Laboratory Review

Review

The College Board provides 12 laboratory exercises for use in AP Biology courses. Completing these labs, or similar labs that your teacher may substitute, provides the laboratory experience typical of a first-year college course in biology. One essay question and approximately 10 percent of the multiple-choice questions are based on these lab exercises.

The laboratory essay question is usually one of two types:

1. **Experimental analysis.** In this type of essay question, you are given some experimental data and asked to interpret or analyze the data. The question usually includes several parts, each requesting specific interpretations of the data. In addition, you are usually asked to prepare a graphic representation of the data. Graph paper is provided. Guidelines for preparing a graph are given in the next section.
2. **Experimental design.** This type of essay question asks you to design an experiment to answer specific questions about given data or an experimental situation. Guidelines for designing an experiment are given in a following section.

Although the data or situation in both of these types of questions will be somewhat different from those you encountered in your AP labs, you will be able to draw from your AP lab experience to analyze data or design experiments.

The material in this section summarizes each of the twelve laboratory exercises with a brief description of its experimental design and conclusions. It is not intended that this material substitute for an actual laboratory experience, but to provide you with a review that will help you answer the AP biology questions.

Graphing Data

The laboratory question in the essay part of the AP exam will often ask you to create a graph using data provided in the question. Include the following in your graph:

1. **Label each axis.** Indicate on each axis what is being measured and in what unit of measurement. For example, "Time (minutes)," "Distance (meters)," or "Water Loss (ml/m^2)" are appropriate labels.
2. **Provide values along each axis at regular intervals.** Select values and spacing that will allow your graph to fill as much of the graphing grid as possible.
3. **Use the *x*-axis for the independent variable and the *y*-axis for the dependent variable.** The dependent variable is the value you are measuring as a result of an independent variable imposed by the experiment. If the graph is plotting the progress of an event, then time is the independent variable and the data you collect that measure the event (such as weight change, distance traveled, or carbon dioxide released) is the dependent variable.
4. **Connect the plotted points.** Usually, straight lines are used to connect the points. Smooth curves are also used, but that usually implies knowledge about intermediate points not plotted or a mathematical equation that fits the experimental results. If the question asks you to make predictions beyond the data actually graphed, extrapolate, or extend, the plotted line with a different line form (for example, dotted or dashed).
5. **In graphs with more than one plot, identify each plot.** If you plot more than one set of data on the same graph, identify each plot with a short phrase. Alternately, you can draw the points of each plot with different symbols (for example, circles, squares, or triangles) or connect the plotted points using different kinds of lines (solid line, dashed line, or dash-dot line) and then identify each kind of symbol or line in a legend.
6. **Provide a title for the graph.** Your title should be brief but descriptive.

Designing an Experiment

The laboratory essay question may ask you to design an experiment to test a given hypothesis or to solve a given problem. In most cases, the question will ask you not only to design an experiment, but also to discuss expected results. Since the form of these questions can vary dramatically, it is not possible to provide a standard formula for preparing your answer. However, the following list provides important elements that you should include in your answer if they are appropriate to the question.

1. **Identify the independent and dependent variables.** The independent variable is the variable you are manipulating to see how the dependent variable changes.
   - You are investigating how the crustacean *Daphnia* responds to changes in temperature. You expose *Daphnia* to temperatures of 5°C, 10°C, 15°C, 20°C, and 30°C. You count the number of heartbeats/sec in each case. Temperature is the **independent variable** (you are manipulating it), and number of heartbeats/sec is the **dependent variable** (you observe how it changes in response to different temperatures).
   - You design an experiment to investigate the effect of exercise on pulse rate and blood pressure. The physiological conditions (independent variable, or variable you manipulate) include sitting, exercising, and recovering at various intervals following exercise. You make two kinds of measurements (two dependent variables) to evaluate the effect of the physiological conditions—pulse rate and blood pressure.
2. **Describe the experimental treatment.** The experimental treatment (or treatments) is the various values that you assign to the independent variable. The experimental treatments describe how you are manipulating the independent variable.
   - In the *Daphnia* experiment, the different temperature values (5°C, 10°C, 15°C, 25°C, and 30°C) represent five experimental treatments.
   - In the experiment on physiological conditions, the experimental treatments are exercise and recovery at various intervals following exercise.
3. **Identify a control treatment.** The control treatment, or control, is the independent variable at some normal or standard value. The results of the control are used for comparison with the results of the experimental treatments.
   - In the *Daphnia* experiment, you choose the temperature of 20°C as the control because that is the average temperature of the pond where you obtained the culture.
   - In the experiment on physiological conditions, the control is sitting, when the subject is not influenced by exercising.
4. **Use only one independent variable.** Only one independent variable can be tested at a time. If you manipulate two independent variables at the same time, you cannot determine which is responsible for the effect you measure in the dependent variable.
   - In the physiological experiment, if the subject also drinks coffee in addition to exercising, you cannot determine which treatment, coffee or exercise, causes a change in blood pressure.
5. **Random sample of subjects.** You must choose the subjects for your experiments randomly. Since you cannot evaluate every *Daphnia*, you must choose a subpopulation to study. If you choose only the largest *Daphnia* to study, it is not a random sample, and you introduce another variable (size) for which you cannot account.
6. **Describe the procedure.** Describe how you will set up the experiment. Identify equipment and chemicals to be used and why you are choosing to use them. If appropriate, provide a labeled drawing of the setup.
7. **Describe expected results.** Use graphs to illustrate the expected results, if appropriate.
8. **Provide an explanation of the expected results in relation to relevant biological principles**. The results you give are your expected results. Describe the biological principles that led you to make your predictions.
   - In the experiment on physiological conditions, you expect blood pressure and pulse rate to increase during exercise in order to deliver more O_2 to muscles. Muscles use the O_2 for respiration, which generates the ATP necessary for muscle contraction.

Laboratory 1: Diffusion and Osmosis

This lab provides exercises that investigate the movement of water across semipermeable membranes. You should review the processes of diffusion, osmosis, dialysis, and plasmolysis discussed in the review section on cells in this book.

In animal cells, the direction of osmosis, in or out of a cell, depends on the concentration of solutes inside and outside the plasma membrane. In plant cells, however, osmosis is also influenced by turgor pressure, the pressure of the cell wall exerted on the contents of the cell. To account for differences in both concentration and pressure, a more general term, water potential, is used to describe the tendency of water to move across a selectively permeable membrane. **Water potential** is the sum of the **pressure potential** (from any externally applied force) and the **solute potential** (osmotic potential) (Figure 16-1):

$$\underset{\text{water potential}}{\psi} = \underset{\text{pressure potential}}{\psi_p} + \underset{\text{solute potential}}{\psi_s}$$

Figure 16-1

Water potential has the following properties:

1. Water moves across a selectively permeable membrane from an area of *higher* water potential to an area of *lower* water potential.
2. Water potential can be positive or negative. Negative water potential is called **tension.**
3. Solute potential results from the presence of solutes and is always negative. The higher the concentration of solutes, the smaller (or more negative) the solute potential.
4. Pressure potential is zero unless some force is applied, such as that applied by a cell wall.
5. Pure water at atmospheric pressure has a water potential of zero (pressure potential = 0 and solute potential = 0).
6. Water potential is measured in bars (1 bar is approximately equal to 1 atmosphere pressure) or megapascals (1MPa = 10 bars).

Think of water potential as potential energy, the ability to do work. The water at the top of a dam has a high water potential, a high potential energy, and a large capacity for doing work, such as the ability to generate electricity as it runs downhill.

At the end of this lab, you should know the following:

1. Dialysis tubing is used to make selectively permeable bags. It will allow small molecules to pass through, such as water and monosaccharides (glucose), but not disaccharides (sucrose), polysaccharides (starch), or proteins.
2. A Benedict's test or a commercial glucose testing tape is used to test for the presence of glucose.
3. Lugol's solution is used to test for the presence of starch. Lugol's solution (iodine and potassium iodide, or IKI) is yellow brown but turns dark blue in the presence of starch.

Designing an experiment to measure water potential: The information learned in this lab can be used to determine water potential, pressure potential, or solute potential. For example, the concentration of solutes (solute potential) in a selectively permeable bag or in living tissues such as potato cores can be determined. Prepare five bags of the unknown solute or five tissue samples. Soak one of each bag or tissue sample in one of five beakers, each of which contains a different solute concentration (different solute potential). If the mass of a bag or tissue sample increases, then the solute concentration in the bag or tissue is **hypertonic** (higher concentration of solutes) to that of the beaker. If the mass decreases, the bag content or tissue sample is **hypotonic** (lower concentration of solutes). When there is no change in mass, the bag content or tissue sample is isotonic with its surroundings and the solute concentration is equal to that of the beaker.

Laboratory 2: Enzyme Catalysis

In this lab exercise, the rate of a reaction is measured in the presence and absence of a catalyst. The catalyst, catalase, is an enzyme in cells that catalyzes the breakdown of toxic H_2O_2. The reaction is

$$2\ H_2O_2 \xrightarrow{catalase} 2\ H_2O + O_2\ (gas)$$

At the conclusion of this lab, you should know the following:

1. The rate of a reaction is determined by measuring the accumulation of one of the products or by measuring the disappearance of the substrate (reactant).
2. The rate of a reaction is the slope of the linear (straight) part of the graph that describes the accumulation of product (or decrease in substrate) as time progresses.
3. Reaction rate may be affected by temperature, pH, substrate concentration, and enzyme concentration.

Designing an experiment to determine reaction rates for an enzyme under various conditions: An experiment can be designed to determine the reaction rates for an enzyme under different conditions (such as pH, temperature, enzyme, or solute concentration). The experiment should incorporate the following:

1. Maintain constant conditions for all reaction tests. Keep substrate and enzyme concentration, pH, and temperature constant unless you are investigating the effects of these variables.
2. Stop the reaction at regular time intervals using a strong acid. Because enzymes are proteins, they are easily denatured (structurally damaged) and disabled by strong acids, such as sulfuric acid (H_2SO_4).
3. Measure the amount of substrate converted or product formed by performing a titration. In the AP lab, the amount of H_2O_2 remaining is determined by titration, that is, a burette is used to gradually add $KMnO_4$ until a pink color persists. By knowing that 2.5 molecules of H_2O_2 react with 1 molecule of $KMnO_4$, you are able to determine how much H_2O_2 remains.
4. Graph the results (with time, the independent variable, on the *x*-axis) and determine the reaction rate by calculating the slope of the linear portion of the plot.

Laboratory 3: Mitosis and Meiosis

As part of the review for this lab, you should read the section on cell division in this book. In particular, review the phases of mitosis and meiosis, the cell structures involved in cell division, and the similarities of and differences between plant and animal cell division.

At the conclusion of this lab, you should know the following (in addition to the material in the section on cell division):

1. In plants, cells dividing by mitosis can be found at the tips of roots and shoots (called **meristems**). In animals, mitotically dividing cells occur in any growing tissue but are most abundant in dividing embryos (especially blastulas).
2. The length of time for each phase of mitosis can be determined by counting the number of times each phase appears under the microscope. The frequency of appearance (number of times it appears, divided by the total number of all phases) equals the relative length of time required for each phase to complete.
3. The results of crossing over during meiosis can be readily visualized under the microscope in the asci of many ascomycete fungi. Asci are hyphae that contain eight haploid ascospores. In most ascomycetes, the asci are embedded in other hyphae that form a fruiting body. One kind of fruiting body, a **perithecium,** surrounds the asci, except for a passageway that allows for the escape of the ascospores. Sexual reproduction begins in ascomycetes when hyphae from two strains fuse. Nuclei from one strain pair with nuclei from the second strain. Subsequently, these pairs of unlike nuclei fuse (**karyogamy**) to produce diploid nuclei, which then undergo meiosis. During meiosis I, homologous chromosomes pair and separate. During meiosis II, each chromosome separates into two chromatids. At the end of meiosis, there are four daughter cells, each possessing one chromatid from each tetrad of homologous chromosomes. Each of the four daughter cells then divides by mitosis to produce two ascospores. The order of ascospores in the ascus corresponds to the chromatids. If no crossing over occurs, then each set of four adjacent ascospores represents a single parent strain and will possess the same traits. This is illustrated on the left side of Figure 16-2 for a trait that determines spore color.

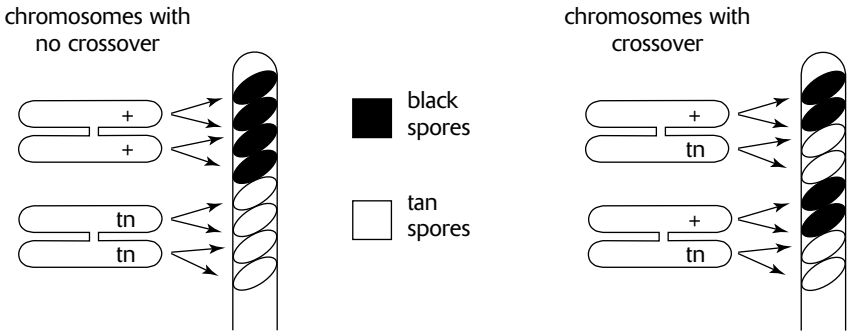


Figure 16-2

If crossing over occurs (Figure 16-2, right), then traits on two nonsister chromosomes exchange, producing alternating patterns of ascospore pairs with and without the traits included in the crossover.

Designing an experiment to determine crossover frequency: The knowledge gained in this lab exercise can be used to determine the crossover frequency in an ascomycete fungus. Include the following steps:

1. Grow two ascomycete fungal strains of the same species on a plate of nutrient enriched agar. Perithecia form at the interface between the two strains.
2. Under the microscope, count the number of asci that contain ascospores with crossovers and the number of asci that contain ascospores without crossovers.
3. Determine the frequency of asci with crossovers (number of asci with crossovers divided by total asci).

Laboratory 4: Plant Pigments and Photosynthesis

In the first part of this lab exercise, photosynthetic pigments are separated using the technique of paper chromatography. In this procedure, an extract from leaves is applied (spotted) near the bottom edge of a sheet of chromatography paper. The paper is then hung so that its bottom edge is just touching the chromatography solvent. As the solvent rises up the paper by capillary action, spotted pigments are carried up the paper with the solvent. Different pigments move up the paper at different rates because they differ in their ability to dissolve in the solvent and their ability to form hydrogen bonds with the hydrophilic, polar molecules of cellulose in the paper. Molecules that dissolve best in the solvent and form the weakest bonds with the cellulose move up the paper the fastest. When the solvent front nearly reaches the top of the paper, the paper is removed from the solvent. Reference front (R_f) values for each pigment are then determined by calculating the ratio of the pigment migration distance to the solvent migration distance.

The following photosynthetic pigments are observed in the following order, from the top of the chromatography paper (fastest moving) to the bottom (slowest moving):

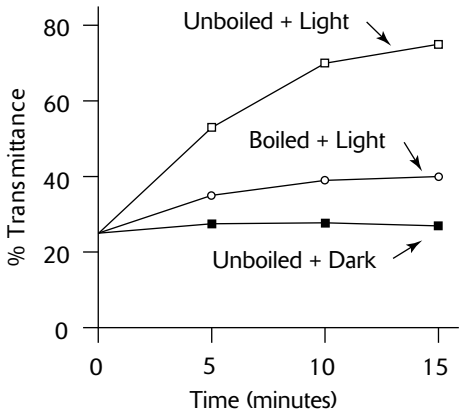
1. beta carotene, a carotenoid (pale yellow)
2. xanthophyll, a carotenoid (yellow)
3. chlorophyll *a* (green to blue green)
4. chlorophyll *b* (yellow green)

In the second part of this lab, the rate of photosynthesis is determined by the amount of dye that changes from blue to clear. The dye, DPIP, acts as an electron acceptor in place of $NADP^+$. DPIP is blue in the **oxidized** state (before accepting electrons) and is clear in the **reduced** state (after accepting electrons). The degree to which the color changes is determined by measuring the transmittance of light through the sample with a spectrophotometer. Transmittance is measured at a wavelength of 605 nm, the wavelength for which DPIP has its greatest absorbance (its darkest color).

The design of the exercise is to compare the rate of photosynthesis in four treatments, as follows:

1. A blank treatment without DPIP for calibrating the spectrophotometer.
2. A control with unboiled chloroplasts, incubated in the dark.
3. An experimental treatment with unboiled chloroplasts, incubated in the light.
4. An experimental treatment with boiled chloroplasts, incubated in the light.

For each treatment, the transmittance of a sample is measured at the beginning of the experiment, and again at 5, 10, and 15 minutes. The results are plotted on a graph with time (the independent variable) on the *x*-axis and percent transmittance (the dependent variable) on the *y*-axis. Typical results are shown below:



Designing an experiment to separate a group of molecules: A group of molecules (such as pigments, amino acids, or sugars) can be separated using the techniques of chromatography. The solvents and the medium (paper or silica gel) may vary with the kinds of molecules being separated. Special stains may be needed to make the separate components visible. After separation, R_f values are calculated and compared to tables of R_f values for known molecules obtained for a similar medium and solvent. If paper is used for the medium, each component can be cut from the paper, dissolved in a solvent, and further analyzed.

Designing an experiment to measure rates of photosynthesis: Light intensity, light wavelength, and temperature may each be varied to determine their effect on photosynthetic rate. Be sure to include the following in an experimental design:

1. Use DPIP as the electron acceptor. It changes from blue to clear as it is reduced.
2. Measure the change in light transmittance with a spectrophotometer set to measure transmittance at a wavelength of 605 nm.
3. Include a blank treatment with no DPIP (in which no reaction occurs) for calibrating the spectrophotometer before each measurement.
4. Include a control treatment to which the experimental treatment(s) can be compared.
5. Include one or more experimental treatments.
6. Measure the transmittance at various intervals so that photosynthetic rate can be determined.
7. Graph the results.

Laboratory 5: Cell Respiration

This lab provides a method for measuring the rate of cellular respiration. Cellular respiration is the breakdown of glucose with oxygen to produce carbon dioxide, water, and energy, as follows:

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

In this lab, respiratory rate is measured in germinating peas by observing the changes in the volume of gas surrounding the peas at various time intervals. Since the volume of gas can be affected by both the consumption of O_2 and the production of CO_2, the CO_2 is removed by using potassium hydroxide (KOH). KOH reacts with CO_2 gas to produce solid K_2CO_3, as follows:

$$2KOH + CO_2 \rightarrow K_2CO_3 + H_2O$$

Since CO_2 gas is removed by its reaction with KOH, volume changes can be attributed only to the following:

1. Consumption of oxygen (due to respiration)
2. Change in temperature
3. Change in atmospheric pressure

The exercise is designed to measure respiratory rates of peas at two temperatures, 10°C and 22°C. Respirometers, consisting of vials with inserted pipettes, are used to incubate the peas and measure respiration. The following treatments are prepared:

1. An experimental treatment at 10°C, consisting of 25 germinating peas. Germinating peas consist of cells actively dividing and growing and exhibit a relatively high respiratory rate.
2. A control treatment at 10°C, consisting of 25 dry peas plus enough glass beads to yield a volume equivalent to the 25 germinating peas in the experimental treatment. Since these peas are not germinating, the respiratory rate will be very low and will provide a comparison for the germinating peas. Since dry peas are smaller and weigh less than germinating peas (which have been soaked in water and are growing), glass beads are added to maintain equal volumes.
3. A control treatment at 10°C, consisting of enough glass beads to yield a volume equivalent to the 25 germinating peas. This treatment measures changes in temperature and pressure not due to respiration.

Another three treatments are similarly prepared for use in a 22°C water bath. Thus, a total of six respirometers are assembled.

The six respirometers are immersed in their respective 10°C and 22°C water baths. Water in the water bath enters the pipettes and travels a short distance.

As respiration occurs, O_2 is consumed in the vials, and pressure in the vials and pipettes drops. As pressure drops, additional water from the water bath enters the pipettes. The amount of water entering can be determined by reading the ml graduations on the pipettes. This reading is a direct measurement of oxygen consumption and thus an indirect measurement of respiratory rate.

Changes in volume observed in the control treatments (beads only) represent responses to external changes in temperature and pressure. Thus, the observed increases or decreases in volume in the control treatments must be subtracted or added, respectively, to the volume changes observed for germinating peas and dry peas.

Designing an experiment to measure respiratory rates: Using the experimental design described, you can determine respiratory rates for seeds germinating at various temperatures or for seeds that have been germinated for different lengths of time. The respiratory rate of insects can also be determined using this experimental design. Since the body size of individual insects will probably vary, volume changes in the respirometers can be plotted against insect body weight to determine the relationship between respiratory rate and insect size.

Laboratory 6: Molecular Biology

The bacterial plasmid pAMP is often used as a vector (or carrier) to transfer genes into bacteria. To insert a gene into a plasmid (or any other piece of DNA), **restriction endonucleases** (restriction enzymes) are used. A restriction enzyme cleaves a DNA molecule at a specific sequence of nucleotides, producing cuts that are usually jagged, with one strand of the DNA molecule extending beyond the second strand. If both the plasmid and the foreign DNA (with the gene of interest) are treated with the same restriction enzyme, the jagged ends, or "sticky ends," of the foreign DNA will match the sticky ends of the plasmid DNA. The foreign DNA fragment can then be bonded to the plasmid DNA by treatment with DNA ligase.

The value of using pAMP as a vector for gene transfers is that pAMP contains a gene that provides resistance to the antibiotic ampicillin. As a result, bacteria that have successfully absorbed the plasmid possess resistance to the antibiotic and can be separated from other bacteria by ampicillin treatment. Only those bacteria that have the pAMP plasmid survive the ampicillin treatment.

In the first part of this lab, the bacterial plasmid, pAMP, is transferred to *E. coli* bacteria. The following steps summarize the procedure:

1. Induce competence in *E. coli* bacteria by treating them with Ca^{2+} or Mg^{2+}. Bacteria are said to be competent when they are most likely to absorb DNA.
2. Facilitate the absorption of DNA by giving the bacteria a heat-shock (short pulse of heat).
3. Test for transformation (the absorption of foreign DNA) by treating the bacteria with ampicillin. Only transformed bacteria survive.

Designing an experiment to observe transformation and bacterial resistance: The following steps should be included:

1. Prepare two tubes of *E. coli* bacteria. One tube will be transformed with pAMP plasmids. The second will be a control.
2. Induce competence in the bacteria in both tubes by adding $CaCl_2$.
3. Transfer pAMP plasmids to one of the test tubes.
4. Induce transformation in both tubes with heat-shock.
5. After incubation (for growth), transfer bacteria from one tube to an agar plate with ampicillin and an agar plate without ampicillin. Repeat for the second tube.

Interpret the results (Table 16-1). Only bacteria transformed with pAMP plasmids can grow in the presence of ampicillin. The control tube of bacteria confirms that without pAMP, growth cannot occur in the presence of ampicillin. It also confirms that the untransformed *E. coli* are not ampicillin resistant.

Table 16-1

	Agar Plate without Ampicillin	***Agar Plate with Ampicillin***
Tube 1: Bacteria with pAMP plasmids	growth	growth
Tube 2: Bacteria without pAMP plasmids (control)	growth	no growth

In the second part of this lab, DNA fragments are separated using gel electrophoresis. The steps are summarized here.

1. The DNA to be analyzed is digested (cleaved) with a restriction enzyme.
2. A tracking dye is added to the sample. This allows the leading edge of DNA migration to be observed.
3. Load the sample into a well (small hole) in the agarose gel of the electrophoresis apparatus.

4. Begin electrophoresis. The electrophoresis apparatus applies a voltage to opposite ends of the gel. The negatively charged DNA molecules migrate from the negative to the positive electrode. Turn off the apparatus when the tracking dye nears the end of the gel.
5. Immerse the gel in a dye that allows the fragments to be observed. Methylene blue is used in this exercise.
6. Record the distance each fragment has migrated from the well.

Designing an experiment to separate and identify DNA fragments: Include the following steps in an experiment to investigate the sizes of fragments produced by a restriction enzyme:

1. Prepare a sample of DNA digested with the restriction enzyme under investigation. (In this exercise, phage lambda DNA molecules are digested with *Eco*RI restriction enzyme.)
2. Prepare a second sample of undigested DNA for use as a control.
3. Prepare a third sample of DNA digested with a standard restriction enzyme. The fragment sizes produced by this standard are known and provide a standard against which fragment sizes produced in step 1 can be compared. (In this exercise, phage lambda DNA molecules are digested with *Hind*III restriction enzyme.)
4. Separate the fragments in the three preparations using gel electrophoresis. Load each preparation into a separate well.
5. Using semi-log graph paper, prepare a standard curve using the observed migration distances and known fragment sizes for the standard restriction enzyme. The standard curve is a plot of fragment size (base pairs) against migration distance. Since migration distance is inversely proportional to fragment size, plotting migration distance against the log of the number of base pairs produces a straight line.
6. Use the standard curve to determine the size of each fragment produced by the sample under investigation.

Laboratory 7: Genetics of *Drosophila*

In this lab, a genetics experiment is conducted using *Drosophila* fruit flies. A summary of important information for conducting these experiments follows:

1. **Life cycle.** Depending upon temperature, *D. melanogaster* requires 10 to 14 days to complete the stages from egg to adult.
   - **Eggs** hatch into larvae after about one day.
   - **Larvae** undergo three growing stages, or instars, over a 4- to 7-day period. They molt (shed their skins) after the first two stages.
   - **Pupae** form after the third larval instar. A hardened outer case (cocoon) forms around the larva. Inside, a larva undergoes metamorphosis and emerges as an adult fly after 5 to 6 days.
   - **Adults** may live for several weeks. Females may begin mating ten hours after they emerge from the pupa.
2. **Sex of fruit flies.** To properly mate parents and count offspring, the sex of each fly must be recognized. The following *typical* (*but variable*) characteristics are used:
   - A female is larger than a male and has four to six solid dark stripes across the dorsal side (top) of her abdomen. The posterior end of the abdomen is somewhat pointed.
   - A male is smaller than a female and has fewer (two to three) stripes on his abdomen. The posterior end of the abdomen is rounded and heavily pigmented (as if two or three stripes have fused). A male also has a small bundle of black hairs, or sex combs, on the uppermost joint of his front legs.
3. **Virgin females.** After mating, female flies store male sperm to fertilize their eggs. To insure that the female does not use sperm from a mating that occurred before the experiment begins, only virgin, or unmated, females can be used. Since a female does not mate until ten hours after emerging from the pupa, isolating the female soon after emergence will ensure a virgin fly.
4. **Fly mutations.** The experiments cross a normal fly (wild-type) with a fly mutant for a particular trait. The following table shows the commonly used traits and mutations. Mutations are recessive if their abbreviations begin with a lowercase letter, dominant if uppercase.

Table 16-2

Trait	*Wild-Type*	*Wild-Type Abbreviation*	*Mutation*	*Mutation Abbreviation*	*Chromosome*
eye color	red	+	white	*w*	chromosome 1 (sex-linked)
eye shape	round	+	bar	*Bar*	chromosome 1 (sex-linked)
wing	present	+	apterous (absent)	*apt*	chromosome 2
wing	normal	+	vestigial (reduced wings)	*vg*	chromosome 2
antennae	normal	+	spineless aristapedia (enlarged and shaped like a leg)	ss^a	chromosome 3
eye color	red	+	sepia (brown eyes)	*s*	chromosome 3

5. **Fly crosses and genetic notation**. Experiments are usually designed so that a fly homozygous for the mutant trait is crossed with a fly homozygous for the normal trait. The wild type genotype is usually denoted by +, and the mutant type is denoted by an abbreviation for the mutation. An allele pair representing the two alleles for a gene (each on one homologue of a homologous pair of chromosomes) is separated by a diagonal. For example, a monohybrid cross between a wild-type fly and a fly homozygous for apterous wings is indicated by +/+ × *ap*/*ap*. The F_1 generation flies are all heterozygous for this trait, or +/*ap*.

6. **χ^2 statistical analysis.** Results of genetic crosses are evaluated using a chi-square (χ^2) statistical analysis. The purpose of this analysis is to determine how well the results of the genetic crosses fit predicted probability ratios. If the experimental results from a cross between two flies are equal to the results expected (from using a Punnett square), the χ^2 value is 0. To determine whether a nonzero value occurs by chance or because something is wrong with the experiment, a χ^2 statistical table is consulted. If chance explains 95% or more of the deviation of χ^2 from zero (a low value for χ^2), then the experimental results are acceptable. If not, the number of flies produced for each trait indicate that something other than chance is influencing the experiment. Crossing over, deleterious mutations, or nonrandom mating (perhaps some traits produce poor breeders) are factors to consider. High values of χ^2 may also result if the expected values used do not account for sex linkage or are otherwise in error.

Designing an experiment to show independent assortment of chromosomes, sex-linkage, or crossing over: To show any of these modes of inheritance, use fruit flies, and employ the following procedures:

1. To show the independent assortment of chromosomes, make a dihybrid cross using flies with two different mutations, each occurring on separate chromosomes. For example, make the cross +/+;*ap*/*ap* × ss^a/ss^a;+/+. If independent assortment occurs, the F_1 generation will be heterozygous for both traits, or +/ss^a;+/*ap*, and the F_2 generation will show a 9:3:3:1 distribution of traits. (Compare the cross of yellow/green, round/wrinkled peas in the section review of heredity.)
2. To show sex-linkage, make a monohybrid cross involving a sex-linked trait, such as white eyes. A white-eyed female (X^w/X^w) crossed with a wild-type (red-eyed) male (X^+/Y) will produce red-eyed females (X^+/X^w) and white-eyed males (X^w/Y). If these traits were not sex-linked, all F_1 flies, males and females, would be red-eyed (+/*w*).
3. To determine crossover frequencies, make a cross between two genes occurring on the same chromosome. Compare the F_2 generation results to those that would be obtained if the genes assorted independently (genes on different chromosomes).

Laboratory 8: Population Genetics and Evolution

This lab demonstrates genetic equilibrium and the use of the Hardy-Weinberg law to describe allele frequencies in populations. Genetic equilibrium (or Hardy-Weinberg equilibrium) occurs when frequencies of alleles remain constant from generation to generation. In particular, note the following conditions that maintain genetic equilibrium:

1. no mutation
2. no selection
3. no gene flow (no migration)
4. large populations (no genetic drift)
5. random mating

The lab compares a population that is in equilibrium to a population undergoing natural selection against the homozygous recessive condition. The following important conclusions can be made from this exercise:

1. For a population in genetic equilibrium, the allele frequencies (p, q) and the genotypic frequencies (p^2, $2pq$, q^2) remain constant (approximately) from generation to generation.
2. For a population in which natural selection eliminates individuals with the homozygous recessive condition, the frequency of the recessive allele (q) declines from generation to generation. However, the allele is not completely eliminated because it is masked in individuals with the heterozygous condition ($2pq$ individuals).

For further review of Hardy-Weinberg concepts, you should read the section on evolution in this book.

Designing an experiment to determine whether a population is in genetic equilibrium: Alleles in the gene pool of a population in Hardy-Weinberg equilibrium remain constant through the generations. In addition, $p + q = 1$ and $p^2 + 2pq + q^2 = 1$. Deviations from these conditions indicate that the population is not in equilibrium, that allele frequencies are changing, and that evolution is occurring.

Laboratory 9: Transpiration

The first part of this lab demonstrates how transpiration rates vary with environmental conditions. Recall from Lab 1 that water moves from areas of higher water potential to areas of lower water potential. Movement of water through a plant is influenced by each of the following mechanisms:

1. **Osmosis.** Water enters root cells by osmosis because the water potential is higher outside the root in the surrounding soil than inside the root. Dissolved minerals contribute to a lower water potential inside the root by decreasing the solute (osmotic) pressure.
2. **Root pressure.** As water enters the xylem cells, the increase in solute pressure produces root pressure. Root pressure, however, causes water to move only a short distance up the stem.
3. **Transpiration.** In leaves, water moves from mesophyll cells to air spaces and then out the stomata. This occurs because the water potential is highest in the mesophyll cells and lowest in the relatively dry air outside the leaf. Evaporation of water from plant surfaces is called transpiration.
4. **Cohesion-tension.** Cohesion (attraction of like molecules) between water molecules occurs because weak hydrogen bonds form between the polar water molecules. As a result, water acts as a continuous polymer from root to leaf. As transpiration removes molecules of water from the leaves, water molecules are pulled up from the roots. The transpirational pull of water through the xylem vessels decreases the pressure potential, resulting in negative water potential, or tension. The cohesion-tension condition produced by transpiration is the dominant mechanism for the movement of water up a stem.

The following factors affect transpiration rates:

1. **Temperature.** When the temperature of liquid water rises, the kinetic energy of the water molecules increases. As a result, the rate at which liquid water is converted to water vapor increases.
2. **Humidity.** An increase in humidity increases the water potential in the surrounding air. In response, the rate of transpiration decreases.
3. **Air movement.** Moving air removes recently evaporated water away from the leaf. As a result, the humidity and the water potential in the air around the leaf drops, and the rate of transpiration increases.
4. **Light intensity.** When light is absorbed by the leaf, some of the light energy is converted to heat. Transpiration rate increases with temperature.

On very hot or dry days, the loss of water by transpiration may exceed the rate by which water enters the roots. Under these conditions, the stomata may close to prevent wilting.

In the second part of the lab, a plant stem is prepared for microscopic study. The following plant cell types are identified. (For a complete discussion of these cell types, see the review section on plants.)

1. **Parenchyma** cells have thin walls and serve various functions including storage, photosynthesis, or secretion. Starch grains are visible in parenchyma cells serving a storage function.
2. **Collenchyma** cells have thick but flexible walls and serve to provide flexible support. In stems, they are often located just inside the epidermis.
3. **Sclerenchyma** cells have thicker walls than collenchyma and provide rigid support. **Fibers** are specialized sclerenchyma cells located toward the outside portion of vascular bundles. They provide support to the vascular bundle and shield the phloem from insect invasion.
4. **Xylem** cells transport water. They have thick secondary walls that prevent them from collapsing under the negative pressure that develops from transpiration. There are two kinds of xylem cells, **tracheids** and **vessel members.** Both are dead at maturity.
5. **Phloem** cells transport sugars. Phloem tissue consist of two kinds of phloem cells, **sieve-tube elements** and **companion cells.** Sieve-tube elements lack nuclei. Companion cells provide physiological support for the sieve-tube elements.
6. **Dermal tissue** consists of **epidermis cells** that cover the outside of plant parts and **guard cells** that surround **stomata.**

Designing an experiment to measure the effects of environmental conditions on the rate of plant transpiration: The transpiration rate in a bean seedling can be measured directly by the methods employed in this lab. In brief, the procedures are as follows:

1. Prepare a potometer by inserting a pipette into one end of a flexible tube. Bend the flexible tube into a U shape so that the open ends of the pipette and tube are pointing up. Fill the tube and pipette completely with water and insert the stem of a freshly cut seedling into the open end of the tube. Apply petroleum jelly to seal the space between the stem of the seedling and the tube.
2. Prepare one potometer for each environmental condition to be investigated. High temperature, high humidity, bright light, or moving air are examples.
3. Prepare one additional potometer for normal conditions, a control to which the transpiration rate at extreme conditions can be compared.
4. Measure the transpiration rate by observing the change in water level in the pipette over several intervals of time.
5. Determine the total leaf surface area.
6. Determine the transpiration rate by dividing the total leaf surface area by the amount of water lost indicated by the pipette.
7. Graph the results.

Laboratory 10: Physiology of the Circulatory System

In the first part of this lab, blood pressure is measured in individuals subjected to various conditions. A **sphygmomanometer** is used to measure blood pressure. The blood pressure cuff is inflated so that blood flow through the brachial artery in the upper arm is stopped. As pressure in the cuff is released, a stethoscope is used to listen for the blood flow entering the brachial artery. When blood first enters the artery, snapping sounds called **sounds of Korotkoff** are generated. Blood pressure is determined as follows:

1. **Systolic blood pressure** is the blood pressure generated by the contraction of the ventricles. It can be read from the sphygmomanometer when the sounds of Korotkoff are first heard in the stethoscope as pressure in the blood pressure cuff is released.
2. **Diastolic blood pressure** is the blood pressure maintained by arterial walls between ventricular contractions. It is read from the sphygmomanometer when the sounds of Korotkoff disappear.
3. A systolic reading of 120 mm Hg and a diastolic reading of 75 is recorded as 120/75.

The following changes in blood pressure and heart rate (pulse) are observed:

1. Blood pressure and heart rate increase when the subject moves from a reclining to a standing position. The increases counteract the gravitational forces on blood movement.
2. Blood pressure and heart rate increase with increase in body activity (exercise). The increases supply additional oxygen to muscle tissue.
3. Individuals who are physically fit require less time than physically unfit individuals for blood pressure and heart rate to return to normal values after exercise. The hearts of fit individuals pump a larger volume of blood with each contraction (**stroke volume**) and deliver more O_2 to muscle tissue than that which occurs in hearts of unfit individuals. As a result, blood pressure and heart rate increases are smaller for fit individuals, and the time required to return to normal conditions is shorter than it is for unfit individuals undertaking the same amount of activity.

In the second part of this lab, the Q_{10} value is calculated for *Daphnia*, the water flea. The Q_{10} value measures the increase in metabolic activity resulting from a 10°C increase in body temperature in ectothermic animals. As environmental temperatures rise, body temperatures and the rates of chemical reactions and physiological processes increase. The Q_{10} value cannot be determined for endothermic animals because body temperatures remain constant regardless of environmental temperatures.

Q_{10} is determined for *Daphnia* by counting the number of heartbeats per minute at two temperatures. The following formula gives the Q_{10} value for two temperatures that differ by 10°C:

$$Q_{10} = \frac{\text{heart rate at higher temperature}}{\text{heart rate at lower temperature}}$$

Ectothermic animals use behavior to help regulate their body temperatures. Moving into the sun to get warm or burrowing into a hole to keep cool are examples. Refer to the review section on animals for a complete review of thermoregulation.

Designing an experiment to measure the effects of drugs on blood pressure: Heart rate changes in *Daphnia* can be measured in response to nicotine, alcohol, caffeine, aspirin, or other drugs by adding varying concentrations of these substances to the *Daphnia* culture fluid. Graph the results showing change in concentration (independent variable on *x*-axis) and heart rate (dependent variable on *y*-axis). Be sure to include a control measurement (drug concentration = 0).

Laboratory 11: Animal Behavior

The objective of this lab is to observe animal behavior in pillbugs (a terrestrial isopod) and fruit flies (*Drosophila melanogaster*). (An older version of this lab examined behavior in the brine shrimp, *Artemia*). Refer to the review section on animal behavior for a complete review of the various kinds of behavior.

For pillbugs, you align two petri dishes side by side with a small section of the touching walls cut away to provide a passageway between the dishes. The lab exercise manipulates substrate moisture and humidity by lining the dishes with filter papers, one damp and one dry. You then count the number of pillbugs (the dependent variable) per unit time (the independent variable) that move from one dish chamber to the other.

In the second part of the lab, you observe courtship behavior of mating fruit flies. Males and females have specific behaviors that often follow a fixed pattern. To reduce the number of variables, you should first observe these behaviors using one male and one female.

Designing an experiment to measure the effects of environmental variables on animal behavior: Select an organism and observe the organism in the absence of applied stimuli. Then, change the environment by introducing a stimulus. Only one stimulus should be applied at a time. Be sure to include the following in your experimental design.

1. One or more experimental treatments.
   - Physical stimuli include moisture, temperature, light, sound, gravity, pH, and chemicals (salt, drugs, nicotine, alcohol, caffeine, aspirin, pesticides).
   - Biotic stimuli include the introduction of members of the same species (males or females) or other species (predators or prey). If members of the same species are introduced, the sex of the introduced individual may influence behavior (mating or agonistic behaviors). Multiple members of the same species may elicit social behaviors.
2. A control treatment to which the experimental treatments can be compared.
3. A graph, histogram, or other graphic representation of the data.
4. An interpretation or discussion of the data.

Laboratory 12: Dissolved Oxygen and Aquatic Primary Productivity

In this lab, the amount of oxygen dissolved in natural water samples is measured and analyzed to determine the primary productivity of the sample. The amount of dissolved oxygen is dependent upon many factors, including the following:

1. **Temperature.** Dissolved oxygen concentration decreases as temperature increases.
2. **Salinity.** Dissolved oxygen concentration decreases as salt concentrations increase.
3. **Photosynthesis.** Photosynthetic activity produces oxygen and increases the amount of dissolved oxygen.
4. **Respiration.** Respiration consumes oxygen and reduces the amount of dissolved oxygen.

The primary productivity of a community is a measure of the amount of biomass produced by autotrophs through photosynthesis (or chemosynthesis) per unit of time. Primary productivity can be determined by measuring the rate at which CO_2 is consumed, O_2 is produced, or biomass is stored. In this lab, primary productivity is determined by the amount of O_2 produced. In addition, the effects of light, nitrogen, and phosphorus on primary productivity are examined.

Primary productivity can be examined with respect to the following factors. (Note that the term **rate** means *per unit time*.)

1. **Gross primary productivity** is the rate at which producers acquire chemical energy before any of this energy is used for metabolism.
2. **Net primary productivity** is the rate at which producers acquire chemical energy less the rate at which they consume energy through respiration.
3. **Respiratory rate** is the rate at which energy is consumed through respiration.

The following four procedures are used to determine primary productivity:

1. The **Winkler method** is used to *measure* dissolved oxygen using a titration technique. Titration is the process of adding a substance of known concentration to a solution containing a substance of unknown concentration until a specific reaction is completed and a color change occurs.
2. The **light and dark bottle method** is used to *compare* dissolved oxygen values in water samples exposed to light and dark. Three samples are required, as follows:
   - **Initial bottle.** The amount of dissolved oxygen in this sample will provide an initial value to which the remaining two samples can be compared.
   - **Light bottle.** Expose the second sample to light. After twenty-four hours, determine the amount of dissolved oxygen in the sample.
   - **Dark bottle.** Store the third sample in the dark. After twenty-four hours, determine the amount of dissolved oxygen in the sample.
3. The dissolved oxygen values determined by the Winkler method or by the light and dark bottle method are used to calculate primary productivity, as follows:
   - **Net primary productivity** is determined from the amount of dissolved oxygen gained in the sample exposed to light. The increase is calculated by subtracting the amount of dissolved oxygen in the initial bottle from the amount in the light bottle.
   - **Respiratory rate** is determined from the amount of dissolved oxygen lost in the sample kept in the dark. The decrease is calculated by subtracting the amount of dissolved oxygen in the dark bottle from the amount in the initial bottle.
   - **Gross primary productivity** is determined by *summing* the absolute values of the net primary productivity and the respiratory rate, or gross primary productivity = net primary productivity + respiratory rate.
4. Convert dissolved oxygen values to mg carbon fixed/L/day. At standard temperature and pressure (0°C and 1 atmosphere pressure), the production of 1 ml of O_2 corresponds to an assimilation of 0.54 grams of carbon into glucose.

Designing an experiment to measure primary productivity in an aquatic community: Follow the four procedures above for determining primary productivity. You can investigate the effect of light, nutrients, pesticides, or other substances. Prepare a bottle of water for each effect you investigate and an initial bottle of water for comparison. You may also investigate the effect of varying concentrations of a particular substance. In this case, prepare additional water samples for each concentration of the substance to be examined. The effect of light intensity can be investigated by exposing additional samples to various intensities of light. Various intensities of light can be produced by using different numbers of screens to filter the light source. Varying the light in this manner can also be used to simulate different depths of water.