Identification of an Unknown Plasmid

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Recent developments in the field of molecular biology and its far-reaching applications have brought biotechnology into the lives and minds of many students. A thorough familiarity with, and understanding of, such topics as recombinant DNA, cloning, and chromosome mapping will be essential for students to adequately tackle the consequent moral and financial concerns brought about by such technology. This laboratory activity is designed to introduce students to some of the strategies and tools employed by molecular biologists using a more problem-solving approach than many other such activities.

Students are presented with an unknown sample of DNA (plasmid, bacteriophage lambda) and are asked to determine its identity. They do this by performing a restriction analysis of the DNA, electrophoretically separating the resultant fragments, staining, and visualizing them. A standard curve is prepared, using lambda H3 marker DNA, which the students then use to determine the fragment sizes of their digested samples. By comparing the restriction profile of their unknown to those of “known” (i.e., determined by the students using actual restriction maps) DNA samples, students can positively determine the identity of their sample.

The restriction maps of two plasmids, pAMP and pKAN, commercially available from Cabisco Technologies (Carolina Biological Supply Company), are shown in Figure 1 (on the next page), along with a stylized representation of their restriction profiles when digested with the endonucleases Bam HI and Hind III. A variety of activities can be prepared through modifications of this approach. I would be interested in hearing about any such modifications.

Bennethum, T. M., J. A. Chiscon, M. O. Chiscon, C. R. Carlin, R. H. Shippee, and J. W. Vanable. 1993. Identification of an unknown plasmid. Pages 129–138, *in* Laboratory manual for *Biology 225-6: The Basic Concepts*. Department of Biological Sciences, Purdue University.

Micklos, D., and Freyer, G. 1990. DNA Science: A first course in recombinant DNA technology. Cold Spring Harbor Press, New York, 477 pages.

Sample citation: Bennethum, T. M. 1994. Identification of an unknown plasmid. Page 347, *in* Tested studies for laboratory teaching, Volume 15 (C. A. Goldman, Editor). Proceedings of the 15th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 390 pages.

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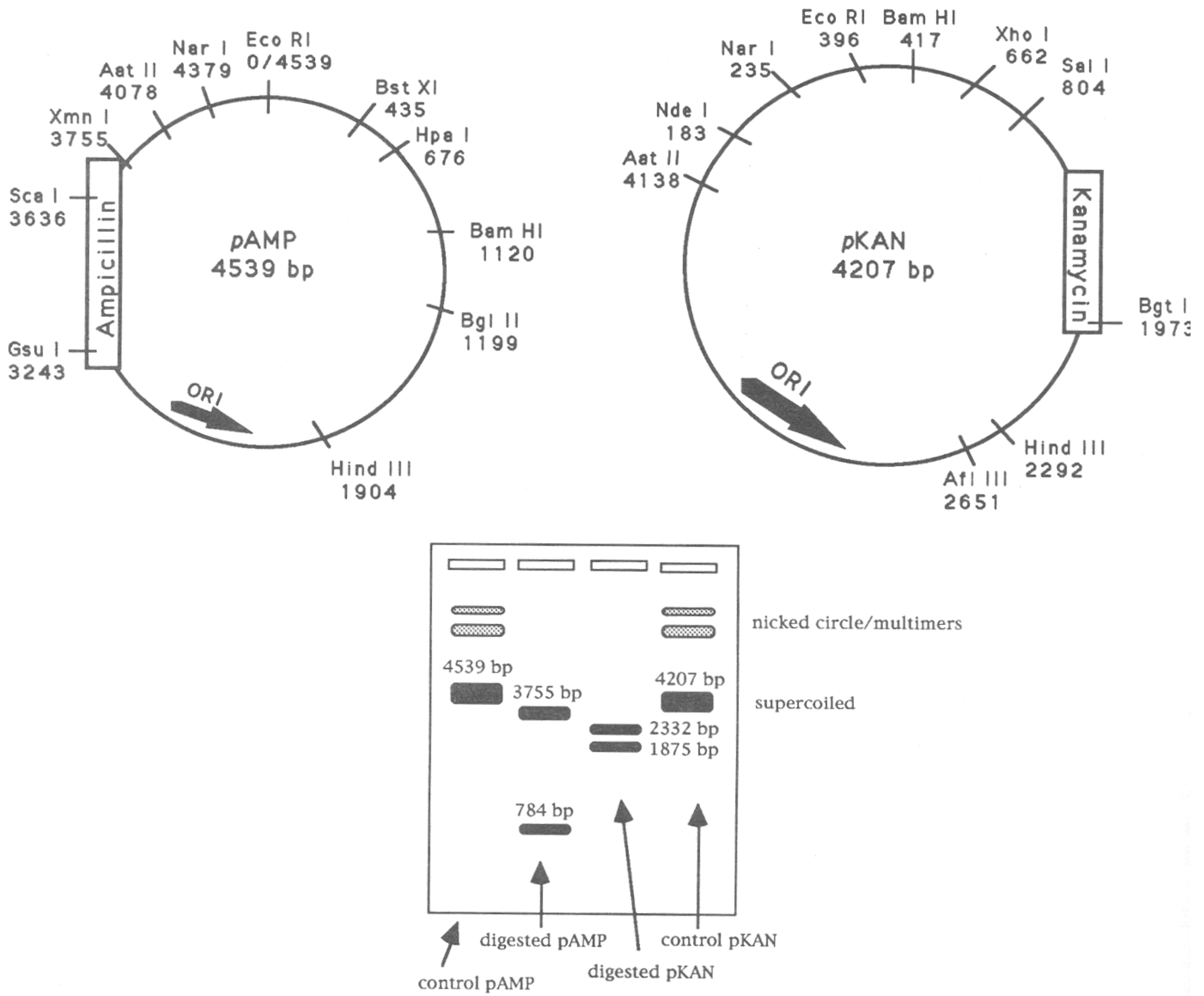

Figure 1. Restriction maps of two plasmids (pAMP and pKAN) and a stylized representation of their restriction profiles when digested with the endonucleases Bam HI and Hind III.

Tissue Printing: A New Way to Study Plant Structure

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When plant tissues are pressed onto nitrocellulose membranes with an even force, an imprint of the tissue is made on the membrane. The imprint of the tissue, called a *physical tissue print*, reveals a great deal of cellular detail that can be viewed with low power magnification. This simple technique for visualizing the anatomy of plant tissues can be used in the laboratory to provide students with a more hands-on laboratory experience in plant structure than is afforded by the usual examination of prepared slides.

Since the proteins (Bickar and Reid, 1992) and nucleic acids within the tissues are also transferred to the membrane, the technique can be used to localize these macromolecules within anatomical structures. Tissue printing, therefore, lends itself to introductory cell biology experiments usually performed in upper-level courses. For a complete guide to the uses and techniques of tissue printing see Reid and Pont-Lezica (1992).

Bickar, D., and P. D. Reid. 1992. A high-affinity protein stain for Western blots, tissue printing, and electrophoretic gels. Analytical Biochemistry, 203:109–115.

Reid, P. D., and R. F. Pont-Lezica (Editors). 1992. Tissue printing: Tools for the study of anatomy, histochemistry, and gene expression. Academic Press, San Diego, California, 188 pages.

A Student-Built Cell Counting Chamber

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It is easy for beginning students to determine the areas encompassed by the fields of view of the various objective lenses of a compound microscope. Such activities are done commonly at the start of a freshman lab. Diameters can be measured accurately by viewing the magnified image of a stage micrometer; acceptable values can be derived using only a millimeter ruler or square-millimeter graph paper. In addition to building familiarity with the microscope, these calibrations contain the seeds of a quantitative way of thinking about laboratory work. The cell counting chamber grew out of a desire to nurture these seeds so that by the time students leave my two-semester introductory lab they are comfortable with a quantitative view of biology and have the skills needed to function quantitatively in upper-level labs.

Simply determining the areas of fields of view involves calculations using exponential notation and the metric system. It is also a first step in dealing with dimensions beyond a student's normal range of perception, an experience that needs to be reinforced. I had them measure one of their coverslips and calculate the proportions of the area of the coverslip observed with each objective. The step from area to volume is then possible by way of finding the volume of water that just “fills” the space under the coverslip. Students did this using microcapillary pipets graduated at 1, 2, 3, 4, 5, and 10 µl. Volumes that are too small are obvious; volumes that are too large are identified by inspecting the edge of the coverslip with the scanning-power lens, looking for extra “puddles.”

Building their own chamber makes a cell counting procedure more transparent to the student than it would be if I gave them a commercial product. Putting the chamber to use brings in the calculations, as well as the pipetting, necessary to master dilutions. The chamber could be used with any non-motile cells visible with the high-dry lens, for example, *Saccharomyces*, *Chlorella*, or *Bacillus*.

I used it to have students estimate the number of red blood cells in 1 ml of sheep's blood. It happens that the classic hemolysis demonstration, which involves adding one drop of blood to 10 ml saline, produces a countable, though high, cell density. I had students sample one of these tubes to derive a preliminary density estimate. They then designed another dilution, which they did in triplicate, providing a good example of true replication. The data are suitable for calculating standard deviations and for graphing as frequency histograms, bringing a great many quantitative skills into use around a simple lab situation that beginning students can comprehend readily.

An Economical Method for Generating and Delivering an Even Flow of CO_2-Gas

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The laboratory exercise "Carbonic Anhydrase" (modified from Maren, 1960) in conjunction with the "Oxygen Dissociation" lab gives the student exposure to various aspects of the gas transport function of blood. The procedure is rather straight forward. The only limiting factor has been the carbon dioxide supply, being too costly for our budget. Two modifications developed in our lab have made it possible to reintroduce this exercise.

The first obstacle to overcome was finding a cost-effective source of carbon dioxide gas. The second hurdle was devising an affordable system which would deliver the gas to the reaction mixture at a controllable even flow rate. The suggestion of sodium bicarbonate and an acid as a low cost source of carbon dioxide gas got us started. The following is a brief outline of the method we now use with the students.

The gas is generated by mixing baking soda and vinegar (1:3, v/v), trapping the gas in a plastic bag, from which most of the air has been removed by flattening it out as much as possible prior to evolving the carbon dioxide gas. (To slow down the rate of the carbonic anhydrase catalyzed reaction, we actually use a 1:1 CO_2-air mixture. This is obtained by taking measured volumes of the generated CO_2-gas and of regular air using a large syringe and transferring these to another plastic bag to arrive at the final gas mixture to be used for the assay.)

With small alterations an aquarium pump is converted to a "closed system." Once alterations are complete the intake tubing of the pump is connected to the plastic bag containing the prepared gas mixture and the outflow tubing is connected to the gas delivery arm of the reaction vessel. Electrically hooked up to a dimmer switch, this pump is transformed into a variable speed pump. The gas mixture can now be delivered to the reaction vessel at an appropriate flow rate, which will remain relatively constant throughout the experiment.

Maren, T. H. 1960. A simplified micro method for the determination of carbonic anhydrase and its inhibitors. Pharmacological and Experimental Therapy, 130(1):26–29.

Teaching Spectrophotometry and Graphing Using Red Cabbage Extract and pH Buffers

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Since students often come to an introductory science course with an insufficient math or science background, we found it useful to teach an exercise where students generate data that they use to construct a table and graph. Cabbage anthocyanins change color at different pH values and we introduce students to this effect of change of pH on these pigments. We also teach use of the Spectronic 20 spectrophotometer as a means of data acquisition, in this case with regard to the electromagnetic spectrum and visible colors. This exercise is performed early in the semester as preparation for later discussions and experiments regarding the effects of changes in pH relative to the action of enzymes during metabolic processes.

The first part of the exercise generates data over the same portion of the visible spectrum for each of four pH solutions. A composite graph is prepared in order to compare the absorption maxima for each of the different colored solutions.

The second part of the exercise deals with the serial dilution of a pigment solution. In this case, absorbance is plotted against pigment concentration relative to the initial amount in solution to demonstrate the linear relationship of pigment concentration to absorbance reading.

Shredded cabbage is boiled in water until grey in color, cooled, and filtered through cheesecloth to remove the cabbage pieces. The purple liquid that results may be diluted with water if the color is too intense. Buffer solutions are prepared from powder capsules and 3 ml of cabbage extract is added to 5 ml of each buffer solution used to prepare color standards. Students are instructed in the proper use of a Spectronic 20. They take a reading for each buffer solution at 40-nm intervals beginning with 400 nm and ending with 600 nm readings. In this process they should realize that different colors absorb more or less light at the same wavelength. The data are collected into table format. Graphing of data is discussed and each student makes a composite graph of the data collected. This task introduces the concept of dependent and independent variables and how to determine one from the other.

Students then select a pH solution and consider the 600 nm reading as the full concentration of pigment in solution. A series of three 1:2 dilutions are made with a reading taken of each dilution as it is made. The independent variable must again be determined and is a bit more subtle. If done correctly, the graph should show the linear relationship between concentration and absorbance at a given wavelength.

Some Effects of pH on Living Systems

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The phenomenon of acid rain is familiar to most students, but the devastating effects are abstract to beginning students. In this exercise we simulated the pH of an acid lake in an aquarium in the lab, and allowed students to measure some of the effects of high acidity on aquatic organisms.

Two aquaria (one at pH 5.0 and one at pH 7.5), each containing specimens of *Elodea* and goldfish (*Carassius auratus*), were maintained in the lab at the two pHs. A daily record of the pH was maintained, and students were encouraged to visually examine the aquaria throughout the semester. On the day of the experiment, photosynthetic rates of *Elodea* were estimated by measuring the release of oxygen bubbles from a cut stem for a period of 6 minutes. Light intensity was varied by making the measurements at different distances from the light source (20 cm, 40 cm, 60 cm, and 80 cm). Students were asked to plot photosynthetic rate as a function of light intensity and compare the curve from acid-exposed plants with that of neutral pH controls. The chlorophyll content of the *Elodea* plants from both aquaria was estimated by determining the area of five leaves, crushing the leaves in 80% acetone to release the chlorophyll and spectrophotometrically measuring the absorbance of the extract at OD_{652} (Witham et al., 1986):

$$mg\ total\ chlorophyll/mm^2\ tissue = \left(\frac{OD_{652}\ x\ 1000}{34.5}\right) x \left(\frac{V}{1000\ x\ A}\right)$$

where OD = optical density at 652 nm, V = volume of chlorophyll extract, and A = total area of leaves in mm^2.

The respiratory rates of goldfish taken from both pH 5.0 and pH 7.5 were estimated by counting the number of operculum movements per unit of time. The ability to maintain respiratory rates at decreasing temperatures was compared by making measurements at room temperature (22°C), 15°C, 10°C, and 5°C. Students compared the rate-temperature plots for fish from pH 5.0 and pH 7.5.

Data indicate a downward shift in the rate-intensity curve for *Elodea* plants from pH 5.0 relative to those from pH 7.5, with no significant change in chlorophyll content. Goldfish from pH 5.0 exhibited an upward shift in the rate-temperature curve relative to those from pH 7.5. Students were required to write a report, including current literature citations, discussing their results with reference to acid rain and the effects that high acidity has on aquatic organisms and ecosystems.

Witham, F. H., D. F. Blaydes, R. M. Devlin. 1986. Exercises in plant physiology. Prindle, Wever, Schmidt, Boston, Massachusetts.