohybrid cross (Parental cross was DD X dd).

10. Determine what the phenotypic ratio is using the Punnett square (Figure UIV-2) for the Rh F_1 cross which will give rise to the F_2 generation.

Figure UIV-2. Punnett square F_1 cross resulting in the F_2 generation.

11. Count the number of Rh+ and Rh- cards in your deck.

12. Fill in Table UIV-5 with the card counts.

Table UIV-5. Number of F_2 offspring cards.

Phenotype	Count data
Rh+	
Rh-	
Total	

13. Calculate (show calculations in space provided) the expected numbers (e_1 and e_2) for the cards.

You expect 3/4 of your sample to be Rh+.
e_1 = your sample (total #) multiplied by 3/4 (or 0.75) = (Round off to the nearest whole number.)

You expect 1/4 of your sample to be Rh-.
e_2 = your sample (total #) multiplied by 1/4 (or 0.25) = (Round off to the nearest whole number.)

Note: subscripts refer to the categories under consideration

Table UIV-6. e_1, e_2, and X^2.

e_1	
e_2	
X^2	

14. Add the values to Table UIV-6.

15. You can now test the validity of the card data using the chi-square test. Once you have calculated (show calculations in space provided) your expected numbers (e_1 and e_2) for your card sample, put your numbers in the chi-square formula and perform the calculations. Calculate the chi-square number (x^2) all class cards. The formula for the chi-square calculation is:

$$X^2 = \frac{(o_1 - e_1)^2}{e_1} + \frac{(o_2 - e_2)^2}{e_2}$$

x^2 = the symbol for the chi-square test statistic

o = observed count (number) (o_1 = the Rh+, o_2 = Rh-)

e = the expected number under H_0. Both e_1 and e_2 must be calculated for your particular sample.

16. Add the values to Table UIV-6.

17. Did you reject or fail to reject the H_0 for your deck? What does this mean?

B. Dihybrid Cross

In a dihybrid cross, **two genes, each on a separate chromosome, with each gene having at least two alleles are studied**. If both parents (P) are homozygous, with one parent homozygous dominant for both traits and the other parent homozygous recessive for both traits, the first generation (F_1) will be heterozygous expressing the dominant phenotype for both traits. As Mendel demonstrated, there will be four different phenotypes in the second generation (F_2) yielding a 9:3:3:1 ratio (9/16 with both dominant traits, 3/16 with one dominant and one recessive trait, 3/16 with the alternative dominant and recessive trait and 1/16 with both recessive traits). This predictions can be validated using the chi-square statistical test.

In this exercise we will create a dihybrid cross of the Rh and ABO blood systems. However, since the ABO system has three alleles with codominance, **we will adjust the exercise to look at the I^A and I^B alleles separately.**

Figure UIV-3. Punnett square of the F_1 cross resulting in the F_2 generation.

☐ You will be given a deck of cards for the F_1 Dihybrid cross of A^+ or B^+ (parental cross was I^AI^ADD X *ii*dd **OR** I^BI^BDD X *ii*dd):

18. Were you assigned the A^+ (I^AiDd X I^AiDd) or B^+ (I^BiDd X I^BiDd) phenotypic trait? _____
19. Using your textbook to help you, construct a Punnett square of the F_1 dihybrid cross (Figure UIV-3) for this phenotypic trait.
20. Fill in Table UIV-7 with the card counts.
21. Calculate the expected numbers (e_1, e_2, e_3, e_4) for all cards (show calculations in space provided); To calculate the expected numbers (e_1, e_2, e_3, e_4) for your 9:3:3:1 dihybrid cross:
    a. You expect **9/16 of your sample (total #) to be A^+ or B^+**
       e_1 = our sample (total #) multiplied by 9/16 (or 0.5625)
    b. You expect **3/16 of your sample (total #) to be A^- or B^-** .
       e_2= your sample (total #) multiplied by 3/16 (or 0.1875)
    c. You expect **3/16 of your sample (total #) to be O^+.**
       e_3 = our sample (total #) multiplied by 3/16 (or 0.1875)
    d. You expect **1/16 of your sample (total #) to be O^-.**
       e_4 = our sample (total #) multiplied by 1/16 (or 0.0625)
22. Add the values to Table UIV-8.
23. You can now test the validity of your data using the chi-square test. Calculate chi-square number (x^2) for all cards (show calculations in space provided). The formula for the chi-square calculation is:

$$X^2 = \frac{(o_1 - e_1)^2}{e_1} + \frac{(o_2 - e_2)^2}{e_2} + \frac{(o_3 - e_3)^2}{e_3} + \frac{(o_4 - e_4)^2}{e_4}$$

    x^2 = the symbol for the chi-square test statistic
    o = observed count (number) (o_1 = **A^+ or B^+**, o_2 = **A^- or B^-**, o_3 = **O^+**, and o_4 = **O**)
    e = the expected number under H_0. All e_1, e_2, e_3, and e_4 must be calculated for your particular sample.
24. Add the values to Table UIV-8.

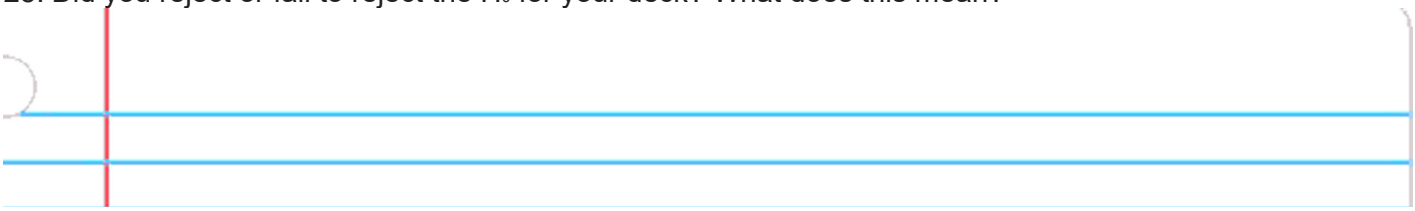
Table UIV-7. Number of F_2 offspring cards.

Phenotype	Count data
_____$^+$	
_____$^-$	
_____$^+$	
_____$^-$	
Total	

Table UIV-8. e_1, e_2, e_3, e_4 and X^2 .

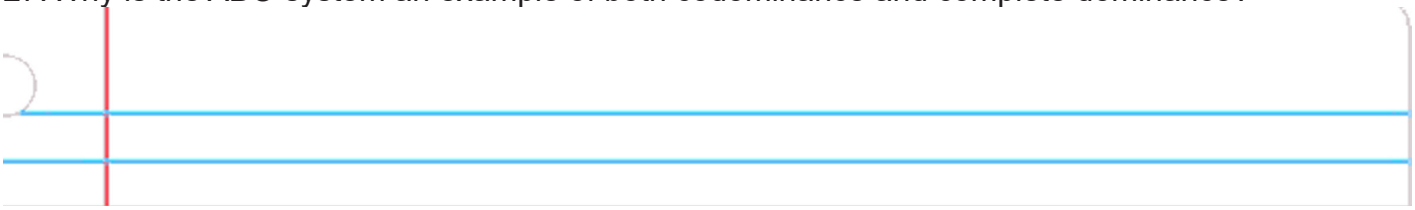
e_1	
e_2	
e_3	
e_4	
X^2	

25. Did you reject or fail to reject the H_0 for your deck? What does this mean?



26. Calculations REQUIRED:

27. Why is the ABO system an example of both codominance and complete dominance?



28. A woman with blood type AB+ and a man with type O- decide to have a child. What are the possible genotypes of the child? **Construct two Punnett Squares to show the genotypes.**

Name:_____ Team #:_____ Section #:_____

Part 4: Population Genetics

Population genetics is the study of genetic variation within populations, and involves the examination and modeling of changes in the frequencies of genes and alleles in populations over space and time. A **population** is a group of organisms of the same species within a specific geographical location. Many of the genes found within a population will be polymorphic - that is, they will occur in a number of different forms (or alleles). How frequently a trait is observed in a population is not related to whether or not it is dominant or recessive. Instead, it is a reflection of how frequently the gene responsible for causing a trait is found. **Allele frequency** is the relative proportion of all alleles of a gene that are of a designated type and **genotype frequency** is the proportion of individuals within a population that are of a prescribed genotype.

Mathematical models are used to investigate and predict the occurrence of specific alleles or combinations of alleles in populations, based on developments in the molecular understanding of genetics, Mendel's laws of inheritance, and modern evolutionary theory. The focus is the population or the species - not the individual. The collection of all the alleles of all of the genes found within a freely interbreeding population is known as the **gene pool** of the population. Each member of the population receives its alleles from other members of the gene pool (its parents) and passes them on to other members of the gene pool (its offspring). Population genetics is the study of the variation in alleles and genotypes within the gene pool, and how this variation changes from one generation to the next.

In natural populations, however, the genetic composition of a population's gene pool may change over time. Mutation is the primary source of new alleles in a gene pool, but the other factors act to increase or decrease the occurrence of alleles. **Genetic drift** occurs as the result of random fluctuations in the transfer of alleles from one generation to the next, especially in small populations formed, say, as the result adverse environmental conditions (the **bottleneck effect**) or the geographical separation of a subset of the population (the **founder effect**). The result of genetic drift tends to be a reduction in the variation within the population, and an increase in the divergence between populations. If two populations of a given species become genetically distinct enough that they can no longer interbreed, they are regarded as new species (a process called **speciation**).

In many cases, the effects of natural selection on a given allele are directional. The allele either confers a selective advantage, and spreads throughout the gene pool, or it confers a selective disadvantage, and disappears from it. In other cases selection acts to preserve multiple alleles within the gene pool and a balanced equilibrium is observed. This situation, labeled balanced polymorphism, can arise because of a selective advantage for individuals heterozygous for a given allele. As a result of balanced polymorphism, the gene pools of most populations contain a number of deleterious alleles that reduce the overall fitness of the population (known as the **genetic load**).

A. Hardy-Weinberg Equations

The Hardy-Weinberg equation describes and predicts a balanced equilibrium in the frequencies of alleles and genotypes. It allows us to calculate the allele and genotype frequencies of Mendelian traits in a population. As we've already learned, Mendelian traits are controlled by one gene with two alternative alleles, one of which is dominant and one of which is recessive. The Hardy-Weinberg principle can be used to predict allele and genotype frequencies in populations that meet the following assumptions:

- ✓ The population is large enough that random statistical events do not affect genotypic frequencies (i.e., there is no genetic drift).
- ✓ Mutations do not occur.
- ✓ Migration into the population (immigration) or out of the population (emigration) does not occur.
- ✓ Natural selection does not occur.
- ✓ Mating is entirely random.

The Hardy-Weinberg equations use the letters *p* and *q* to represent alternative alleles for a particular gene. The letter ***p* represents the frequency of the dominant allele**, and ***q* represents the frequency of the recessive allele**. Because all diploid individuals possess two alleles, the sum of *p* plus *q* equals one, shown in Equation 1:

Equation 1: $p + q = 1$

The second Hardy-Weinberg equation can be used to calculate the frequencies of genotypes in a population that result from combinations of two alternative alleles, shown in Equation 2:

Equation 2: $p^2 + 2pq + q^2 = 1$

In this equation, ***p*² represents the frequency of homozygous dominant genotypes**, **2*pq* represents the frequency of heterozygous genotypes**, and ***q*² represents the frequency of homozygous recessive genotypes**. These are the three possible genotypic combinations with two alternative alleles.

These equations are useful because if the frequency of one of the alleles (*p* or *q*) within a population is known, or the frequency of one of the homozygous genotypes (either p^2 or q^2) within a population is known, the frequencies of the other allele and other genotype can be calculated.

For example, assume that coat color in cats is controlled by one gene locus with two possible alleles (B and b). If a population of cats (*n* = 1000) has 910 individuals with the dominant coat color (B_), and 90 individuals with the recessive coat color (bb), the frequency of the homozygous dominant genotype is 0.49, the heterozygous genotype is 0.42, and the homozygous genotype is 0.09. Calculate as follows:

Step A1 - determine q^2 of Equation 2 (90 cats have the recessive coat color, bb, out of a total 1000 cats:

$$p^2 + 2pq + 90/1000 = 1$$
$$p^2 + 2pq + 0.09 = 1$$

Step A2 - solve for *p* of Equation 1 by first taking the square root of q^2 from Equation 2 (Step 1):

$$p + \sqrt{0.09} = 1$$
$$p + 0.3 = 1$$
$$0.7 + 0.3 = 1$$

Step A3 - fill in Equation 2 with the *p* and *q* calculated from Equation 1 (Step 2):

$$0.7^2 + 2(0.7)(0.3) + 0.3^2 = 1$$
$$0.49 + 0.42 + 0.09 = 1$$

B. Using Hardy-Weinberg Equations with THREE Alleles

Hardy-Weinberg equations can also be used to understand frequencies in a population where three alleles exist for a single locus. In this case, ***p* represents the frequency of the dominant allele 1**, ***q* represents the frequency of dominant allele 2**, and ***r* represents the frequency of the recessive allele**, shown in Equation 3:

Equation 3: $p + q + r = 1$

The second Hardy-Weinberg equation for three alleles can be used to calculate the frequencies of genotypes in a population that result from the combinations of the three alleles, shown in Equation 4:

Equation 4: $p^2 + 2pr + 2pq + q^2 + 2qr + r^2 = 1$

In this equation, ***p^2 and q^2* represent the frequency of homozygous dominant genotypes**, ***2pr, 2pq, and 2qr* represent the frequency of heterozygous genotypes**, and ***r^2* represents the frequency of the homozygous recessive genotypes**. These are the six possible genotypic combinations with three alternative alleles.

Like the previous equations if the frequency of one of the alleles (*p*, *q* or *r*) within a population is known, or the frequency of one of the homozygous genotypes (either p^2, q^2 or r^2) within a population is known, the frequencies of the other alleles and other genotypes can be calculated.

For example, shell color in a species of *Helix* snail is controlled by three alleles at a single locus: C^B (brown), $C^P C^B$ (brown and pink), C^P (pink) and C^Y (yellow). The brown and pink alleles are codominant and dominant to yellow; yellow is completely recessive. A population of snails (n=1000) has 463 brown individuals, 289 pink individuals, 182 brown/pink individuals, and 66 yellow individuals. You can calculate the allele and genotype frequencies as follows:

Step B1 - If the population size is *n*, then we also know that:

$n(p^2 + 2pr)$ = the number of brown snails in the population,
$n(q^2 + 2qr)$ = the number of pink snails in the population,
$n(2pq)$ = the number of brown/pink snails in the population, and
$n(r^2)$ = the number of yellow snails in the population.

Notice that we fit both homozygote and heterozygote frequencies into the brown and pink equations but the brown/pink and yellow equations are due to either heterozygotes or homozygotes.

Step B2 - solve for *r* of Equation 3 by first taking the square root of r^2 from Equation 4 which will give us the allele frequency for yellow in a snail population:

$$r^2 = 66/1000 = 0.066$$
$$p + q + \sqrt{0.066} = 1$$
$$p + q + 0.257 = 1$$

Step B3 - set up a quadratic equation for the number of brown snails.

$$n(p^2 + 2pr) = 463 = 1000\ (p^2 + 2pr)$$
$$p^2 + 2pr = 463/1000$$
$$p^2 + 2pr = 0.463$$
$$p^2 + 2p(0.257) = 0.463$$
$$p^2 + 0.514p = 0.463$$
$$p^2 + 0.514p - 0.463 = 0$$

Quadratic Equation: $ax^2 + bx + c = 0$

Step B4 - using the quadratic formula solve the quadratic equation for *p* determined in step B3 (p^2 + 0.514*p* - 0.463 = 0 where $a = 1$, $b = 0.514$, and $c = -0.463$:

Quadratic Formula: $$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

$$p = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} = \frac{-0.514 \pm \sqrt{(0.514)^2 - 4(1)(-0.463)}}{2(1)} = \frac{-0.514 \pm \sqrt{0.264 - (-1.85)}}{2}$$

$$p = \frac{-0.514 \pm \sqrt{2.114}}{2} = \frac{-0.514 \pm 1.454}{2} = -0.984, 0.47$$

There are two solutions for *p*. Since *p* cannot be negative, we know that $p = 0.47$.

Step B5 - solve for *q* using *p* and *r*:

$$p + q + r = 1$$
$$q = 1 - (p + r)$$
$$q = 1 - (0.47 + 0.257)$$
$$q = 1 - (0.727) = 0.273$$

Step B6 - determine the allele and genotype frequencies and population estimates (Table UV-8A and B) now that we have solved for:

$p = 0.47$
$q = 0.273$
$r = 0.257$

Table UIV-9. A) Allele and **B)** Genotype frequencies and population estimates for the color of *Helix* shells (*n*=1000).

A	Alleles	Dominance	Allele Frequencies
p	C^B	dominant	0.47
q	C^P	dominant	0.273
r	C^Y	recessive	0.257
		Total =	1

B	Genotype	Phenotypic Trait	Genotype Frequencies	Population Estimates
p^2	C^BC^B	brown homozygous dominant	$(0.47)^2$	221
$2pr$	C^BC^Y	brown heterozygous dominant	2(0.47)(0.257)	241
$2pq$	C^BC^P	brown/pink heterozygous codominant	2(0.47)(0.273)	257
q^2	C^PC^P	pink homozygous dominant	$(0.273)^2$	75
$2qr$	C^PC^Y	pink heterozygous dominant	2(0.273)(0.257)	140
r^2	C^YC^Y	yellow homozygous recessive	$(0.257)^2$	66
		Total =	1	1000

Part 5: Calculating Frequencies

A population of ISU students taking a certain course typed their blood. In lab today, we will use their results to predict the allele and genotype frequencies in the course population.

☐ Divide into 12 separate groups with **one to two people per group**.

☐ Your instructor will project the data.

1. Determine the total number of students that typed their blood; *n* =_____.
2. Fill in Table UIV-10 with the number of students expressing the different phenotypic traits.

Table UIV-10. ABO and Rh phenotypes expressed.

ABO Phenotype	Number of Students
A	
B	
AB	
O	
Rh Phenotype	**Number of Students**
Rh +	
Rh -	

A. Rh (D antigen) Frequencies

Remember the Rh Blood Group System has one genetic locus that exhibits two (2) alleles: D and d and two phenotypes: Rh+ and Rh-. The Rh blood groups are determined by the presence (+) or absence (-) of the D antigen on surface of red blood cells. There are two possible alleles for the Rh factor: a dominant allele (D) which encodes for the D antigen protein on red blood cells, and a recessive allele (d) which does not encode for the D antigen.

3. What are the possible genotype(s) for the dominant Rh trait:_____ _____
4. What are the possible genotype(s) for the recessive Rh trait:_____
5. Calculate the allele and genotype frequencies and the population estimates for D antigen (Rh factor) and show the calculations in the space provided.

6. Fill in Table UIV-11 with the frequencies and estimates calculated using the Hardy-Weinberg Equations 1 and 2.

Table UIV-11. A) Allele and **B)** Genotype frequencies and population estimates for D antigen (*n* =_____).

A	Alleles	Dominance	Allele Frequencies
p	D		
q	d		
		Total =	

B	Genotype	Phenotypic Trait	Genotype Frequencies	Population Estimates
p^2	DD			
$2pq$	Dd			
q^2	dd			
		Total =		

Calculations REQUIRED

Step A1:

Step A2:

Step A3:

B. ABO Frequencies

The ABO Blood Group System has one genetic locus that exhibits three (3) alleles: I^A, I^B, and i and four phenotypic traits: A, B, AB, and O. Because there are three alleles on one locus and the I^A and I^B are **codominant** over i, we will use the Hardy-Weinberg equations for three alleles.

7. What is the codominant phenotypic trait? _____ What is the possible genotype for this trait?_____

8. What is the recessive phenotypic trait? _____ What is the possible genotype for this trait?_____

9. What are the other possible phenotypic traits? _____ _____ What are the other possible genotypes for these traits?_____ _____ _____ _____

10. Calculate the allele and genotype frequencies and the population estimates for the A and B antigens and show the calculations in the space provided.

11. Fill in Table UIV-12 with the frequencies and estimates calculated using the Hardy-Weinberg Equations 3 and 4 along with the quadratic equation and formula.

Table UIV-12. A) Allele and **B)** Genotype frequencies and population estimates for the A and B antigens (n =_____).

A	Alleles	Dominance	Allele Frequencies
p	I^A		
q	I^B		
r	i		
		Total =	

B	Genotype	Phenotypic Trait	Genotype Frequencies	Population Estimates
p^2	I^AI^A			
$2pr$	I^Ai			
$2pq$	I^AI^B			
q^2	I^BI^B			
$2qr$	I^Bi			
r^2	ii			
		Total =		

Calculations REQUIRED:

Step B1:

Step B2:

Step B3:

Step B4:

Step B5:

Step B6:

Unit V - Evolution & Ecology

Objectives

- Learn about biological classification, taxonomy, and systematics.
- Distinguish between primitive and derived characters.
- Learn about the biological species concept.
- Use fossil evidence to construct a tree.
- Introduce evidence that links modern humans with ancient and modern primates.
- Define biological diversity and its importance.
- Use simple observations to estimate biodiversity.
- Compare levels of biodiversity.
- Learn about Ecological Footprints.

Terms & Definitions

Adaptation - the process and structures by which organisms adjust to changes in their environment.

Adaptive radiation - the process by which a single species evolves into a wide array of descendant species that differ greatly in their habitat, form, or behavior.

Ancestor - a plant, animal, or object that is related to one existing at a later point in time. Autotroph - an organism that has a metabolic pathways that use energy either from inorganic molecules or light to make organic molecules.

Archaea - one of the three domains of life that encompasses those one-celled organisms called archaeans.

Axes of anatomy - a hypothetical axis used to transect an anatomical entity in a straight line through space:
A. Anterior-posterior (AP) - extends longitudinally from head to tail.
B. Dorsal-ventral (DV) - ventral typically faces toward, and dorsal away, from a substrate (meaning towards the ground for land-dwelling organisms or towards the ocean or river/lake bottom for marine or aquatic organisms).
C. Left-right (LR) - to a plane running along the anterior-posterior midline.

Bacteria - one of the three domains of life that encompasses those one-celled organisms called bacteria.

Biological classification - is a system for comparing and grouping organisms, and the naming of those groups.

Biogeography - the study of geographic distribution of extinct and modern species.

Biological diversity (Biodiversity) - variety within and among living organisms.
A. Diversity - a measure of biological diversity that incorporates both the number of species in an area and the relative distribution of individuals among species.
B. Richness - the numbers of species in a community.

Dentition - the development of teeth and their arrangement in the mouth
A. Dental formula - the number of teeth of each type is written as a dental formula for one side of the mouth, or quadrant, with the upper and lower teeth shown on separate rows. The number of teeth in a mouth is twice that listed, as there are two sides.
B. Elodont - teeth that continuously grow
C. Incisors - slicing teeth
D. Canines - tearing teeth
E. Premolar - grinding teeth
F. Molars - grinding teeth

Evolution - the phenomenon that populations of organisms change from one generation to the next. As a result, some organisms become more successful at survival and reproduction.
A. Convergent - the process whereby two different species from different lineages show similar characteristics because they occupy similar environments.
B. Divergent evolution - the process whereby two different species from the same lineages show different characteristics because they occupy different environments.

Evolutionary trees - a model of evolutionary relationships among groups of organisms that is based on similarities and differences in their DNA, physical features, biochemical characteristics, or some combination of these. It maps the relationships between ancestral groups and their descendants, and it clusters the most closely related groups on neighboring branches.
A. LUCA - last universal common ancestor is the most recent population of organisms from which all organisms now living on earth are descended.
B. Most recent common ancestor - the most immediate ancestor that two lineages shares.
C. Node - the point in an evolutionary tree indicating the moment in time when an ancestral group split,or diverged, into two separate lineages. The node represents the most recent common ancestor of the two lineages in question.
D. Root - in an evolutionary tree a root represents the ancestral lineage, and the tips of the branches represent the descendants of that ancestor.

Gene - a unit of heredity that contributes to the characteristics or traits of an organism. At the molecular level, a gene is composed of organized sequences of DNA. Every person has two copies of each gene, one inherited from each parent.
A. Equilibrium - is a condition where a gene pool is not changing in frequency across generations.
B. Expression - gene function both at the level of traits and at the molecular level.
C. Flow - occurs when individuals migrate between different populations and results in changes in the genetic composition of the resulting populations.
D. Pool - all of the genes found in a population.
E. Sex-linked - refers to genes that are found on one sex chromosome but not the other.
F. X-linked - a gene found on the x chromosome but not on the y.
G. Y-linked - a gene found on the y chromosome but not on the x.

Genetic drift - the random changes in a population's allele frequencies from one generation to the next that is attributed to chance. It occurs more quickly in small populations.
A. Bottleneck - an effect caused by adverse environmental conditions.
B. Founder - an effect caused by geographic separation of a subset of the population.

Genotype - the alleles or variants an individual carries for a particular gene:
A. Allele - one of two or more versions of DNA sequence (a single base or a segment of bases) at a given gene locus. An individual inherits two alleles, one from each parent, for any given gene where such variation exists. If the two alleles are the same, the individual is homozygous for that allele. If the alleles are different, the individual is heterozygous.
B. Heterozygous - two different alleles at the same gene.
C. Homozygous genotype - two identical alleles at the same gene.

Life - a monophyletic group (refers to a group that consists of an ancestor and all of its descendants) that includes all known organisms. Characterized by a nucleic acid based genetic system (DNA or RNA), metabolism, and cellular structure. Some parasitic forms have secondarily lost some of these features and rely on the cellular environment of their host.

Lineage concept:
A. General – a species is an independently evolving lineage which is defined by morphology, reproductive isolation, DNA sequences, and ecology among other things
B. Evolutionary – a species is defined by its separate evolutionary lineage.
Species concept - different approaches for distinguishing species.
A. Biological - a species is a group of individuals that can interbreed and produce fertile offspring but typical cannot breed with members of another species.
B. Ecological - a species is defined by the ecological niche to which it belongs in its native environment and its influence on its environment and other species.
C. Morphological - definition of a species that relies on differences in physical characteristics among them.

Natural selection - the process that eliminates those individuals that are less likely to survive and reproduce in a particular environment, while allowing other individuals with traits that confer greater reproductive success to increase in numbers.
A. Balancing - a type of natural selection that maintains genetic diversity in a population.
B. Diversifying - natural selection for individuals at both ends of a range of phenotype but against the "average" phenotype.
C. Sexual - a type of natural selection that is directed at certain traits of sexually reproducing species that make it more likely for individuals to find or choose a mate and/or engage in successful mating.
D. Stabilizing selection - a pattern of natural selection that favors survival of individuals with intermediate phenotypes.

Phylogeny - evolutionary history of a group of organisms.
A. Monophyletic – a group of species, a taxon, consisting of the most recent common ancestor and all of its descendents.
B. Paraphyletic – a group of organisms that contains a common ancestor and some, but not all, of its descendants.

Population - a group of individuals of the same species that occupy the same environment and can interbreed with one another.
A. Biological - individuals of the same species that live and breed in the same geographic area.
B. Ecological - the study of how populations grow and what factors promote or limit growth.
C. Genetics - study of the factors in a population that determine allele frequencies and their change over time.

Speciation - formation of new species
A. Allopatric - evolution of a new species from biological populations that have become geographically isolated from each other to an extent that prevents or interferes with gene flow.
B. Sympatric - evolution of a new species from a surviving ancestral species while both continue to inhabit the same geographic region.

Systematics - the study of biological diversity and evolutionary relationships among organisms, both extinct and extant.

Taxon(a) - a group of species that are evolutionarily related to each other. In taxonomy, each species is placed into several taxons that from a hierarchy from large (domain) to small (genus)

Taxonomy - the field of biology that is concerned with the theory, practice, and rules of classifying extinct and extant organisms and viruses

Trait/character - a characteristic of an organism, such as the appearance of seeds, flowers, or stems; an identifiable characteristic; refers to a variant:
A. Adaptive - a genetic trait that helps an organism to maximize its reproductive success
B. Homologous - a feature that is similar across species because of common decent. Homologous traits may begin to look different form one another over time.
C. Quantitative - a trait that shows continuous variation over a range of phenotypes.
D. Shared derived - a trait that is shared by a group of organisms but not by a distant common ancestor.
E. Shared ancestral - a trait shared with a distant ancestor.

Background

Biological classification is a system for comparing and grouping organisms, and the naming of those groups. It encompasses both taxonomy and systematics relying heavily on many other fields of biology. How many species are there? This question is easier to ask than to answer, because we have described only a fraction of the earth's biota. Most large terrestrial organisms, such as birds and mammals, are well inventoried. However, at the other extreme are soil bacteria, often impossible to culture in standard media, and so poorly understood that there are probably a number of undescribed species thriving beneath the campus grounds. Over the last 250 years, biological classification has made it possible for the description, naming, and organization of 1.2 million species into three domains (Figure UV-1) with those species catalogued into a central database. However, it is estimated that there are another 7.5 species that have yet to be described. With the current rate of 6,200 eukaryote species described per year, describing Earth's remaining species could take as long as 1,200 years and would require 303,000 taxonomists at an approximated cost of $364 billion. With extinction rates now exceeding natural background rates by a factor of 100 to 1,000, species will become extinct before we know they even existed.

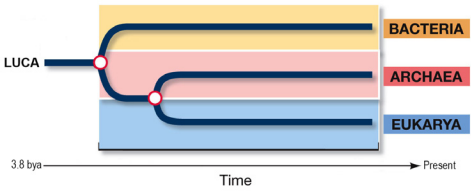


Figure UV-1. A phylogenetic tree of the three domains of life.

Name:_____ Team #:_____ Section #:_____

Part 1. Systematics

The study of the diversity of organism and of the relationships between them is the scientific field of **systematics**. Phylogenetic systematics provides methods for inferring evolutionary relationships. Relationships are inferred by distinguishing between traits that represent an **ancestral** condition for the organism in question and those that represent the **derived** condition. Organisms are grouped together on the basis of common ancestry; this classification represents patterns of evolutionary diversification and the **Tree of Life**. **Shared derived traits** among organism are evidence of common ancestry. A **phylogenetic tree** (Figure UV-2) is a graphical representation of the evolutionary relationship between taxa. Each **node** along a branch of the phylogenetic tree represents a population that lived at a particular point in time. The **root** is the original population. Nodes mark the population that split to produce two daughter populations or two separate species (known as **speciation**). The tips represent the populations that are currently living (extant).

The **last universal common ancestor** (LUCA) is the most recent population of organisms from which all organisms now living on Earth are descended. LUCA should not be assumed to be the first living organism on Earth but the most recent common ancestor of all current life on Earth. The LUCA is estimated to have lived some 3.5 to 3.8 billion years ago. The composition of the LUCA is not directly accessible as a fossil, but can be studied by comparing the genomes of its descendents, all organisms living today.

A. NOVA Evolution 101

- Before attending lab, go to Canvas and complete the PRE-LAB Assignment.
- Make sure to take a screen shot (PrtScr key) of the page showing all six completed missions. YOU MUST open the login tab (yellow icon) that shows your account name. If your account name is not shown in the uploaded screen shot, you will not receive any points. Upload the screen shot into the Canvas assignment and answer all the questions.

B. Tree Diagram Anatomy

☐ Using Figure UV-3, answer questions 1-9.

1. Draw and arrow that depicts the direction time is moving. Label the arrow with *past populations* and *present(extant) populations*.
2. Which arrows (x, y, or z) point to the most recent common ancestor of A and B? _____
3. Which arrow points to the most recent common ancestor of A and C? _____
4. Is B or C more closely related to the A?_____
5. Which node represents a speciation event? _____

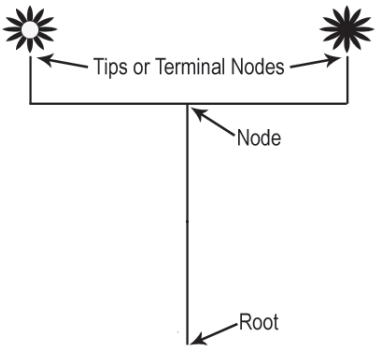


Figure UV-2. Phylogenetic tree diagram with two extant populations.

6. Which traits do A and B share? Which traits do B and C share?
   A & B_____
   B & C_____
7. Which lived most recently, the most recent common ancestor of A and B, or the most recent common ancestor of A and C?_____
8. Why are A and B more closely related to each other than B and C?
9. Which tree(s) below show the same relationship as Figure UV-3.

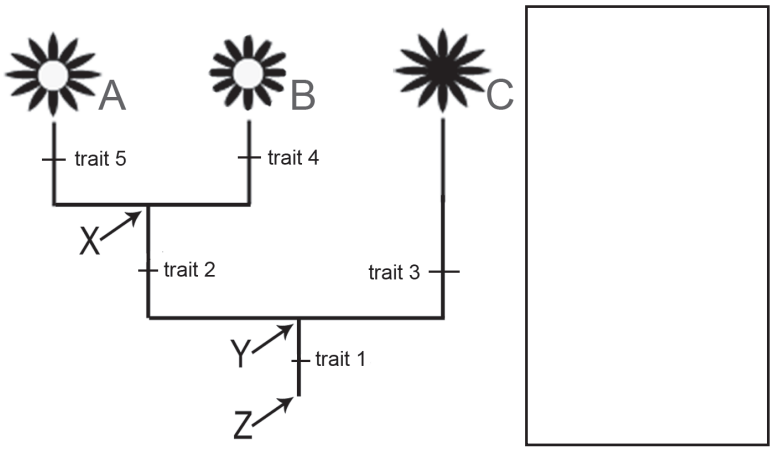
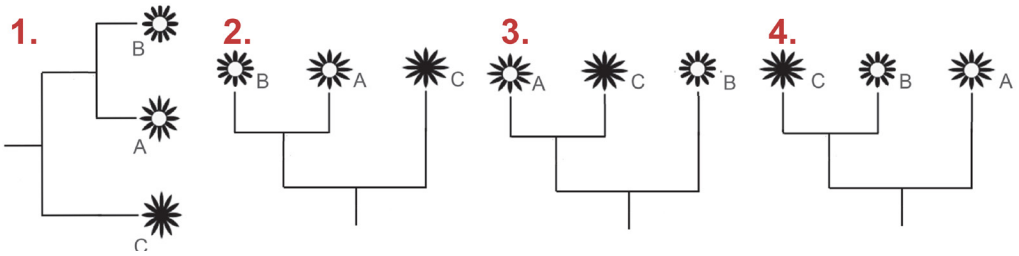


Figure UV-3. Phylogenetic tree diagram with three extant populations.



C. The Tree of Biol 1101L

Over the semester, we have observed or experimented with specimens from ten different genera. Using our knowledge of systematics, we are going to construct a tree that includes all ten species.

10. Write in the **species name** on the tip of the correct branch of the tree (Figure UVI-4). Use the traits to help you.

Table UV-1 . Specimens observed in Biol 1101L.

Species Name	Specimen	Unit
Homo sapiens	Human urine & blood	UI & UV - basic biochemistry & blood types
Scenedesmus sp.	Pond water	Lab 1 - microscopy
Solanum tuberosum	Potato tuber	UII - osmosis & diffusion
Saccharomyces cerevisiae	Baker's yeast	UIII - respiration & fermentation
Spinacia oleracea	Spinach leaves	UIII - photosynthesis & respiration
Coregonus clupeaformis	Whitefish blastodisc	UV - mitosis
Allium cepa	Onion root-tip	UV - mitosis
Lithobates catesbeianus	Frog blood	UV - blood genetics

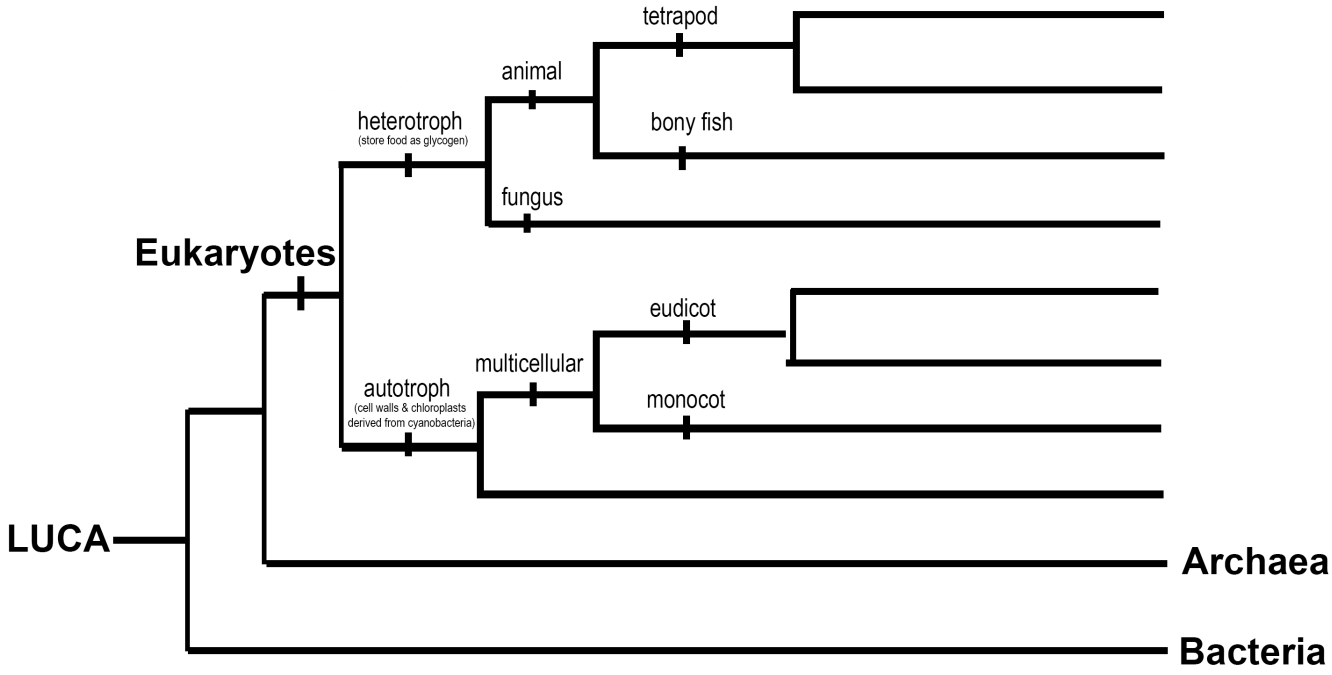


Figure UV-4. Tree created from the species observed in Biol 1101L.

Part 2. Taxonomy

The discipline of classification encompasses the field of **taxonomy** which is the classification, description, and naming of groups of organisms. Since the 18th century, biologists have subscribed to a standard protocol for the description, naming, and classification of organisms. In formal **b**iological classification, species are grouped according to estimates of their similarity or relatedness. Such groups are called taxa (singular, **taxon**). The taxa are listed in a hierarchical pattern. The most commonly used groups in the system of biological classification are shown in Table UV-2 (listed from the most inclusive to the most exclusive). In this system, the animal kingdom is divided into a number of phyla (singular, phylum). Each phylum is divided into classes, classes into orders, orders into families, families into genera (singular, genus) and genera into species. A classification developed for a taxon will be affected by the particular characters used, the relative weight given, and how they are analyzed. If different characters or weighting is used a different classification will arise. Animals have two types of names, common and scientific. Every animal taxon has a unique scientific name that is used throughout the world. Common names are less precise and can cause confusion because the name can be used for several different species. Also, the majority of species do not have a common name. The scientific name of a species is binomial and is always italicized. The name consists of two words the generic name and the specific name. For example the American Elk's scientific name is *Cervus canadensis*. A dichotomous key (Figure UV-2) is a device used to identify an organism through several steps. At each step (called a couplet) a choice must be made between two alternatives based on the presence of certain characters. Usually the characters are morphological (that is they are based on the form or shape of an organism). Each alternative will lead to another couplet or to the name of the identified organism.

Table UV-2. Example of a taxonomic classification.

Taxa		**Syringa**	**American Elk**
Domain		Eukarya	Eukarya
Kingdom		Plantae	Animalia
Phylum or Division		Magnoliophyta	Chordata
Class		Magnoliopsida	Mammalia
Order		Rosales	Artiodactyla
Family		Hydrangeaceae	Cervidae
Genus		*Philadelphus*	*Cervus*
Generic name + specific name	= *Species name*	*Philadelphus lewisii*	*Cervus canadensis*

11. Use the key in Figure UVI-5 to determine the phyla of unknowns A-D:
    A._____
    B._____
    C._____
    D._____

Figure UV-5. Dichotomous key to some of the animal phyla. Adapted from a key made available by the UCLA Marine Science Center.

1. Radial symmetry or asymmetry……2
   Bilateral symmetry……4

2. Highly porous surface, no true tissues, cellular level of organization.…**Phylum Porifera**
   Surface is not highly porous, true tissues present……..3

3. Exhibits pentaramous symmetry and tube feet……**Phylum Echinodermata**
   Lacks pentaramous symmetry and tube feet, possesses tentacles (with nematocysts)……**Phylum Cnidaria**

4. Macroscopic colony of sessile, microscopic individuals, individuals ‹ 0.5 mm in size……**Phylum Ectoprocta**
   Solitary or colonial in form, individuals of colony › 0.5 mm in size……5

5. Gelatinous……..6
   Not gelatinous……7

6. Solitary individuals with 8 rows of comb plates……**Phylum Ctenophora**
   Solitary and/or colonial with incurrent and excurrent siphons, and a gelatinous exterior called a tunic……**Phylum Chordata**

7. Possesses segmentation……..8
   Lacks segmentation……9

8. Exoskeleton with jointed appendages……**Phylum Arthropoda**
   No exoskeleton, appendages, if present, not jointed, segmented worm-like body, possibly in a tube (if in a tube, may have tentacles)……..**Phylum Annelida**

9. Possesses a foot, radula, arms and/or shell……**Phylum Mollusca**
   Lacking all of above, dorso-ventrally flattened to a thickness of less than 1 mm……..**Phylum Platyhelminthes**

Part 3: Human Evolution

Fossils are a vital source of information concerning the evolutionary relationships of both living and extinct organisms. This exercise is designed to give you experience looking at specific anatomical features and describing them in a way that allows you to look at the evolutionary relationships between the organisms. An organism's dentition (number and type of teeth) can provide us with important information about its life history and evolution. To find the dental formula look at the mandible (lower jaw), split the mandible in half length wise and count the number of incisors, canines, premolars and molars, report these numbers in a list, (i.e. 2, 1, 2, 3).Hominids seem remarkable for the sheer diversity of the fossil record. No other mammal has spread over as large a geographic and ecological range, and evolved so many new forms of behavior, within just a few millions years. The origins of this variability are behavioral as well as genetic. As human ancestors evolved, the accumulation of hominid technology and culture gave our biological variability an accelerating push. Before technology could have much impact, our evolution was helped along by the human tendency to migration and the resulting geographic isolation of different hominid groups. Separated in space, hominids evolved into regional variants that are sometimes treated as different species. Genetic variability within hominid species, and uncertainties in fossil reconstruction or geological dating, make these distinctions controversial. They are also somewhat beside the point: early humans were a restless species evolving at a rapid pace. As a further complication, fossils document the coexistence of different hominid species over the last two million years. Exactly how these different species coexisted or interacted is unclear. Hominid fossil remains are precious. Complete skeletons are extraordinarily rare before recent times. Teeth and lower jaws, and the facial and upper cranial bones of the skull, are the most common fossils to survive from any period and as such are particularly valuable as evidence for hominid evolution. Throughout time, no issue has interested humans more than learning about our origins. Where did we come from? What did our ancestors look like? Where did they live? Today, we are beginning to understand those questions, thanks to evidence provided by biologists and anthropologists. Nevertheless, the topic remains controversial.

☐ Your instructor will introduce you to a number of skull traits and their functions.

12. As these traits are described to you, fill in the Table UV-3 with the location and function, and whether they are present in modern humans, chimps, and gorillas.

Table UV-3. The location and function of primate skull traits; presence or absence in modern humans, chimps, and gorillas.

Structure	Location and Function	Modern Human	Chimp	Gorilla
Cranium				
Mandible				
Supraorbital torus				
Zygomatic Arches				
Temporal lines				
Sagittal crest				
Nuchal crest				
Dentition: Incisors Canines Premolars Molars				
Prognathism				
Foramen Magnum				

☐ View each skull and in your group discuss which traits are more derived and decide which you think were the most important with respect to the evolution of *Homo sapiens sapiens*.

13. Record in Table UV-4 column 2 'Trait'.

☐ Rank the skulls into an order that moves from most ancestral to the derived state of *Homo sapiens sapiens*.

14. Record the rank in Table UV-4 column 4 'Skull Number'.

☐ Using the ranking from Table UV-4 column 4 'Skull Number' and Figure UV-6, determine which tree branch tip (A-K) each species belongs.

15. Write the corresponding branch tip letter in Table UV-4 column 5 'C. Branch letter'.

☐ Your instructor will show you the completed tree of the current hypotheses for the evolutionary relationships of both extant and extinct primates.

16. Fill in the **scientific species or genus name AND numbers on Figure UV-6**.

Table UV-4. Rank of; **A)** Skull traits arranged from most important with respect to the evolution of *Homo sapiens sapiens* to least important, **B)** Species skulls arranged from the most ancestral state to the derived state of *Homo sapiens sapiens*, and **C)** your team's decision as to which branch tip each skull belong (letter).

A. Rank	Trait	**B.** Rank	Skull Number	**C.** Branch Letter
Most important		Most ancestral		
1		10		
2		9		
3		8		
4		7		
5		6		
6		5		
7		4		
8		3		
9		2		
10		1		
Least important		Most derived		

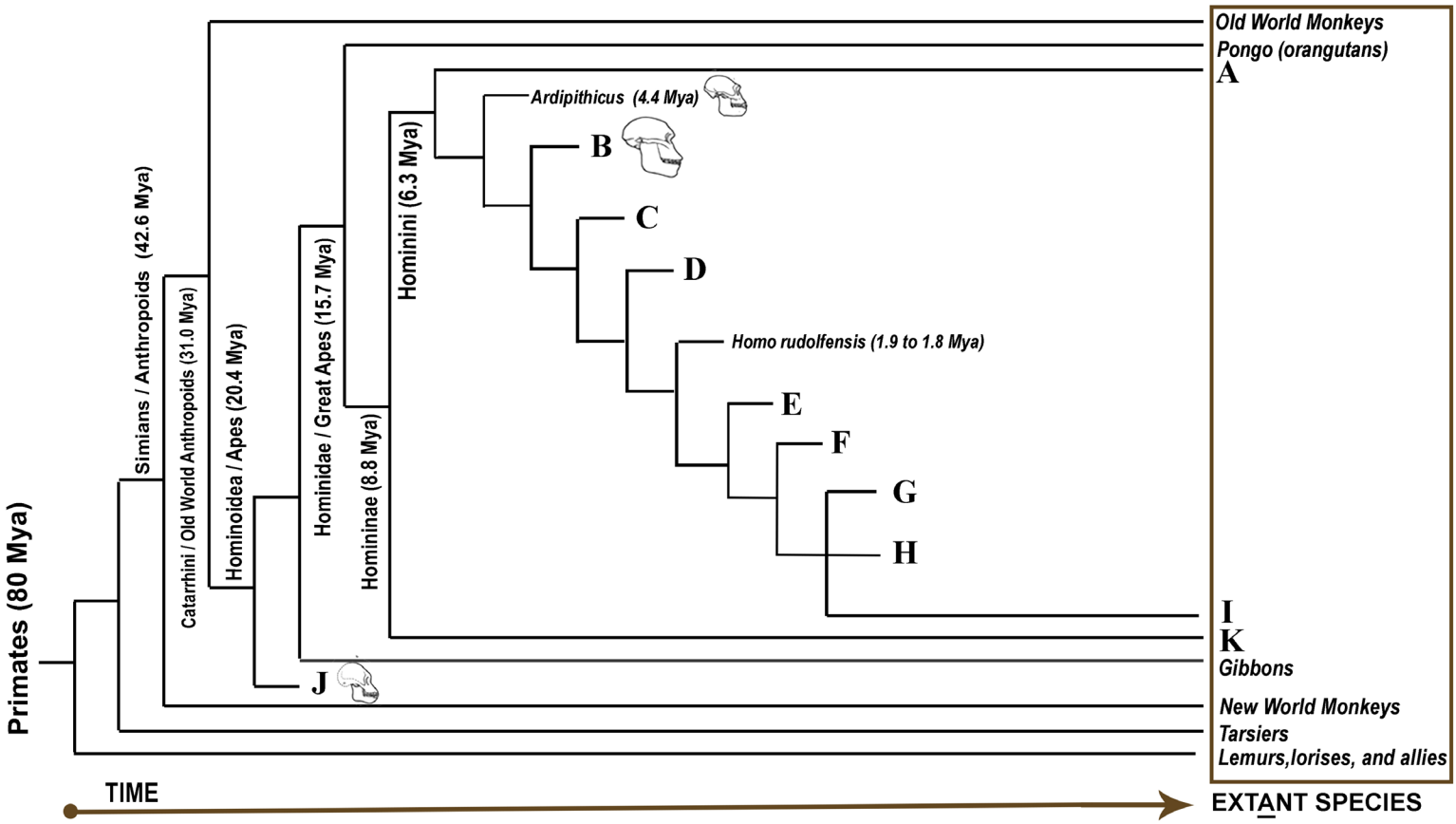


Figure UV-6. Current hypotheses for the evolutionary relationships of both extant and extinct primates

17. Based on the primate phylogeny which extant group is the:

A. Closest relative to modern humans?

B. Least related to modern humans?

C. How do you know?

18. Is it likely that the discovery of any new hominid fossil will result in solving the mystery of the “missing link” and be designated as the direct common ancestor of modern humans and other primates?

Name:_____ Team #:_____ Section #:_____

Part 4. Biodiversity

Biodiversity (biological diversity) is defined as the variety of life on Earth at all its levels, from genes to ecosystems, and can encompass the evolutionary, ecological, and cultural processes that sustain life and is important for utilitarian and intrinsic reasons. Utilitarian reasons include our basic human needs (food, fuel, shelter, medicine), crucial services (pollination, seed dispersal, climate regulation, water purification, nutrient cycling, control of agricultural pests, control of pathogens and disease), and cultural (spiritual or religious). Intrinsic reasons mean that the life of other organisms has inherent worth, which is independent of its value to anyone or anything else. They have inalienable rights to exist.

Biodiversity, which Darwin described as "endless forms most beautiful and most wonderful," remains an object of scientific curiosity and passion. A contemporary biology student, snorkeling over a coral reef for the first time is likely to share Darwin's wonderment at the diversity of life. Coral reefs are incredibly species-rich communities. Surrounded by hard and soft corals, echinoderms, annelids, molluscs, arthropods, and fishes of all shapes, sizes, patterns, and colors it is hard to believe that so many different species can coexist in the same place.

How many species are there? This question is easier to ask than to answer, because we have described only a fraction of the earth's biota. Most large terrestrial organisms, such as birds and mammals, are well inventoried. However, at the other extreme are soil bacteria, often impossible to culture in standard media, and so poorly understood that there are probably a number of undescribed species thriving beneath the campus grounds. In his book, The Diversity of Life, ecologist E. O. Wilson (1999) reports an estimate of 1.4 million described species. As for the numbers of living species not yet described, estimates range from 5 million to 30 million.

Why preserve biodiversity? Although extinction is a natural process, unnecessary loss of a species is drastic and irreversible. For example, many medicines such as quinine, morphine, digitalis, taxol, and codeine were discovered initially in natural populations. Unknown numbers of pharmaceutical products perhaps lie undiscovered in the habitats of earth. In addition, all of our domestic plants and animals are descended from wild ancestors. Today's wild growing relatives of domesticated species could prove useful in providing disease resistance. We continue to depend on natural populations of fish, shellfish, and trees for food, wood, rubber, and other products. In addition to contributing materially to human welfare, biodiversity contributes immeasurably to the quality of life on earth. Every time a species becomes extinct, the quality of our lives and that of our descendants also diminishes.

A. Experimental Design

You will compare the diversity of arthropod terrariums (Figure UV-7). To calculate diversity biologists use indices that are based on mathematical equations. For this lab you will use the diversity spreadsheet linked to Canvas to calculate the Shannon-Wiener diversity index (*H'*) which is calculated as -Σ(pi ln pi). This index is an indicator of the evenness and richness (i.e. number of genera/species and the abundance within each genera/species) within an environment. *H'* ranges upwards from 0. The 0 value indicates a single genera/species and increases as richness and evenness increases.

The niche is a set of environmental factors necessary to the continued existence of a species. Arthropods are a major component of all terrestrial ecosystems and their behavior has been the object of many famous ecological studies. All arthropod species are in the **Kingdom Animalia** and **Phylum Arthropoda** but they are in many different classes, orders, and families. A large proportion of arthropods are plant detritivores, i.e. organisms that feed on dead and decaying plant material. These organisms hasten the conversion of biomass to soil, speed up rates of nutrient cycling, and as a result, increase the productivity of ecosystems.

☐ Decide as a class four distinct niches that would be good to use. All groups of students should use the same niches.

☐ Divide into eight (Poc) or six (IF) separate groups with **two to three people per group**.

☐ Open Word document, answer questions 1 and 2, and then save the document.

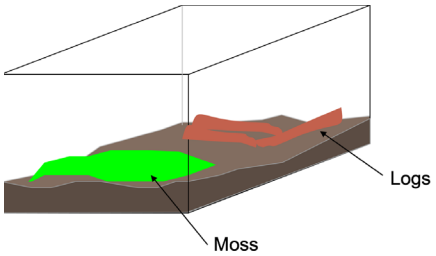


Figure UV-7. Diagram of an arthropod terrarium.

1. At the top right of the document type:
   - Biol 1101L
   - Unit V - Ecology
   - Your section #
   - Your team #
   - Names of everyone on your team
2. Type the answers to the following questions into the Word document.
   A. Background: Define the Shannon-Wiener Index (*H'*). Complete sentences required.
   B. Question?
   C. Determine your variables: Which is your response (dependent) variable? Which is your predictor (independent) variable? Are the variables categorical and/or quantitative?
   D. Describe the control and experimental groups for your experiment.
   E. Describe the type of relationship between the variables (positive, negative, or neutral).
   F. Develop a hypothesis.
   G. What is your prediction as to the outcome of your experiment.

B. Data Collection

☐ What is your assigned terrarium: _____

☐ Look at the niches that do not require you to disturb the terrarium. Do you see any of the arthropods (dead ones don't count).

☐ Look at all of the rest of the niches disturbing the terrarium as little as necessary. Be very gentle and careful (we don't want to harm any of the arthropods).

☐ In Table UV-6 record the number of arthropods you see in a each niche. AND to the Ecologys workbook on the instructor computer.

☐ Fill in Tables UV-7 and 8 with the class results.

Table UV-6. Abundance of arthropod types from terrarium #_____.

Arthropod	Niche			
	1	2	3	4
cricket				
isopod				
millipede				
bess beetle				
darkling/tenebrio beetle				
other 1				
other 2				
Total Abundance				

Table UV-7. Average abundance (n=____) of arthropod types from each niche type.

Arthropod	Niche			
	1	2	3	4
cricket				
isopod				
millipede				
bess beetle				
darkling/tenebrio beetle				
other 1				
other 2				
Total Average Abundance				

C. Interpretation

☐ Your instructor will send your lab section's completed workbook to each student's ISU Google Account Gmail.

☐ Open the Word document from Section A

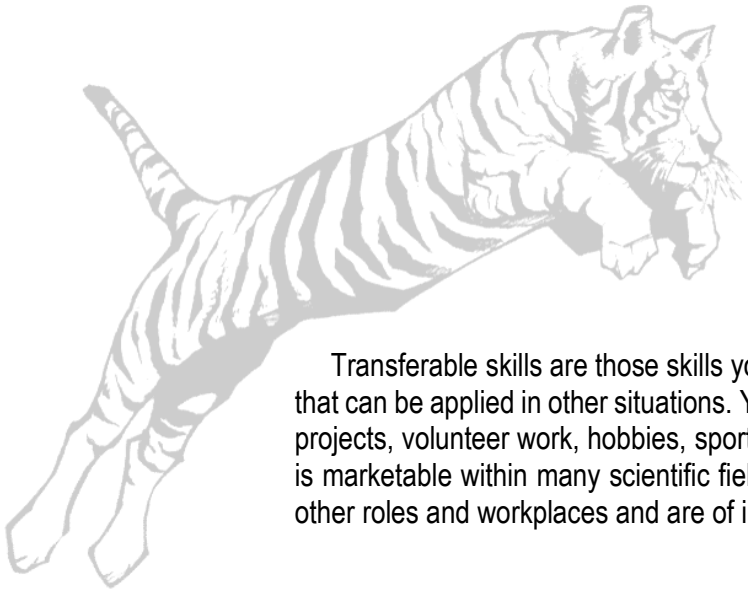
3. Insert the final table and graph from the Ecology workbook into the document.

Table UV-8 Shannon-Wiener Diversity Index (*H'*) for each niche.

Niche	*H'*
1	
2	
3	
4	

4. Create a table caption ABOVE the table.
5. Create a figure caption BELOW the figure.
6. Type the answers to the following questions into the Word document you inserted your tables and graphs into:
   H. Which niche was most diverse? Why do you think this is the case? Complete sentences required.
   I. What was your hypothesis and prediction?
   J. Did you reject or support your hypothesis? WHY? Complete sentences required.

☐ **Send the document to your lab instructor using your ISU Google Account Gmail**



TRANSFERABLE SKILLS

Transferable skills are those skills you acquire during any activity in your life - not just your studies - that can be applied in other situations. You can acquire skills through all sorts of activities: employment, projects, volunteer work, hobbies, sports, virtually anything. The knowledge you will develop in biology is marketable within many scientific fields. You will also gain skills that are transferable to a variety of other roles and workplaces and are of interest to a wide variety of employers.

Four types of skills that **all undergraduates** (regardless of major) are expected to develop:

- ✓ **Intellectual** – comprehension, critical reasoning, analytical, evaluation, planning and information-gathering, report writing.
- ✓ **Communication** – clarity of writing, layout and presentation of oral and written material, referencing, use of appendices, bibliographies, glossaries, indexes, and figures/tables.
- ✓ **Organizational** – prepare for exams, organize and complete assignments, time management, working under pressure.
- ✓ **Interpersonal** – negotiation, diplomacy, flexibility, adaptability, teamwork as well as independent work, delegation, and self-motivation.

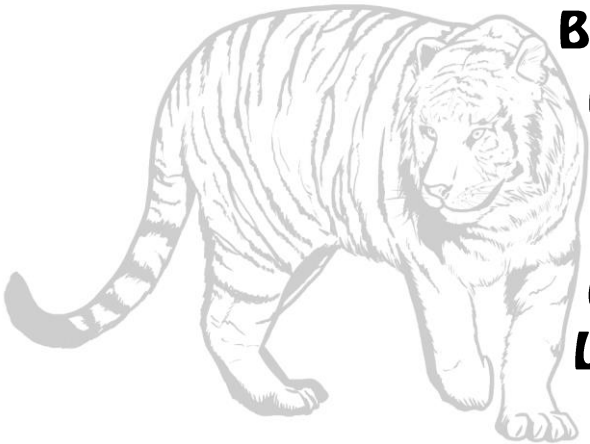
Four types of skills (in addition to those above) that **all biology students** are expected to develop:

Foundational knowledge – structure/anatomy, function, physiology, reproduction, growth/development, origin, ecology, evolution, and distribution of organisms.

Applied knowledge

- ✓ **Research** – use primary sources (read, understand, and cite scientific literature) to develop questions that are innovative, novel, and creative; use the scientific method to answer these questions by constructing hypotheses and predictions, design experiments to test the hypotheses and predictions; monitor, record, and manage data; statistical analysis of the data; and conduct a critical analysis of the results.
- ✓ **Numeracy** – mathematical ability is necessary in most fields, and it is important that all students maintain at least a rudimentary comprehension of numeracy.
- ✓ **Computer literacy** – typing speed and accuracy, text formatting, spreadsheet use, formal presentation construction, academic and professional use of search engines, email, and other types of software and web applications.

BENGAL SURVIVAL SKILLS



BE PREPARED AND RESPONSIBLE
EMBRACE POSITIVE CHOICES
NURTURE A POSITIVE ATTITUDE
GIVE RESPECT TO SELF AND OTHERS
ACT ON TIME AND ON TASK
LABOR FOR SUCCESS