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# **Isolation of DNA from Gels**

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 The technique of isolating DNA from a gel has many applications including the isolation of individual DNA fragments to be used for subcloning a specific piece of DNA. DNA isolated from bands in gels can also be digested with additional restriction endonucleases to generate restriction endonuclease maps. Individual bands of DNA can also be labelled and used as specific probes in hybridization experiments.

 As gel electrophoresis is used more often in the undergraduate laboratory, there is a need for experiments demonstrating further applications of gel electrophoresis. Gels can be used in Southern blotting experiments (Karcher, 1991). Gels can also be used to isolate specific DNA fragments. This mini workshop demonstrated how to isolate DNA from agarose gels by electroelution.

 To isolate specific fragments of DNA from an agarose gel by gel electroelution, the DNA is first digested with the appropriate restriction endonuclease and subjected to electrophoresis through an 0.8% agarose gel. After electrophoresis, the gel is stained in ethidium bromide  $(0.5 \text{ µg/ml})$  and viewed under ultraviolet light to visualize the DNA bands. Using great care to minimize exposure to the ultraviolet light, the specific DNA band of interest is cut out of the gel with a razor blade or scalpel. Care is taken not to scratch the surface of the ultraviolet light box. Placing the gel on a sheet of plastic wrap on top of the light box can minimize scratches to the light box. Using forceps and gloved hands, the gel slice with the DNA of interest is placed inside a dialysis tubing bag. The bag is filled with a small amount of gel electrophoresis running buffer (0.5X TBE; 0.0445 M Tris— Trizma base—pH 8.0, 0.0445 M boric acid, 0.01 M EDTA—ethylenediamine tetraacetic acid) and tied or clamped shut. The dialysis bag is then placed in an electrophoresis chamber and subjected to electrophoresis until the DNA migrates out of the gel piece. The DNA migrates out of the agarose gel fragment and remains in the buffer within the dialysis tubing. The dialysis bag is then opened and the buffer (which contains the DNA) within the dialysis bag is removed with a pipet. The buffer-DNA sample can be used as is or the sample can be precipitated with alcohol to concentrate the DNA. The DNA sample can be further purified by phenol extraction. The DNA isolated this way can be used in additional restriction endonuclease digestion reactions or in labelling reactions. For more details, see Karcher (1991:12).

 Electroelution is a useful method to isolate DNA from gels in the teaching laboratory because it is an easy procedure that allows the students to visualize the DNA readily during the procedure. There are numerous other methods to isolate DNA from gels, including centrifugation through filter paper. For some examples, see the references listed below.

Ericson, M. L. 1990. Quick DNA recovery from agarose gels by ultracentrifuge run. Trends in Genetics, 6:278.

Heery, D. M., F. Gannon, and R. Powell. 1990. A simple method for subcloning DNA fragments from gel slices. Trends in Genetics, 6:173. (Centrifugation through glass wool.)

**Sample Citation:** Karcher, S. J. 1993. Isolation of DNA from gels. Page 209, *in* Tested studies for laboratory teaching, Volume 14 (C. A. Goldman, Editor). Proceedings of the 14th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 240 pages.

- Copyright policy: http://www.zoo.utoronto.ca/able/volumes/copyright.htm

Although the laboratory exercises in ABLE proceedings volumes have been tested and due consideration has been given to safety, individuals performing these exercises must assume all responsibility for risk. The Association for Biology Laboratory Education (ABLE) disclaims any liability with regards to safety in connection with the use of the exercises in its proceedings volumes.

- Karcher, S. J. 1991. Non-radioactive DNA hybridization experiments for the undergraduate laboratory: The Southern blot analysis. Pages 1–31, *in* Tested studies for laboratory teaching. Volume 12. (C. A. Goldman, Editor). Proceedings of the 12th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 218 pages.
- Koenen, M. 1989. Recovery of DNA from agarose gels using liquid nitrogen. Trends in Genetics, 5:137.
- Peloquin, J. J., and E. G. Platzer. 1991. A simple inexpensive electroelution device for the recovery of nucleic acid fragments from agarose gels. BioTechniques, 10:159–160. (Electroelution using a microfuge tube instead of dialysis bag.)
- Vogelstein, B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. Proceedings of the National Academy of Sciences, U.S.A., 76:615–619. (Use of chaiotropic salt NaI.)
- Weichenhan, D. 1991. Fast recovery of DNA from agarose gels by centrifugation through blotting paper. Trends in Genetics, 7:109.

# **Teaching the Electron Transport Chain**

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 We have used the technique presented here to successfully teach electron transport to groups of from 12 to 40 students in both lecture and laboratory settings. The goal is to emphasize the concept that energy is released and stored as bonds are broken in one molecule and formed in another. This technique also helps students to connect laboratory experiments (which often focus simply on the beginning and end-products of respiration) with the more complex pathways they are learning in lectures.

 The materials required for each student are one small paper cup, one piece of candy, and one sign. We make one sign for each carrier molecule in the electron transport chain, and enough signs with "oxygen" printed on one side and "water" printed on the other to give one sign to every member of the class. It is important that the signs be large enough to be read by all members of the class. We make our signs using the poster option in the computer program Printshop. The posters are enlarged to  $11'' \times 17''$  on a copy machine. We attach yarn to the signs representing the carrier molecules, so that the students wearing these signs are able to put them around their necks and free their hands.

 As students enter the room, hand each student a sign and a cup. The signs assign students to represent the carrier molecules in the electron transport chain (NAD, FMN, C<sub>o</sub>O, cytochromes), or to become oxygen. Have the students who represent the carrier molecules line up in the correct order. The remaining students line up on one side of the room with their signs showing oxygen. (We sometimes have to "tease" the students into doing this). Explain to the students that the candy represents electrons, and at any one time a student may only have one piece of candy in his or her cup. Drop a piece of candy into the cup of the student representing NAD (this is an appropriate time to review REDOX), have the students act out a "bucket brigade" ending with one of the "oxygens" receiving a piece of candy and becoming "water." Repeat until all the "oxygen" has become "water." We bring a few extra pieces of candy to the class and allow the chain to backup, leaving all the electron carriers in the reduced state. This lends itself to a discussion of the relationship between aerobic and anaerobic respiration.

## **Membrane Permeability: A Quantitative Approach**

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 The processes of diffusion and osmosis are vitally important to all living organisms. Most general biology courses include laboratories studying these processes but few of them utilize a truly quantitative approach. This laboratory exercise allows students to use living cells (sheep erythrocytes and *Elodea*) to examine the processes of diffusion and osmosis and collect empirical data. Students then analyze this data to better understand the relationships involving a membrane's permeability to various solutes.

 By first placing fresh red blood cells in solutions of varying saline tonicity (e.g., distilled water, 0.15 M NaCl and saturated NaCl), students observe, under the microscope, the effects of osmosis on a typical animal cell. This is also an appropriate opportunity to review concepts such as solution concentration and terms like solute/solvent, hypo-/iso-/hypertonic, plasmolysis/turgor, and hemolysis/crenation.

 The relationship between molecular size and solute permeability is easily studied by adding, to test tubes each containing 3 drops of erythrocytes, 5 ml of 0.3 M solutions of nonelectrolytic solutes with differing molecular weights (e.g, water, urea, glycerol, glucose, and sucrose). Students can hold these tubes up to a printed page and count the number of seconds before the tubes clear, allowing them to read the page through the tube. These values can then be graphed, and the relationship between size and permeability of each of the solutes can be determined quantitatively.

 Using the principles discussed above, students can use *Elodea* cells to approximate the isoosmotic concentration of various solutes in the vacuolar sap. First, *Elodea* cells are introduced to varying concentrations of a given solute. A field of 100 or so cells is examined, and the number of plasmolyzed and normal cells is counted. Plasmolyzed cells (i.e., % cells plasmolyzed) is graphed versus solute concentration (in M); the subsequent solute concentration which corresponds to 50% plasmolysis is roughly equivalent to the iso-osmotic concentration of that solute in the plant's vacuolar sap. Graphical comparisons of these values can be made for a variety of solutes (e.g., electrolytes vs. nonelectrolytes, electrolytes of differing valence, nonelectrolytes of varying molecular size) to better understand the principles of membrane permeability and how different solutes affect the rates of diffusion/osmosis in living cells.

Bennethum, T. M., J. A. Chiscon, M. O. Chiscon, C. R. Carlin, R. H. Shippee, and J. W. Vanable, Jr. 1992. Laboratory manual for *Biology the Basic Concepts.* Department of Biological Sciences, Purdue University. [pages 9–15]

# **Teaching Enzyme Kinetics Using a Commercial Diagnostic Assay for Glucose in Plasma**

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 Introductory biology students often have difficulty with the concepts involved in enzyme kinetics. One would hope that the laboratory would be a source of clarification for these problems but, depending on the enzyme system being utilized, that may not be the case. While having students extract the enzyme that will be used in the assay has many merits, the data obtained from this method are often skewed or fluctuate from one run to the next which can be both confusing and frustrating to the introductory level student. I have found that performing a colorimetric assay using a commercial preparation of an enzyme mixture used for the diagnostic determination of glucose in blood plasma eliminates the disparity between runs and is virtually foolproof. This assay also has the added advantages of requiring very little preparation time, it can easily illustrate a number of principles relating to enzymes, and the experiment length can be tailored to meet one's needs.

 The enzyme mixture (Sigma Diagnostics Glucose Trinder #315, Sigma Chemical Co., St. Louis, MO 63103) once reconstituted with deionized water is stable for up to 3 months if refrigerated. The basic assay I have worked out using this enzyme is the following: 0.5 ml glucose  $(0.2 \text{ mg/ml}) + 1.5$ ml  $dH_20 + 1.0$  ml enzyme. Once mixed (time zero), spectrophotometric readings at 510 nm are taken at 15, 30, 45, 60 120, and 180 seconds. This gives a nice linear graph.

 I have the students perform this assay several times, changing parameters as they go. They test the specificity of the enzyme for its substrate by performing the assay not only with glucose, but also with the isomers of glucose, mannose and galactose (it is readily evident from the data that this enzyme complex is specific for glucose and the relative position of an -OH on the ring does indeed make a difference). The students also test the effect temperature has on the enzymatic reaction (room temperature,  $4^{\circ}C$ ,  $37^{\circ}C$ , and  $65^{\circ}C$  are adequate to demonstrate the changes), and I also introduce the students to the concept of Michaelis-Menton kinetics by having them perform an experiment where the enzyme concentration is kept constant and the glucose concentration is varied (I have them use 0.1, 0.3, 0.5, 0.7, and 1.0 ml of 0.2 mg/ml glucose). They may then determine for the enzyme the maximal velocity for the enzymatic reaction (Vmax) and the Michaelis constant (Km, a measure of the strength of the enzyme-substrate complex).

 This assay, by virtue of its simplicity, also lends itself quite nicely to independent projects. Students can be given the general assay procedure and be asked to hypothesize the effects of temperature, pH, or substrate concentration on the enzyme's effectiveness. They can then test their hypotheses quickly and build upon them. The quick, positive results they obtain from experiments using this assay tend to elicit a positive attitude toward future experiments, which is indeed an added benefit!

# *Marsilea* **– The Fast Fern**

### *Leland G. Johnson*

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 The aquatic fern, *Marsilea* (sometimes called "water clover"), is a useful teaching organism because of the speed with which gametophyte development proceeds.

 Development is initiated by hydration of the contents of the sporocarp, which consists of a hard capsule enclosing numerous megaspores and microspores. When the capsule is cut open and placed in a fern culture medium, clean pond water, or even bottled spring water, a gelatinous ring swells within a few minutes and carries the spores out of the sporocarp. Archegonium and antheridium development occurs quickly. At room temperature, sperm release occurs 6–8 hours after hydration, or in as little as 5 hours at  $30^{\circ}$ C. Though the period of active sperm swimming is relatively brief, students often succeed in observing swimming sperm in sample drops taken from the culture.

 Development of the sporeling (young sporophyte generation plant) is also quite rapid. Within 48 hours the embryo, which is faintly visible inside the gametophyte, has leaf and root primordia. By 4 days, a small green-sheathed leaf and a primary root emerge from the gametophyte. The sporeling can be cultured for further observation.

 Several major biological supply companies offer *Marsilea* sporocarps and include informative instruction bulletins with shipped orders. Though *Marsilea* sporocarps are relatively expensive, *Marsilea* gametophyte development can be a very informative and interesting addition to labs in general biology, developmental biology, or any of several plant biology courses.

## **Grocery Store Botany**

### *Laura K. Thompson*

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 This laboratory exercise has been used to introduce students to taxonomic keys and the names given to various plant fruits. Materials needed for the laboratory include kitchen knives, dinning hall trays, and a large variety of fruits and vegetables from the local store or collected in the field. Figure 1 is the key used by the students for this laboratory, while Table 1 is used by the instructor during preparation for the laboratory.

 Before the laboratory session the fruits are distributed on one table in the front of the laboratory room. Student interest is stimulated as they arrive for class by letting them handle the fruit. Usually the students are not told what will happen in this laboratory, so they are always a little curious. The laboratory session begins with an explanation of the taxonomic key: why scientists use keys, how keys are used, and differences between keys. Also, some of the terminology used in the key is explained, for example, the difference between simple fruits *(2a)* and fruits derived from more than one pistil *(2b)*. The particular key shown in Figure 1 is simplified for use with non-biology major students, thus some of the technical taxonomic vocabulary has been simplified, for example, the term accessory fruits *(4b)* is usually explained not as superior and inferior ovaries, but as to the location of the flower in relation to the fruit.

 After all introductory remarks have been made, the laboratory exercise is started by the instructor tossing one of the fruits to a student and asking them to key the fruit "out-loud," while the other students are asked to agree or disagree with the "keying" student. This process is usually started with a fruit that is easily keyed, for example a peach or cherry. Students are not allowed to make assumptions. For instance, if they come to a question "Fruits with more than one seed...*(3b)*" and they answer "Yes," the instructor then counters with "How do you know that?!" At this point the student is requested to prove their answer by cutting open the fruit and displaying the seeds. Once the fruit has been keyed, the student can then distribute the fruit to the other students for consumption. The laboratory is usually ended with the keying of a pineapple, which all the students seem to enjoy eating.

 This laboratory allows for a great deal of creativity. The variety of fruits available can change with the season. Discussions can be built around the different fruits such as seedless oranges, hybrid corn, and pineapple production. In conclusion, this laboratory can be quite fun, noisy, and tasty.

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### **Table 1.** Key to common fruits.





## Figure 1. Common types of fruits.

# **A Problem Solving Approach to Animal Physiology**

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 A problem solving (PS) emphasis was used in a course for 15 students, sophomore through doctoral. Our working definition of PS included the ability to resolve a situation by knowing and recognizing pertinent facts, and being able to find at least one defensible alternative cause or outcome consistent with the student's state of knowledge. Activities of PS included PS strategy handouts, instructor modelling of PS, whole class PS activities in which the instructor acted only as a recording secretary, written PS assignments for the weekly laboratory activities, and periodic individual PS assignments. Lecture and textbook materials established a knowledge base upon which PS activities were affixed for summary and review. The evaluation criteria used for the PS activities were based on the identification of the issue, important factors associated with the problem, and alternative solutions, as well as evaluation of alternative solutions, relevancy of outcomes, and defense of student's preferred outcome. Written examinations also included 30% PS essay questions.

 For laboratories, students were divided into small groups that enabled peer instruction in content and PS. Each group should be small, not include friends, and reflect different ages, sexes, backgrounds, and abilities. Group-generated written responses to content and PS questions were turned in at the beginning of each laboratory. Laboratory exercises required specific activities followed by an inquiry component, which progressively became a larger portion of the laboratory. For the inquiry part, the groups cleared their experiment designs with the instructor, carried them out, and shared their data with other groups. Groups also answered post-lab PS questions which often concerned the data from the inquiry portion of the laboratory.

 An initial laboratory involved formulating, testing, and application of appropriate statistics to a hypothesis concerning the length of student fingers. Groups devised their procedures, wrote them up, and gave them to another group to perform. Group written directions often had to be revised for the other group to understand. Results were presented to the class, with discussion. At a later date an entirely inquiry-type laboratory required the students to determine what kinds of environmental conditions were perceived by adult *Tenebrio* (meal worms), and the neural receptors possibly involved. The groups presented their experimental design to the instructor for review, revised, and performed their experiments. Each group presented procedures, results (including appropriate tables and graphs), and conclusions to the class, followed by discussion. Group results were occasionally "negative" and conflicting, as is true of science. The students clearly improved their experimental design and PS skills during the semester.

# **Following Directions: A No Fuss, Paper and Pencil Lab Activity**

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 This no-fuss exercise is one of my favorite labs and has been adapted from an *American Biology Teacher* article by Brett (1989) — who is Chair of the Life Sciences Department at Indiana State University. It is a seemingly simple activity that challenges students to read and reread the provided directions and to translate the language into a visual figure. Terms and definitions are listed with requisite directions for constructing a two-dimensional hypothetical insect. The only materials required are pencil, eraser, paper, metric ruler, protractor, simple geometry aid, and the instructions.

 I use this exercise as an introductory activity to impress upon the students the importance of reading to extract the most precise meaning of terms. Students work together in groups of two to four and while it encourages cooperative learning, this type of project enables them to informally become acquainted and to expose their varied thinking skills and resources. It enables the instructor to passively observe the different abilities and approaches as the students tend to think out loud while discussing their interpretations of the directions.

 The instructions in the original article entitled *Insect singularis* do indeed challenge the students. The ambiguities that students criticize in the outline emphasize the need for clear descriptions using clean language. When students complain, I usually nod agreeably and suggest they produce an improved version which I will gratefully adapt. As the wordy blueprint becomes graphically visible, the students respond by accelerating their efforts and smiling in satisfaction. With non-majors, this exercise usually requires a 3-hour laboratory period; about half the time with majors.

 I have rewritten this exercise to allow more space between the directions and to enable the students to pause between instructions. While the text is identical to the original article, the style is more student-friendly. I will be glad to share a copy upon request.

Brett, W. J. 1989. Insect singularis. American Biology Teacher, 51:43–45.

## **Using Flow Diagrams to Learn Cell Biology**

### *Thomas Fogle*

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 Beginning students who are learning basic features about eukaryotic cell biology often have difficulty integrating concepts. Synthesis of knowledge presupposes an understanding of the basics. Flow diagrams, together with an accompanying list of questions, promote review and encourage students to search out new conceptual relationships that are not otherwise obvious. Using a sequential set of diagrams, each one adding more detail, students develop a richly dynamic view of cellular biology. In the process, there is a sense of self-discovery about how ideas fit together. Students can be challenged with "thought experiments" in which they follow paths in the flow diagram to hypothesize outcomes. That is, flow diagrams have heuristic properties.

 Flow diagrams are most successful when used as a capstone for review and synthesis rather than the initial introduction to the concepts. An introductory flow diagram on cytological/physiological aspects of the cell becomes the framework for building additional knowledge. Linked to the initial flow diagram are the processes of cell respiration, photosynthesis, and gene expression, in that order. Completion of the series of diagrams results in a complexly integrated view of the cell that has been constructed by a stepwise linking of concepts and terms.

 The advantages of flow diagrams are that: (1) class time is spent laying the groundwork and student time is spent on developing higher-order understanding; (2) students find this to be a very helpful way of pulling it all together; and (3) new conceptual relationships are discovered that are not evident from classroom or text learning.

## **Growlab: A Gardening-Based Curriculum Guide**

### *Denise Martin*

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 Growlab is a gardening-based program designed by the National Gardening Association (Burlington, VT). The curriculum guide, *Activities for Growing Minds* (Cohen and Pranis, 1990), places a strong emphasis on the experimental and dynamic aspect of science for kindergarten through eighth grades. Instead of being cast in the role as the sole source of information, teachers are encouraged to be facilitators and co-learners with their students. Emphasis is placed on helping students develop valuable skills, such as making observations, posing testable questions, designing and conducting sound experiments ("fair tests"), and interpreting results.

 The curriculum guide is designed to be flexible with student input as a key ingredient. While intended to be the springboard for further inquiry within science, and for interdisciplinary activities, the usually open-ended lab exercises are sufficiently structured so they can be tailored for a variety of competency levels. Even inexperienced gardeners can benefit greatly from the lab exercises and, in fact, the program is a tremendous confidence builder. At St. Michael's College, the Growlab curriculum guide is an important part of the laboratory in our Biology for Elementary Education Majors course, a required sophomore-year course.

 The are numerous reasons for using the Growlab curriculum guide in a biology course for Elementary Education majors. The curriculum guide places a strong emphasis on the importance of making careful observations, designing fair tests and posing testable questions, and interpreting data. The guide is currently being used in many K–8 classrooms throughout the United States and can be used in future teaching endeavors. There are many opportunities to incorporate language arts, mathematics, and social skill development into the program, thus emphasizing the interrelationship of science and the liberal arts. Although geared for the K–8 age group, many of the exercises can be valuable learning experiences for college-aged students. As part of the project, students are asked to develop more sophisticated questions and approaches to the exercises that are appropriate for their own educational background. Finally, most of the materials are readily available, relatively inexpensive, and do not require sophisticated environmental conditions beyond a lighted space.

 For more information about the National Gardening Association's educational materials and programs write or call: National Gardening Association, 180 Flynn Ave., Burlington, VT 05401, 802/863-1308, FAX: 802/863-5962.

Cohen, J., and E. Pranis. 1990. Growlab: Activities for growing minds. National Gardening Association, Burlington, Vermont, 307 pages.

# **Science Olympics: Helping Biology Compete**

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 The annual event known locally as the "Science Olympics" draws over 200 secondary school students to the University campus as competitors in various events that challenge their scientific knowledge and creativity in the areas of biology, chemistry, and physics.

 A Science Olympics Manual providing background information, rules, and hints for all events is available to schools 2 months prior to the Olympics. Teams are assembled and begin training. Whenever more detailed instruction or coaching is required, one member of each team meets with the event coordinator in a "workshop." The Grade 9 and 10 events emphasize team work and participation; teams are large and the events tend to involve physical activity. Upper-level events focus more on individual knowledge and problem solving skills.

 The Olympics format requires events that: (1) are derived from the secondary school curriculum, (2) can be prepared or practised in advance, and (3) can be completed reasonably rapidly on the day of competition. It has been my experience that developing biology-based events requires extra creativity in order to conform to this format. Examples from past and present biology events are listed below.

**Amazing Rodents:** Students construct a standard maze and train a mouse, hamster, or gerbil to run it in the minimum time. A training log must be kept to ensure compliance with the Guiding Principles of the Canadian Council on Animal Care.

**"... and** *this* **Little Pig ...":** Students are expected to develop careful dissection technique and become familiar with the name, location, and function of the major organs and physiological structures of the fetal pig. Competition involves location and/or identification of structures on partially dissected male and female specimens.

**Fermi Event:** Students develop their best estimates of quantities that are difficult or impossible to determine exactly. Teams must also provide an estimate of the likely uncertainty in their answers. Fermi questions often involve biological scenarios and/or biophysical problem solving. For example: What is the combined horsepower of all the honeybees from one hive? Project how large Canada's population would be in 250 years if present trends were to continue.

**Stick 'em Up:** Students are expected to become familiar with normal and abnormal human karyotypes. On the day of competition, teams must "cut and paste" and interpret a diagnostic karyotype from a poster-sized enlargement of a human metaphase cell.

**If the Key Fits:** Students are expected to become familiar with the use of dichotomous keys for the identification of organisms such as trees, wildflowers, insects, algae, etc. Another part of this event requires part of each team to construct a key that the remaining part of the team can then use.

Add 'em Up: Students are expected to develop proficiency with the use of the microscope, various micrometers, and cell counting chambers in order to estimate characteristics of cell populations such as cell type, concentration, volume, relative proportions of mixed populations, etc. Protozoans, yeast, and pollen have all been used in this event.

# **Writing Exam Questions That Promote Critical Thinking**

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 In an effort to promote critical thinking among general biology students, teachers may first ask students to define certain key terms like interpretation, conclusion, observation, and assumption. Then students are given problem-solving situations and are asked to apply these terms to the situations given. In order to encourage critical thinking skills among general biology students, one problem-solving situation is incorporated as a part of each unit test. Students may be given experimental data, a graph, or a situation. They are then asked to identify the correct conclusion, interpretation, observation, or assumption. In addition students explain why one answer is correct and the other answers are incorrect. These problems enhance not only critical thinking skills but also integrative writing skills.