Enzyme Activity Guided Inquiry Lab Turnip Peroxidase

Introduction

Peroxidase enzymes are widely distributed in plants and animals, including bacteria, to protect cells against the effects of oxidative stress and cell damage due to hydrogen peroxide. Peroxidases are easily extracted from turnips and other root vegetables and provide a model enzyme for studying enzyme activity—how the rate of an enzyme-catalyzed reaction depends on biotic and abiotic factors. Enzyme activity studies reflect enzyme structure and function and provide the foundation for understanding the mechanism or theory of enzyme action.

Background

The term *peroxidase* refers to both a class of oxidoreductase enzymes and to specific enzymes within that class. As a general class of enzymes, peroxidases catalyze the oxidation–reduction decomposition reaction of hydrogen peroxide. There are two general types of peroxidases—catalase and peroxidase. Catalase catalyzes the disproportionation reaction of hydrogen peroxide to water and oxygen gas (Equation 1). In reactions mediated by catalase, hydrogen peroxide substrate molecules act as both oxidizing agent (electron acceptor) and reducing agent (electron donor). In contrast, peroxidase acts in the presence of other naturally occurring organic reducing agents, such as ascorbic acid and glutathione, to catalyze the decomposition of hydrogen peroxide. Organic reducing agents, abbreviated AH_2, transfer hydrogen atoms and electrons to hydrogen peroxide, resulting in the formation of water and oxidized organic substrates such as A_2 in Equation 2. Oxygen gas is not produced in this latter reaction.

Catalase-catalyzed reaction $2H_2O_2 \rightarrow 2H_2O + O_2$ *Equation 1*

Peroxidase-catalyzed reaction $2H_2O_2 + 2AH_2 \rightarrow 4H_2O + A_2$ *Equation 2*

The differences in the two equations shown above provide a basis for studying the enzyme activity of turnip peroxidase in this guided-inquiry laboratory investigation. Many endogenous organic compounds may be used as reducing agents in Equation 2. One of the most common and convenient reducing agents for this purpose is guaiacol, a colorless compound having the formula $C_7H_8O_2$. Oxidation of guaiacol according to Equation 2 converts it to a dark orange compound called tetraguaiacol. The rate of the reaction may be followed by measuring the absorbance or color intensity of the orange product as a function of time.

Materials

Buffer capsules, pH 3–8, 100 mL each
Distilled or deionized water
Guaiacol solution, $C_7H_8O_2$, 0.2%, 1 mL
Hydrogen peroxide, H_20_2, 3%, 3 mL
Isopropyl alcohol, $(CH_3)_2CHOH$ 70%, 100 mL
Phosphate buffer, pH 7, 500 mL, NaH_2PO_4 and Na_2 HPO_4
Turnip (root/tuber)
Ice and water baths
Blender
Erlenmeyer flask, 500-mL
Filter paper and funnel
Hot plate
Knife, paring
pH paper, narrow range
Pipets, serological, 2- and 5-mL
Spectrophotometer
Test tubes, 13 × 100 mm, 6, and rack
Thermometer
Timer

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Safety Precautions

The guaiacol solution contains isopropyl rubbing alcohol (70%) and is a flammable liquid. Keep away from heat, flames, and other sources of ignition. Dilute hydrogen peroxide solution (3%) may be irritating to the eyes and skin. Exercise care when using a knife to peel and cut the turnip. Avoid contact of all chemicals with eyes and skin. Pure guaiacol is toxic by ingestion. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Preparation

1. Extraction Buffer: Prepare 500 mL pH 7 phosphate buffer by mixing equal volumes, 250 mL each, of 0.1 M sodium phosphate monobasic and sodium phosphate dibasic solutions.
2. Reaction Buffers: Dissolve one each pH 3–8 buffer capsules in 100 mL distilled or deionized water according to instructions.
3. Hydrogen Peroxide: Dilute 3 mL of 3% hydrogen peroxide to a final volume of 500 mL using distilled or deionized water. Store in a dark bottle protected from heat and light.

Enzyme Extraction

Peel and cut a turnip root into small cubes, about 1 cm on each side. Measure approximately 2 g (about 2 pieces) in a weighing dish and add to 300 mL of pH 7 phosphate extraction buffer in a blender. Pulse the turnip root in 1–3 minute bursts three times, with 2-minutes rest between pulses, to homogenize and extract the enzymes. Filter the *enzyme extract* through filter paper and store the extract over ice or in the refrigerator.

Baseline Activity—Peroxidase-Catalyzed Oxidation Decomposition of Hydrogen Peroxide

Read the entire procedure before beginning. Pay special attention to the requirements for mixing the tubes and timing the reaction. Accurate timing is crucial for rate studies.

1. Turn on the spectrophotomer, adjust the wavelength setting to 500 nm, and allow the instrument to warm up for 15–20 minutes.
2. Prepare separate 13 × 100 mm test tubes containing substrates (tube S) and enzyme (tube E) in pH 5 buffer as shown below. The presence of pH 7 extraction buffer in tube E makes it possible to vary the enzyme concentration while maintaining the overall buffer composition constant. The concentration of the diluted hydrogen peroxide is described in the *Preparation* section.

Tube S:	2 mL pH 5 buffer	*Tube E:*	2 mL pH 5 buffer
	2 mL dilute H_2O_2		1.5 mL pH 7 phosphate extraction buffer
	1 mL 0.2% guaiacol		0.5 mL enzyme extract

3. Prepare a "blank" by combining 4 mL pH 5 buffer, 2 mL dilute H_2O_2, 1 mL guaiacol solution, and 2 mL pH 7 phosphate buffer in a 13 × 100 mm test tube.
4. Zero the spectrophotometer (zero absorbance, 100% transmittance) at 500 nm using the blank solution.
5. When ready to begin a kinetics run, carefully pour the contents of tube S into tube E and ***immediately start timing.*** Carefully pour the combined contents back into tube S, wipe the outside of the tube with lab tissue, and place the test tube in the spectrophotometer cell holder.
6. Measure and record the absorbance as a function of time every 20 seconds. Ideally, the elapsed time between mixing the tubes and recording the first absorbance measurement should be no more than 20–40 seconds!
7. Graph absorbance versus time to obtain the reaction rate from the slope of the line.

Enzyme Activity—Opportunities for Inquiry

Vary the enzyme and substrate concentrations and investigate the effects of pH, temperature, and possible inhibitors on the rate of the peroxidase-catalyzed decomposition of hydrogen peroxide. Analyze and graph the results and explain in terms of the mechanism of enzyme action and structure–function relationships involving biological protein molecules. Precise volume transfers and accurate "time zero" measurements are essential for meaningful results and analysis.

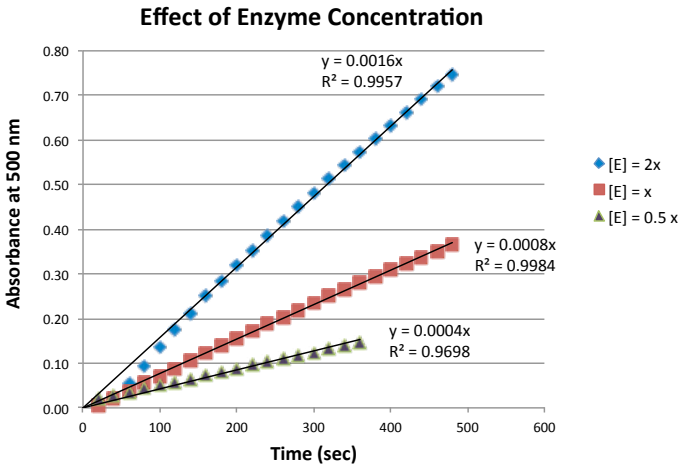
AP Biology Curriculum Framework (2012)

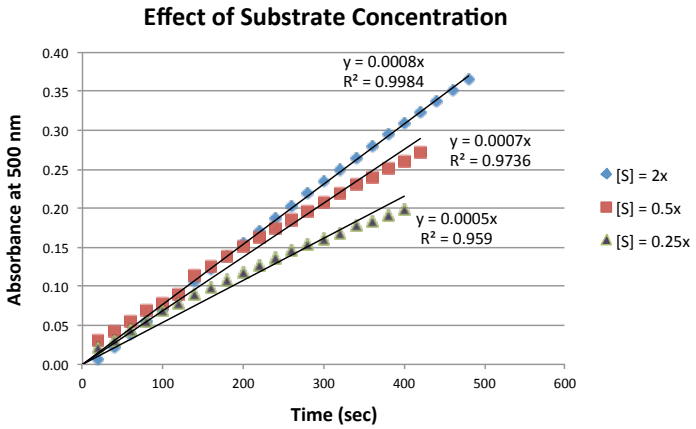
Essential Knowledge 2.D.1. All biological systems from cells and organisms to populations, communities and ecosystems are affected by complex biotic and abiotic interactions involving exchange of matter and free energy.

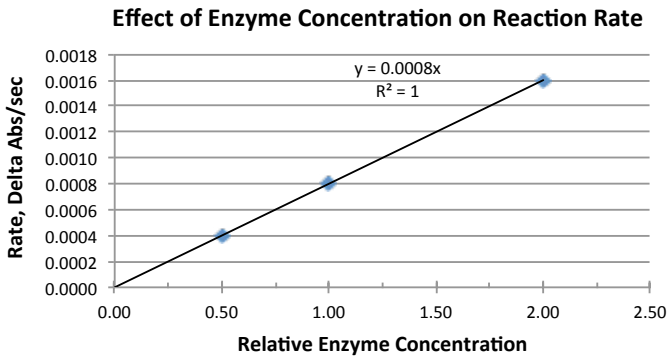
Essential Knowledge 4.A.1. The subcomponents of biological molecules and their sequence determine the properties of that molecule.

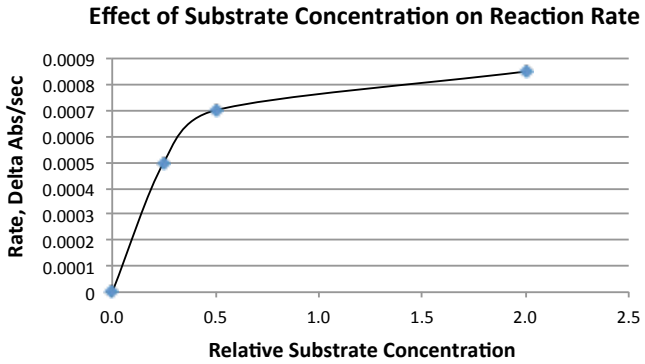
Essential Knowledge 4.B.1. Interactions between molecules affect their structure and function.

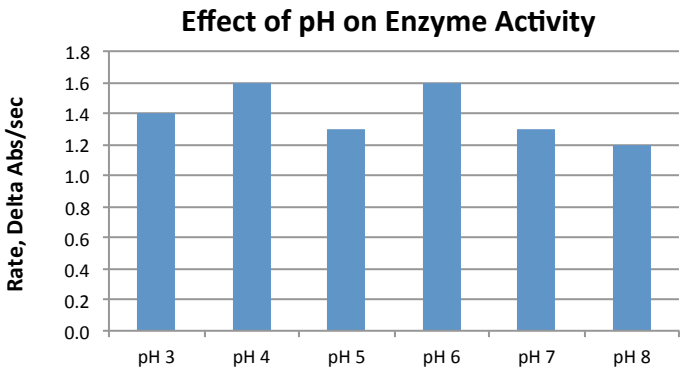
Sample Results

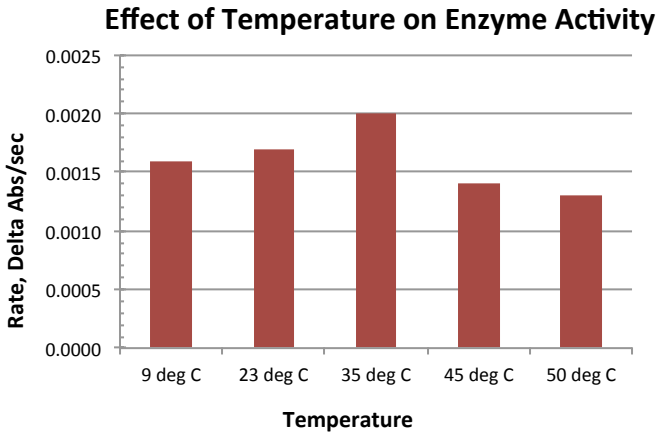












Materials for *Enzyme Activity Guided Inquiry Lab* are available from Flinn Scientific, Inc.

Catalog No.	Description
B0227	Buffer Capsules, pH 2–12
B0099	Buffer Solution Concentrated, pH 7, 500 mL
G0054	Guaiacol Solution, 0.2%, 100 mL
H0009	Hydrogen Peroxide, 3%, 473 mL
I0021	Isopropyl Alcohol, 70%, 500 mL
AP4869	Blender, Single-Speed
FB2039	Peroxidase Enzyme Activity—Advanced Inquiry Lab

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

Investigating Energy Flow in an Ecosystem

Introduction

Energy flows from one organism to another as food. Energy enters into a food web either as solar energy captured as part of photosynthesis or as chemical energy captured by chemosynthetic bacteria in specialized ecosystems. No matter the source, this energy is used to create complex energy rich macromolecules which are either used immediately to maintain homeostasis or are stored for later use. Consumers feed on organisms in order to acquire complex energy rich macromolecules for their own needs. This investigation demonstrates how ecologists determine the flow of energy along a simple food chain.

Concepts

- Community modeling
- Ecological pyramid
- Net primary productivity

Background

Food chains and food webs are pictorial representations of the flow of energy from one organism to another (see Figure 1). Most often these diagrams focus on a food chain based on the Sun's energy being captured by photosynthesis. A similar chain forms in some of the deepest areas on Earth where chemosynthetic bacteria capture energy from sulfur vents on the sea floor and other harsh environments. Since the Sun food chain is the most common, that is the one we will focus on in this investigation.

In order to determine the actual amount of solar energy captured by producers, scientists measure the dry mass of all life within that ecosystem. The mass is converted to energy using calories per gram, a known constant for each organism. Since ecosystems are complex, scientists harvest part of the ecosystem or use a simplified model system to make an estimate of the whole.

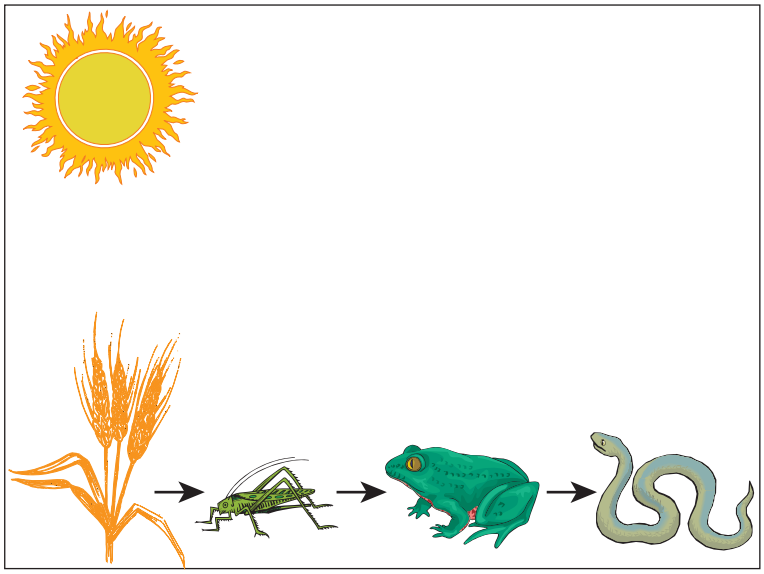


Figure 1. Food Chain

Plants use water, carbon dioxide, trace nutrients, and light to grow and carry out metabolic functions. Plants convert these raw materials into macromolecules, which have mass and store energy. *Gross primary productivity* is a measure of the total amount of energy converted by plants during photosynthesis and includes accounting for the energy in the waste products of photosynthesis and respiration. This is not easily measured because the waste products are oxygen and carbon dioxide. Scientists are generally interested in the amount of energy available to the next trophic level, or net primary productivity. The total mass of all the plants in an ecosystem at a given time is the biomass of the ecosystem. The added dry biomass that grows within a measured area over a specific amount of time is the *net primary productivity*. This is reported in grams per square meter per week, or as kilograms per square meter per year, depending on the type of ecosystem and nature of the study.

When plants grow from tiny seeds to large organisms, it may seem that they create mass from nothing. However, the law of conservation of mass states that mass cannot be created or destroyed, simply rearranged into different molecules. Where does the dry mass come from? Living things are carbon-based organisms; fats, carbohydrates, and proteins are primarily carbon, hydrogen, and oxygen. Therefore, the mass of the plant mainly comes from carbon dioxide and, to a lesser extent, water.

Primary consumers, those that eat plants, are not able to capture 100% of the plant's biomass for growth. They use most of the energy they acquire from plants just to maintain homeostasis. In addition, not all of the plant is digestible and a large fraction is lost as fecal waste, heat, and waste gases. Only a fraction of the energy acquired is used to make more cells

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(growth). By massing the animal over time, the *net secondary productivity* can be determined.

Biomass is typically reported as a *dry mass* because water content can vary greatly and does not contribute energy. Consider the differences in the mass of the prairie grass in a one square meter area of prairie in a drought year versus a rainy year. By drying the prairie grasses the mass of the macromolecules from one year to the next can be compared. Therefore, calculations must be done on dried plants and animals. The percent dry weight can be calculated by massing the plants when they are harvested then drying them in a controlled environment and reweighing the same plants.

In this activity, the plant used is wheat, which is a monocot cereal grain and an important food crop. The type used in this experiment is a hard red winter wheat. The grain seeds are harvested for bread and other foodstuffs. Young blades of wheat, called wheatgrass, can also be used as food. Wheatgrass has an overall dry weight energy value of about 4 kcal per gram. The wheat seed contains the endosperm, the embryo called the wheat germ, and the hard outer layers called the wheat bran. In this activity, wheat bran will be used as the food source for mealworms. Wheat bran has an overall dry weight energy value of about 4 kcal per gram.

Mealworms are the larval form of the *Tenebrio molitor* beetle. The mealworm is easily cultivated in wheat bran and water to survive. The larvae have an overall energy value of 6.5 kcal/g and are 36% dry weight. The pupae have an overall energy value of 6.4 kcal/g and are 35% dry weight. The adult beetles have an overall energy value of 5.8 kcal/g and are 34% dry weight.

Experimental Overview

The *Baseline Activity* explores the net productivity for wheat and one of its predators, the mealworm. Daily observations allow for the study of the life cycle of a metamorphic animal and the growth of a monocot plant. The results of this baseline activity provide a procedure and model for open-inquiry and student-designed experiments .

Materials

- Mealworm diet, 10–20 g
- Mealworms, 10–15
- Wheat seeds, approx. 200
- Aluminum foil
- Apples
- Camera (shared)
- Containers, 3
- Dissection needle or pin
- Fertilizer solution, 0.1% solution in aged tap water
- Heat-resistant gloves
- Laboratory oven (shared)
- Lid
- Marker
- Paper towels
- Planting trays
- Plastic wrap
- Ruler
- Spoon, plastic (reused)
- Weighing dish (reused)
- Vermiculite

Safety Precautions

No parts of this laboratory are considered hazardous. Do not handle living animals unnecessarily. Wash hands thoroughly with soap and water before leaving the laboratory. Please follow all laboratory safety guidelines.

Baseline Activity Setup

Part A. Wheat

1. Use a dissecting needle or a dissection pin to poke at least 30 holes into the bottom of two of the containers. Move the dissection needle or pin around when forming each hole to ensure each hole remains open.
2. Label the container as instructed so it can be identified later.
3. Measure the area of the container in square meters.
4. Fill the two containers about halfway with vermiculite.

5. Thoroughly wet the vermiculite with the dilute fertilizer solution.
6. In each container, distribute 100 wheat seeds evenly on top of the wet vermiculite.
7. Cover the seeds with a light layer of vermiculite.
8. Place a piece of plastic wrap over the top of the container while the wheat plants sprout.
9. Place the containers in the planting trays in the grow area. The grow lights should be raised as the plants grow so they are always about 6 inches above the wheat plants.

Part B. Mealworms

1. Use a dissection needle or a dissection pin to poke at least 30 holes into the lid of the container. Move the dissection needle or pin around when forming each hole to ensure each hole remains open for air exchange.
2. Measure the area of the container in square meters.
3. Mass the container and write this on the side of the container.
4. Mass 10–20 g of mealworm diet (wheat bran) in a weighing dish. Record the mass. Transfer to the empty container. Wipe out the weighing dish with a paper towel.
5. In the weighing dish, mass 10–15 mealworms; record the mass. Transfer to the container with the wheat bran. Keep the weighing dish for future measurements.
6. Record the combined mass of the container, wheat bran, and mealworms.
7. Label the container as instructed so it can be identified later.
8. Place the aerated lid onto the container and place the mealworm culture in a designated area.

Baseline Activity

1. Make daily observations and maintain the wheat and mealworms for 2–3 weeks.
   a. Plant maintenance:
      i. Water the wheat as necessary.
      ii. Add vermiculite as needed to the wheat containers. The vermiculite holds water but compresses over time. This is especially important over the weekend when plants may dehydrate and die.
      iii. Remove the plastic wrap once the wheat seeds have grown enough to touch it.
   b. Animal care:
      i. Once adults emerge add small pieces of apple to the culture. The adult *Tenebrio* do not eat wheat bran and will cannibalize the pupa instead.
      ii. As the culture progresses it may be necessary to add more wheat bran and to collect deceased animals and sheds. Keep track of the mass of any added wheat bran and anything removed from the culture.
2. Once a week:
   a. mass the entire mealworm container, wheat bran, and *Tenebrio*.
   b. gently collect all of the *Tenebrio* (mealworms. pupae, and beatles) into a weighing dish and mass them.
   c. determine the mass of the solids within the container, including wheat bran, fecal matter, and eggs (if adults are present).
3. Once the wheat reaches a height of 4 inches, harvest the entire wheat crop from one of the two containers:
   a. Record the number of days since planting.
   b. Gently remove all of the vermiculite from the roots. *Note:* This can be a time consuming process. Remove as much vermiculite as possible, then use a tub of water to rinse off most of the remaining vermiculite. Use gently running water to completely remove the vermiculite. *Note:* Do not rinse large amounts of vermiculite down the drain. It will clog the pipes.
   c. Pat the wheatgrass dry and mass.
   d. Calculate the average mass per plant.
   e. Create an aluminum tray using the aluminum foil (see Figure 3).

f. Mass the aluminum tray.

g. Place the wheatgrass onto the tray and place into a 105 °C laboratory oven. Allow the wheatgrass to dry for 24–48 hours.

h. After the wheatgrass has dried, mass again to determine the dry weight.

i. Calculate the average dry mass per plant.

4. Allow the remaining container of wheatgrass to grow one or two more weeks, and then harvest the remaining wheat crop using the same procedure as in step 3.
5. Calculate the net primary productivity and net secondary productivity of this model ecosystem.
6. Evaluate the model and make adjustments as necessary to determine how productivity is affected by various factors.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Never release living specimens into the local ecosystem. Mealworms make excellent food for many amphibians, birds, fish, and reptiles. Otherwise the mealworms or adult beetles must be euthanized prior to disposal. The wheat can be considered Type VI Biological Waste and disposed of in the normal garbage.

Lab Hints

- This activity was adopted from *Energy Dynamics—Advanced Inquiry Laboratory Kit* (Flinn Catalog No. FB2049).
- This lab will last for several weeks. Most days only a few minutes are needed to maintain the plants and animals. Setup, harvesting, drying and planing days will take most or all of a 60-minute lab period.

The Materials for *Investigating Energy Flow in an Ecosystem* and supporting supplies are available from Flinn Scientific, Inc.

Catalog No.	Description
FB2049	Energy Dynamics Advanced Inquiry Lab
LM1113	Mealworms, Tenebrio larvae, pkg. 100
FB0582	Monocot Seeds, Wheat, 1 oz, pkg. 700
FB0674	Vermiculite, 8-qt bag
FB1617	Flinn Mealworm Diet, 800 g
FB0676	Plant Fertilizer, Liquid, 32 oz
AB1456	Planting Tray, Plastic, 11″ × 22″

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

Lactose Intolerance

Enzyme Digestion Demonstration

Introduction

Intestinal gas, bloating, and stomach cramps—oh my! This can be a common concern for a majority of the world's population who lack the enzyme to digest certain foods. Milk and dairy products, for example, cause problems for many people who lack the enzyme required to digest lactose, the main carbohydrate found in milk. This demonstration illustrates the use of a commercial enzyme product called Lactaid™ as an aid in milk digestion.

Concepts

- Enzyme
- Disaccharide
- Monosaccharide

Materials

Balloons, 4
Glucose, 10 g
Galactose, 5 g
Lactose, 10 g
Water bath, 35–40 °C
Yeast, active dry, 12 g
Lactaid™, ½ tablet
Erlenmeyer flasks, 125-mL, 4
Mortar and pestle
Water bath, 35–40 °C

Safety Precautions

Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory. Follow all laboratory safety guidelines. Please review current Safety Data Sheets for additional safety, handling, and disposal information.

Procedure

1. Prepare a warm water bath (35–40 °C) for use in the demonstration—a clear glass baking pan with hot tap water will work nicely. The water bath is needed to allow the dramatic balloon expansion to occur within a typical 50-minure class period.
2. Weigh out the dry ingredients prior to the demonstration, and grind ½ tablet of Lactaid™ in a mortar with a pestle.
3. Review the summary diagram of the demonstration setup shown in Figure 1.
4. Clearly label each flask as shown in Figure 1.

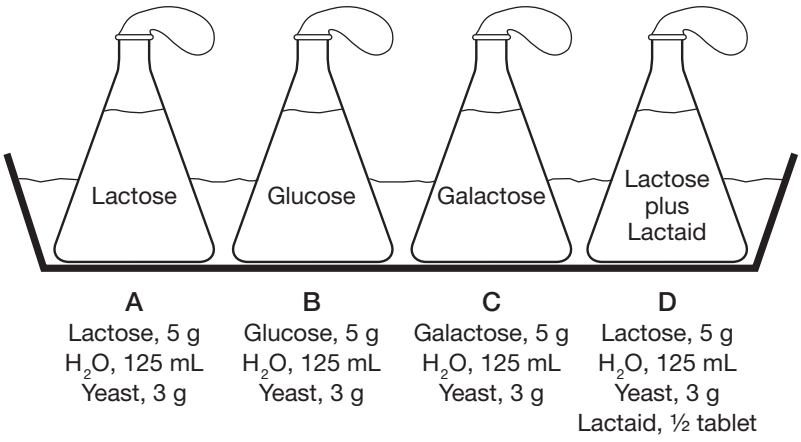

Figure 1.

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5. Place 5 g of the appropriate dry sugar into each flask, as shown in Figure 1.
6. Add pre-ground Lactaid™ tablet to one flask containing 5 g Lactose, as shown in Figure 1.
7. Add about 110 mL of warm (30–35 °C) tap water to the sugar in each flask and swirl each flask until all the sugar has dissolved.
8. Add 3 g of yeast to each flask. Swirl each flask gently to mix in the yeast.
9. Place a balloon securely over the lip of each flask. *Note:* Be sure each balloon is flexible and not stuck together. Inflate each balloon at least once before placing it on the flask.
10. Place all four flasks in the water bath (35–40 °C).
11. Observe the flasks for 15–30 minutes, checking for the production of gas as observed in the balloons. Discuss the results and the effectiveness of Lactaid™ in the experiment.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. The leftover solutions may be disposed of down the drain with excess water according the Flinn Suggested Disposal Method #26b.

Tips

- Yeast lacks the enzymes necessary to digest lactose or galactose, but it does contain the enzyme needed to digest glucose. See Figure 2 for a summary of the demonstration results.
- The balloon in Flask B inflates rapidly due to the production of carbon dioxide from digestion of glucose.
- In Flask D, Lactaid™ effectively breaks down the lactose to give glucose and galactose. The yeast then further digests the glucose to give alcohol and carbon dioxide, which causes the balloon to inflate.
- Flasks A and C should reveal no activity, as the yeast cannot easily digest these sugars. The balloons should remain uninflated for the duration of the demonstration.
- This demonstration can serve as a springboard for lab project extensions. How much lactose can a Lactaid tablet digest? How long does the Lactaid remain active? What factors influence the rate of the reaction?

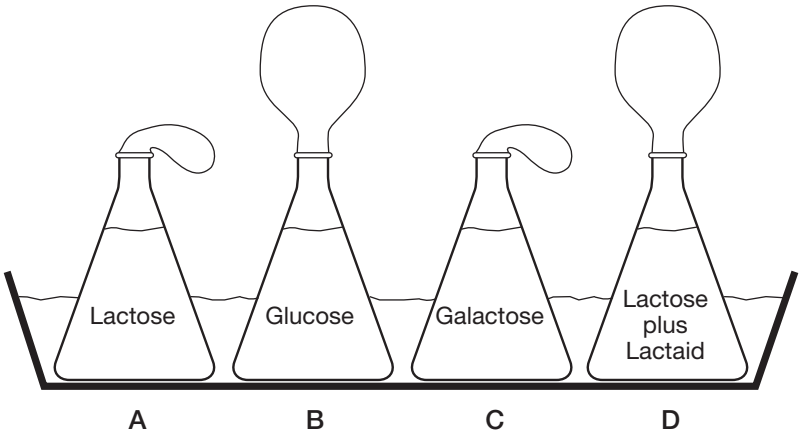

Figure 2.

- This demonstration can also be used as an introduction to topics of evolution. Why is it that some continue to produce lactase after infancy while others do not? What environmental factors could have led to this evolutionary event for humans?

Discussion

Lactose (also called milk sugar) is the principal carbohydrate in milk. It is a *disaccharide*, which means that is it composed of two simple sugars or monosaccharides—glucose and galactose. Some individuals produce insufficient quantities of lactase, the enzyme required to break the bond between the two monosaccharide units in lactose. The condition in which lactase is not produced so that lactose cannot be broken down into the two simpler sugars is referred to as *lactose intolerance*. With this condition, the lactose from milk and various other milk products remains undigested and causes an increase in the osmotic pressure in the intestinal contents. Consequently, water is "drawn" from the tissues into the intestine. At the same time, intestinal bacteria may act upon the undigested lactose and produce organic acids and gases. As a result, the person may feel bloated and suffer from intestinal cramps, diarrhea, and gas.

Researches at Lactaid, Inc. discovered a way to mass produce the enzyme lactase. Lactase converts lactose into glucose and galactose, both easily digestible monosaccharides. The mass-produced lactase is formulated into the tablet product—Lactaid™. The enzyme units in Lactaid are eaten by the lactose-intolerant person before or simultaneous to ingesting lactose. The Lactaid breaks down the lactose allowing the person's system to successfully utilize the resulting glucose and galactose.

An external test for the effectiveness of Lactaid is to find an organism that will only digest the simple sugars and not lactose. In this experiment, yeast is used as a test organism.

Yeast does not produce lactase and therefore cannot digest lactose. (It is lactose intolerant!) Yeast does, however, digest glucose very efficiently. When it digests glucose, the yeast breaks down the glucose and produces carbon dioxide gas as a waste product (Equation 1). This production of gas can easily be monitored. The absence or presence of gas production is used as evidence of digestion by the yeast.

$$C_6H_{12}O_6(aq) \rightarrow 2CH_3CH_2OH(aq) + 2CO_2(g) + \text{energy} \qquad \textit{Equation 1}$$

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Evidence, models, and explanation
Content Standards: Grades 9–12
Content Standard B: Physical Science, structure and properties of matter
Content Standard C: Life Science, matter, energy, and organization in living systems

Reference

This activity was adapted from *Flinn ChemTopic™ Labs, Vol. 20, Biochemistry—The Molecules of Life*.; Cesa, I., Editor; Flinn Scientific, Inc.; Batavia, IL (2002).

Materials for *Lactose Intolerance* are available from Flinn Scientific, Inc.

Catalog No.	Description
FB1570	Lactose Intolerance Demonstration Kit
G0018	Galactose, 100 g
D0005	Glucose (Dextrose), 500 g
L0002	Lactose, 500 g
Y0008	Baker's Yeast, Pkg. of 3 Packets (7 g each)
GP3040	Erlenmeyer flasks, 125-mL
OB2059	Balance, centigram (0.01-g precision)
AP1900	Balloons, Latex, 12", Pkg. of 20
AP6066	Mortar and pestle

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

Altering the Rate of Mitosis

Introduction

All new cells come from previously existing cells. New cells are formed by the process of cell division, which involves the replication of the cell's internal structures and the division of the cytoplasm (*cytokinesis*). What factors affect the rate of mitosis?

Concepts

- Mitosis
- Abiotic and biotic factors
- Plant hormones

Background

The health of a plant or animal depends upon both biotic and abiotic factors. Imagine the parking lot of your school. A few plants may be growing in cracks and crevices of the pavement. In these cracks there is at least a subsistence level of nutrients and water for a plant to survive. A few meters away an unpaved area with soil and little foot traffic may have more plants. The plants compete for space, water, nutrients, and light. If you were to compare plants from the paved and soil areas you would likely see differences in the height of the stems, the number of leaves, and the number and length of the roots. This is a simple example of abiotic factors in the environment affecting plant growth.

Many biotic factors also affect plant growth. A classic example of a beneficial biotic effect is the mutualistic relationship between legumes (beans, peas, clover, and alfalfa) and the nitrogen-fixing bacterium, rhizobia. Rhizobia (singular=rhyzobium), live in nodules on the roots of beans and other plants. Bean plants with rhizobia nodules are typically larger and healthier than plants that are not infected.

Not all biotic interactions benefit a plant. Parasitic interactions may harm a plant by increasing mitosis. For example, the plant pathogen *Agrobacterium tumefaciens* (now called *Rhizobium radiobactor*) causes plant cancer or galls. It does this by triggering the plant to produce certain plant hormones that promote cell division. By forcing the plant to expend more energy in that location and not in the roots, stems, and leaves, the pathogen weakens the plant and may cause death.

The plant of choice for studying mitosis is the common onion. Onions germinate easily without soil so the chemicals provided to the plant can be easily controlled. Onion root tips also grow quickly and are only a few cells thick. A stain is used to dye condensed chromosomes—like those undergoing mitosis—a very dark color. By viewing the onion root tip using a light microscope, it is easy to determine if a particular cell is in interphase or mitosis. See Figure 1 for a graphical representation of the anatomy of an onion root tip. Note that cell division occurs only in the meristem region, not in the other regions of the root tip. Recall also that 90% of the time a cell in the zone of cell division will be in interphase, since mitosis typically makes up only 10% of a full cell cycle. Onions are alive and therefore the onion slide preparation will have more than one layer of cells present in each preparation. In order to reduce the total depth of the slide preparation, the onion root tip needs to be treated and then squashed between the cover slip and the microscope slide.

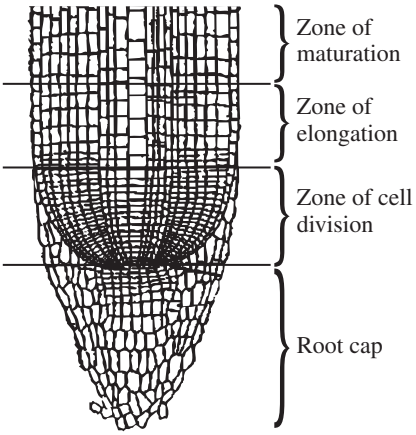

Figure 1. Apical meristem

Materials

Ethyl alcohol, 95%, 20 mL
Gibberellic acid, 2 mg
Hydrochloric acid solution, HCl, 1 M, 2 mL
Indole-3-acetic acid, 2 mg
Toluidine blue stain, 1%, 2 mL
Water, deionized (DI)
Onions, green or onion sets, 6
Dissection scissors
Forceps
Glass slides, 6
Paper towels
Pencil with eraser
Permanent marker or wax pencil
Pipets, disposable, 2

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Compound microscope with 40X objective
Cover slips, 6
Cups, plastic, 8
Sand, white
Spot plate

Safety Precautions

Toluidine blue stain causes skin and eye irritation and is a permanent stain on many objects. Ethyl alcohol is harmful if swallowed and causes skin and serious eye damage. It is a highly flammable liquid and vapor. Keep away from heat, sparks, open flames, and hot surfaces. Hydrochloric acid solution causes severe skin burns and eye damage, may cause respiratory irritation and may be harmful if swallowed. Gibberellic acid and indole-3-acetic acid cause serious eye irritation. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Avoid contact of all chemicals with eyes and skin and wash hands thoroughly with soap and water before leaving the laboratory. Please follow all laboratory safety guidelines.

Pre-Lab Preparation

1. Prepare the indole-3-acetic acid or gibberellic acid solution (2 mg/L)
   a. In a 1-L volumetric flask, dissolve 2 mg of either indole-3-acetic acid or gibberellic acid in 10 mL of ethyl alcohol.
   b. Fill the volumetric flask to the 1-L mark with tap water and mix well.
   c. The solution is stable at room temperature for several weeks.
2. Prepare the (control) water solution.
   a. Add 10 mL of ethyl alcohol to a 1-L volumetric flask.
   b. Fill the volumetric flask to the 1-L mark with tap water and mix well.
   c. The solution is stable indefinitely if stored at room temperature.
3. Germinate root tips.
   a. Fill a plastic cup about ⅓ full with fine white sand.
   b. Pour the sand into a tared weighing dish on a balance. Use the same mass of sand in each cup.
   c. Fill 8 plastic cups with the same massed amount of fine white sand.
   d. Label 4 of the cups "control" and the remainder "treatment."
   e. Remove any long roots or dried roots from the bottom of each onion.
   f. Insert onions into the cups. Place several small onions per cup.
   g. Add enough control solution to the "control" cups to completely wet the sand. Depending upon the size of the cup used the amount varies from 20 to 40 mL.
   h. Add the same amount of the treatment solution to the "treatment" cups to completely wet the sand.
   i. Loosely place plastic wrap on top of each cup to prevent excess evaporation.
   j. The onions do not need to be placed in direct light but the room should be 65–75 °F. Check for root growth every day. Roots are typically ready after 36–48 hours.

Procedure

1. Fill the spot plate as follows: top row with 1 mL of the hydrochloric acid solution, second and fourth rows with deionized water, and the third row with 1 mL of the stain. See Figure 2.
2. Cut three roots from onions treated with the control group and three from the treatment group. Only harvest roots that are less than 1.5 cm long.
3. Trim the tapered end of each root to 0.5 cm. Only the tapered end of the root tip will be used. The remainder may be dis-

carded in the trash.

4. Place each tip into the hydrochloric acid solution for 5 minutes. The HCl begins to break down the cell walls.
5. Remove the tips from the hydrochloric acid and place in the DI water for 1 minute.
6. Remove the tips from the water rinse and place in the stain for 2–3 minutes.
7. Remove the tips from the stain and place in the second DI water rinse for at least 1 minute. The water may need to be changed. The final color of the rinse water should be a clear blue. This is a key step. Too much or too little stain will inhibit analysis. Achieving the correct depth of color may take some practice.
8. Place a single root tip onto a microscope slide.
9. Place a cover slip onto the root tip and gently press down on the cover slip with the eraser of a pencil. *Note:* The cover slip may be gently spun or moved back and forth to reduce the thickness of the prep. If the cover slip breaks use forceps to remove the pieces and replace with a new cover slip. If more solution needs to be added to the prep, use a pipet to add a partial drop of water to the edge of the cover slip. The water should move under the cover slip by capillary action.

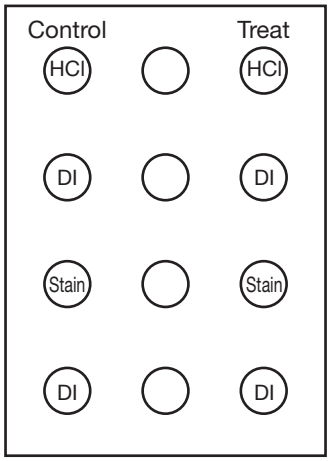

Figure 2. Spot Plate

10. Locate the meristem area of the root tip and identify mitosis or interphase for a minimum of 300 cells or completely count two fields of view, whichever is greater. *Note:* Count all cells in the meristem region. Take care not to count the same cell more than once. Also, do not only count cells in mitosis. This would skew the results.
11. Repeat with the remaining root tips.
12. Pool data with that of the other lab groups.
13. Complete a chi-square statistical analysis of the data (optional).

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Excess hydrochloric acid may be neutralized with base and then flushed down the drain with plenty of excess water according to Flinn Suggested Disposal Method #24b. Excess Toluidine blue solution, control solution and IAA or gibberellic acid solution may be flushed down the drain with an excess of water according to Flinn Suggested Disposal Method #26b.

NGSS Alignment

This laboratory activity relates to the following Next Generation Science Standards (2013):

Disciplinary Core Ideas: High School
HS-LS1 From Molecules to Organisms: Structures and Processes
LS1.A: Structure and Function
LS1.B: Growth and Development of Organisms

Science and Engineering Practices
Planning and carrying out investigations
Analyzing and interpreting data
Using mathematics and computational thinking

Crosscutting Concepts
Cause and effect
Structure and function
Stability and change

Tips

- A minimum of 2,000 cells should be counted for each treatment.
- Although only IAA and gibberellic acid are mentioned in the procedure, other plant hormones will also affect plant growth. Each has a concentration range that promotes plant growth and affects mitosis. Concentrations above this range will inhibit plant growth, while lower concentrations will not show a statistically significant effect. These plant hormones are:

  Abscisic acid (Dormin) (Catalog No. A0298) 0.1–10 mg/L
  Gibberellic acid (Catalog No. G0042) 0.01–5.0 mg/L

Indole-3-acetic acid (Catalog No. I0001) 1–3 mg/L
Indole-3-butyric acid (Catalog No. I0003) 0.1 –10 mg/L

- Red kidney bean lectin (*Phytohemagglutinin*) is used in AP Biology Investigation 7 by the College Board. Lectin is a hlyophilized powder that must be kept refrigerated. The recommended concentration is 10 mg in 200 mL of deionized water. Once diluted, it must be stored in the refrigerator. It is only active for a few days. Lectin significantly increases mitosis in the roots. Please read the SDS before using lectin. Lectin is available from Flinn Scientific (Catalog No. L0114).
- Caffeine inhibits mitosis. Onions treated with 1% aqueous caffeine failed to produce any roots after several weeks. A 0.1% aqueous caffeine solution did produce short roots. However, when stained the cell cycle phase is difficult to determine and may not produce quantifiable results. Caffeine is very toxic with an oral (rat) LD_{50} of 192 mg/kg. Please read the SDS before using caffeine. Caffeine is available from Flinn Scientific (Catalog No. C0344).
- The auxin 2,4-dichlorophenoxyacetic acid (2,4-D) was tested at a concentration of 1 mg/L using the above procedure. Numerous root tips germinated but they remained very short and made it difficult to quantify the results. 2,4-D is available from Flinn Scientific (Catalog No. D0056).
- Other factors that may affect the mitotic rate are salinity, temperature, mineral limiting factors, pH, roundworms, soil bacteria or fungus, other plant hormones, amount of light, acetaminophen, aspirin, ibuprofen, vitamins and minerals, heavy metals, antibiotics, and certain plant competitors that excrete inhibiting chemicals.
- Store bought onions may be treated with rooting inhibitors. Onion sets are available from Flinn Scientific (Catalog No. FB1468).
- This write-up is based on the kit, *Environmental Effects on Mitosis—An Advanced Inquiry Lab* (Catalog No. FB2031).

Materials for *Altering the Rate of Mitosis* are available from Flinn Scientific, Inc.

Catalog No.	Description
AP6399	Spot Plate, Polystyrene, Pkg. of 12
FB1468	Onion Sets, Pkg. of 100
G0042	Gibberellic Acid, 0.5 g
H0013	Hydrochloric Acid Solution, 1 M, 500 mL
I0001	Indole-3-acetic Acid, 1 g
S0004	Sand, Fine White, 2 kg
T0048	Toluidine Blue O Solution, 100 mL

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

Add Up and Cross Over

Sordaria Genetics Simulation

Introduction

Crossing over occurs during meiosis I. During crossing over, homologous pairs of chromosomes exchange sections of DNA that contain the same genes. It is important to note that crossing over does not have to occur during each generation, nor does it always take place at the same point of exchange. Over time, however, crossing over leads to a greater variety of genes in a population and contributes to a diversity of characteristics and increased fitness of the population. This is reflected in the ability of the population to adapt to changes in the environment.

Concepts

- Genetic diversity
- Gene mapping
- Crossing over
- Meiosis

Background

During sexual reproduction hyphae of different haploid *S. fimicola* come into contact allowing cells in the hyphae to fuse and form a single cell with two nuclei, one from each individual. This fused cell is called a *dikaryon*. The dikaryon is not considered diploid since the two nuclei are from separate fungi and the nuclei are not fused together. The dikaryon cells undergo multiple rounds of mitosis to form a mass of cells. This mass of cells can exist for years without undergoing fusion of the nuclei. Sexual reproduction occurs when some of the dikaryon nuclei fuse. After fusion the fruiting body forms and meiosis occurs, creating the asci and ascospores, which are haploid cells.

The ascospores form inside the tight confines of the tube-like ascus. The ascospores actually line up in order based on which cell produced that particular ascospore. In 1956, a geneticist named Lindsay S. Olive (1917–1988) published an article about crossing over in *S. fimicola*. Dr. Olive used ultraviolet light to cause mutations in the genes of *S. fimicola*. After numerous trials, Dr. Olive produced a mutation in the gene that produced the pigment in the ascospore. The production of the black pigment is either greatly reduced or completely repressed in the mutated strain of *S. Fimicola*. A reduction in the amount of black pigment results in gray asco spores. An absence of black pigment results in tan ascospores. By collecting the gray or tan ascospores, Dr. Olive was able to produce true breeding fungi much like Mendel's peas.

Recall that each ascospore can be traced back to the parent chromosome. The pattern of black and tan ascospores shows whether crossing over occurred during meiosis. Observe Figure 1. Note that the diagram of the ascus indicates eight ascospores in each ascus, not the expected four cells. This is because each of the four haploid daughter cells undergoes a single mitosis after the end of meiosis II. So, each daughter cell produces a clone of itself. These clones reside next to each other within the ascus. If the cells come from parents with identical pigment genes, the ascus will contain eight spores that are the same color whether black or tan. If the cells come from parents with each pigment type, but crossing over did not occur, the spores will appear as four black, wild-type and four tan, mutant spores (4b:4t) (see Figure 1a). If crossing over of the pigment gene between a black wild-type and a tan mutant occurred during meiosis I, the four spores will have one of two possible patterns: 2:2:2:2 or 2:4:2. Each of the numbers can be either tan or black. This is written out as 2b:2t:2b:2t or 2t:2b:2t:2b and 2b:4t:2b or 2t:4b:2t. (See Figure 1b and 1 c).

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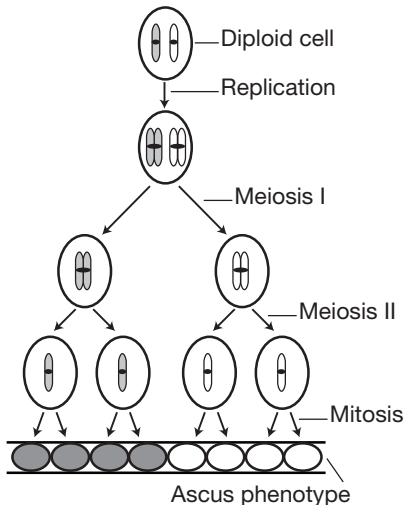

Figure 1a.
Non-crossover ascus—4b:4t

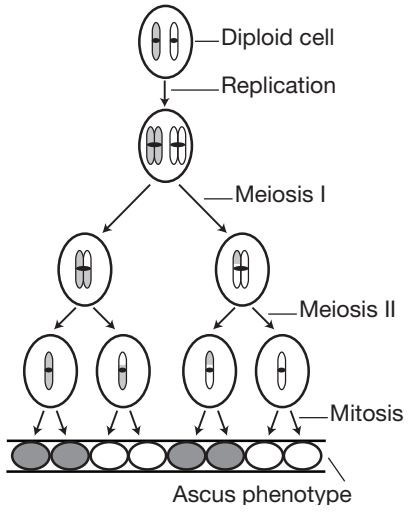

Figure 1b.
Crossover ascus—2b:2t:2b:2t

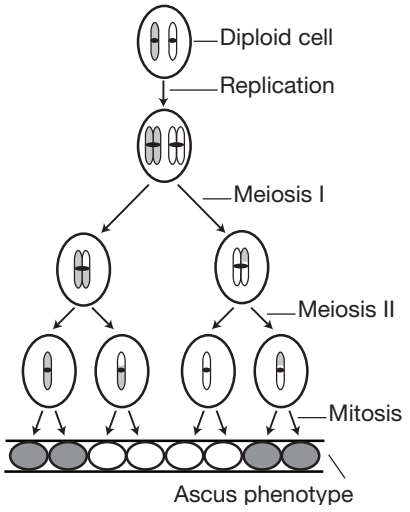

Figure 1c.
Crossover ascus—2b:4t:2b

Materials

Sordaria Cards, 5

Pencil

Safety Precautions

Although this activity is considered nonhazardous, please follow all laboratory safety guidelines.

Procedure

1. Begin with one of the five *Sordaria* cards assigned. Categorize each ascus containing both black and tan ascospore beginning at the twelve o'clock position. *Note:* Do not categorize any single-colored asci. single-colored asci are not the result of sexual reproduction between a tan mutant and a black wild type *S. fimicola* and will not be used to calculate the frequency of crossing over or the map distance.
2. Tally the results on the Counting Crossing Over Worksheet corresponding to the correct color arrangement for each ascus. *Note:* Refer to Figures 1a–1c for schematics of possible two-colored asci.
3. Categorize all of the two-colored asci on the first card.
4. Repeat steps 1–3 with the four remaining cards, compiling all the data.
5. Complete the calculations and data analysis on the Counting Crossing Over Worksheet.

AP Biology Curriculum

Enduring Understanding

Essential knowledge 3.A.3: The chromosomal basis of inheritance provides an understanding of the pattern of passage (transmission) of genes from parent to offspring.

Essential knowledge 3.C.1: Changes in genotype can result in changes in phenotype.

Essential knowledge 3.C.2: Biological systems have multiple processes that increase genetic variation.

Tips

- Published sources cite between 26 and 28 map units as the distance between the tan mutant gene and the centromere.
- Use this activity as an introduction for the Advanced Placement Biology Investigation 7 Part 5.

References

Olive, L. S. Genetics of *Sordaria fimicola; American Journal of Botany*, **1956**, 43, 97–107.

Cassell, P., Mertens, T. R., A Laboratory Exercise on the Genetics of Ascospore Color in *Sordaria fimicola; The American Biology Teacher*, **1968**, 30, 367–372.

The Tree of Life Web project (accessed August 2010). http://tolweb.org/Ascomycota

Biology: Lab Manual; College Entrance Examination Board: 2001.

Kits covering the same topic as *Add Up and Cross Over* are available from Flinn Scientific, Inc.

Catalog No.	Description
FB1973	Counting Crossing Over— Sordaria Genetics Student Activity Kit
FB2001	Sordaria Genetics—Student Laboratory Kit

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

Name: _____

Add Up and Cross Over Worksheet

Data Table

Color combinations	Tally Marks	Total
Non-crossover Asci		
4b:4t		
4t:4b		
Crossover Asci		
2b:2t:2b:2t		
2t:2b:2t:2b		
2b:4t:2b		
2t:4b:2t		

Post-Lab Questions and Calculations

1. Take the sum of the tally marks for each combination. Record each result in the *Total* column.
2. Determine the total number of non-crossover asci counted.
3. Determine the total number of crossover asci counted.
4. Determine the total number of hybrid asci counted.
5. Recall that each ascus contains eight spores because the four haploid spores underwent an additional mitosis. a correction to the totals must be performed to compensate for that extra mitotic event. Divide each of the numbers calculated in steps 3–4 by 2. Record the corrected values below.
5. Determine the map distance between the gene for spore color and the centromere using Equation 1. Report the result in map units. However, keep in mind that each ascus contains 8 spores because the four haploid spores underwent an additional mitotic event after meiosis. To account for this, the map distance found in Equation 1 needs to be halved (Equation 2).

$$\text{Map distance} = \frac{\text{corrected number of crossover asci}}{\text{corrected total number of asci counted}} \times 100 \qquad \textit{Equation 1}$$

$$\frac{\text{Map distance}}{2} \qquad \textit{Equation 2}$$

6. Was the number of each type of crossover phenotype observed relatively constant or equal? Explain why you would expect these numbers to be constant.

7. A similar technique can be used to determine the distance between two genes on a single chromosome. In this laboratory a color mutation was used as the gene of interest. What is the benefit for using a color mutant gene for learning about map units.