Digital Resources for Simulating Protein Acrylamide (PAGE) Electrophoresis

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Biography

Marianne Niedzlek-Feaver received her Ph.D. from the University of Michigan. As an evolutionary ecologist, she is interested in identifying factors that shape the mating systems of grasshoppers and katydids. She currently teaches Evolution, Invertebrate Zoology and Introductory Biology courses. She has received various grants to improve the laboratory experience, and is a member of the NCSU Academy of Outstanding Teachers

Betty L Black received her Ph.D. from Washington University (St. Louis). She conducts research on development of embryonic intestine in birds and mammals, and teaches a course in Developmental Anatomy plus distance education courses in Histology and Animal Diversity. She has received two University awards for "Innovative Excellence in Teaching and Learning with Technology."

Introduction

This paper introduces digital resources that can be used by instructors to supplement or extend the laboratory experience. Web animations for a distance "lab" to accompany our distance introductory biology course were developed to recreate a laboratory exercise that was always well received by students and instructors and emphasized proteins as products of genes that are turned on or off in different tissues and at different times in development. Students separated proteins by electrophoresis, comparing, the banding patterns produced by denatured proteins extracted from green and white corn seedlings, different parts of the same plant (such as flowers and leaves) and finally different stages of the life cycle of the fruitfly *Drosophila virilis*. Since students had already investigated the Mendelian ratios produced when different corn plants are crossed, this exercise served to introduce upcoming laboratories, which treat development in animals and plants. More importantly, this exercise distinguishes between differences in phenotype resulting from differential gene inheritance and differences among tissues and stages in the life cycle of multicellular organisms that result from differential gene activity in genetically identical cells. This lab on "gene products" as it was dubbed, was reluctantly abandoned when our enrollments grew to 800 students per term, because the preparation to implement it was so demanding. Consider for example the vast amount of larvae and pupae or various types of plant material that would have to be harvested at the exact stage required and well in advance of the laboratory for this number of students. Problems also arose from the different materials needed to prepare plant versus animal tissue.

The greatest problem to overcome, however, is that although students did take photographs of their gels, having them quantify their results in some meaningful manner was almost impossible. Obtaining consistent and usable results with polyacrylamide gel electrophoresis requires some experience and in the hands of first-time and freshmen users, the results varied. Almost all groups of students did obtain discernable differences in banding patterns across lanes. However, bands in some lanes often proved too faint to resolve. Too much protein could be loaded in other lanes resulting in a protein smear from the top to the bottom of the lane instead of bands. Polyacrylamide gels are thin and students often tear them as they try to remove them from the carrier or photograph them. These gels are also difficult to photograph. Although differences could easily be seen on a transilluminator, there was no good way for students to leave the lab with data they could analyze. Even when gels were photographed, students had difficulty, given the number of bands obtained, in distinguishing "major" bands from "minor" bands and band positions among lanes on a fairly small Polaroid. Ultimately students were given with a stylized drawing of the expected banding patterns to analyze for homework.

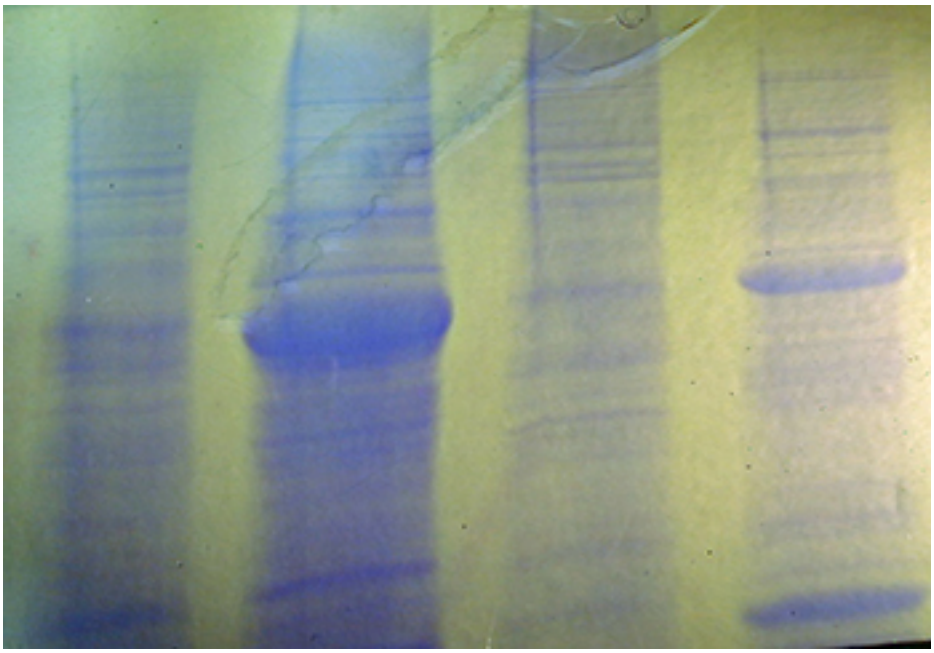

Figure 1: Magnified 4x Portion of a student produced gel as viewed on a light box. Note that several bands in each lane can be resolved

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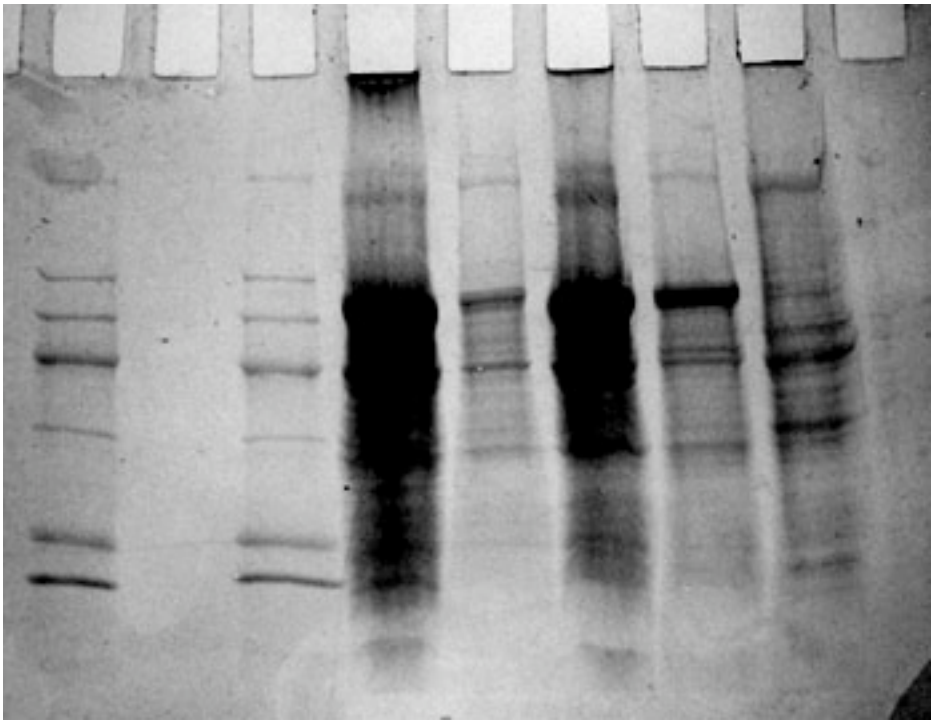

Figure 2: Photograph of student gels showing problems encountered by inexperienced students with loading, determining proper protein concentration, etc. Note also that the small Polaroid preserved only the most major bands and made even these bands difficult to score and compare across lanes.

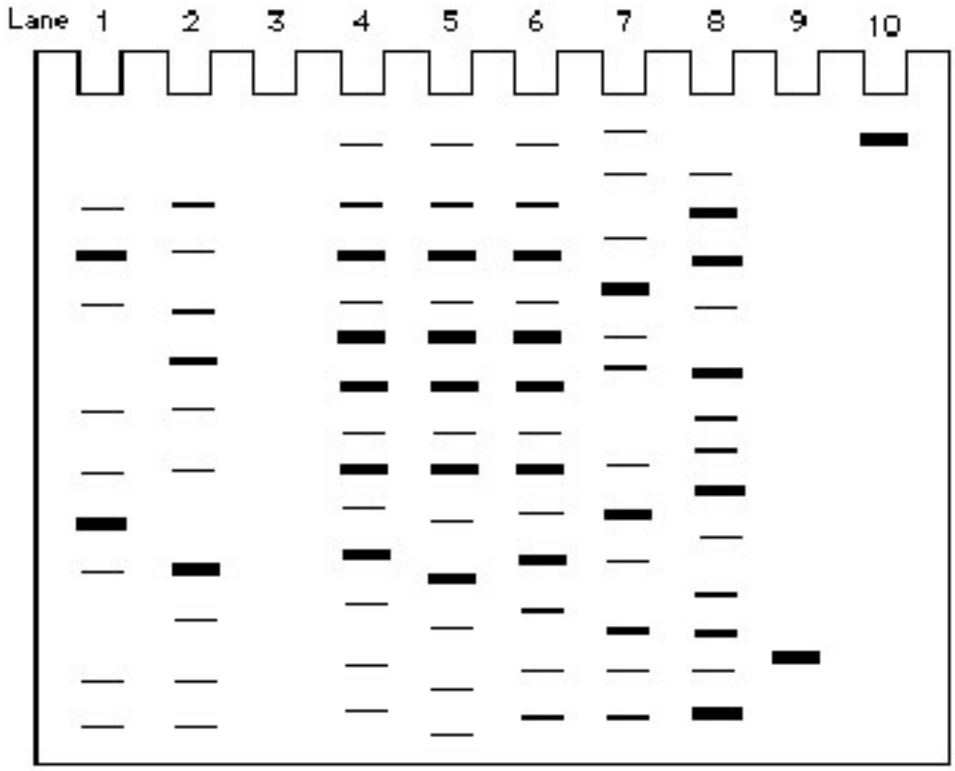

Figure 3: Diagram used for homework. Students were told they were working with a stylized diagram of a gel obtained for species Z from planet X. They compared this diagram in some general ways (number of bands, etc.) to the photographs they obtained for the lab gels.

The Animations

The web animations that we developed provide the students with a more realistic diagram of the expected gel. Students can access the animations on course web sites to extend the laboratory experience. Screenshots of the "gel" produced by the animations can be included in homework assignments. More importantly, other instructors are not wedded to the material that we used to illustrate differential gene activity. In conjunction with these animations, we have developed what we term a “customizable” gel animation, in which instructors can themselves determine where bands appear and the thickness of those bands.

Gene expression animations

Three animations were developed for our distance education lab. One recreates the patterns obtained when ground cuttings of albino corn seedlings are compared with ground cuttings of green corn seedlings. The second compares the patterns obtained from flowers versus leaves. The third recreates the banding patterns obtained when stages in the life cycle of *Drosophila virilis* are compared.

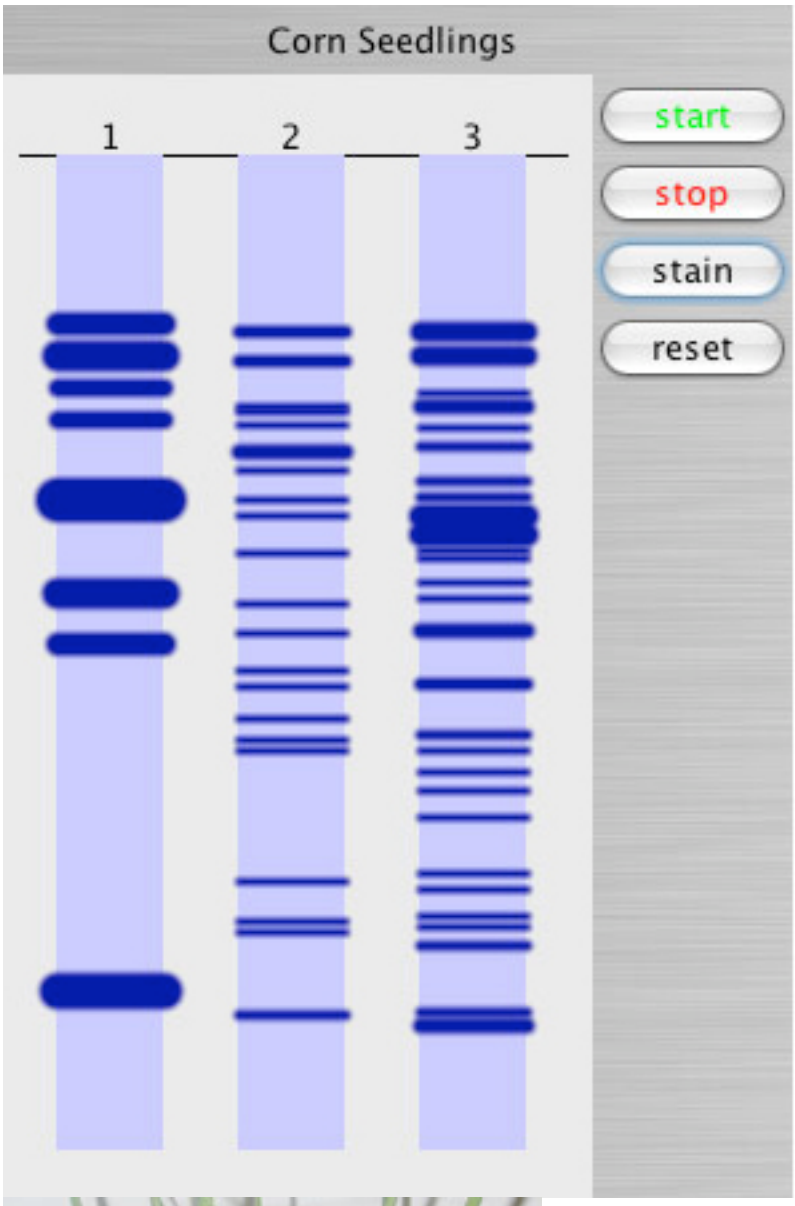

Figure 4: Animation of banding patterns obtained when approximately 5 grams of leaf material is ground in buffer from albino (lane2) and green (lane 3) corn seedlings.
Lane one contains a standard. The standard consists of proteins of the following weights (in Daltons): Beta-galactosidase 116,250, Phosphorylase b 97,500, Bovine Serum Albumin 66,200, Ovalbumin 46,000, Carbonic anhydrase 31,000, Trypsin inhibitor 21,000, Lysozyme 14,400, Apotinin 6500.

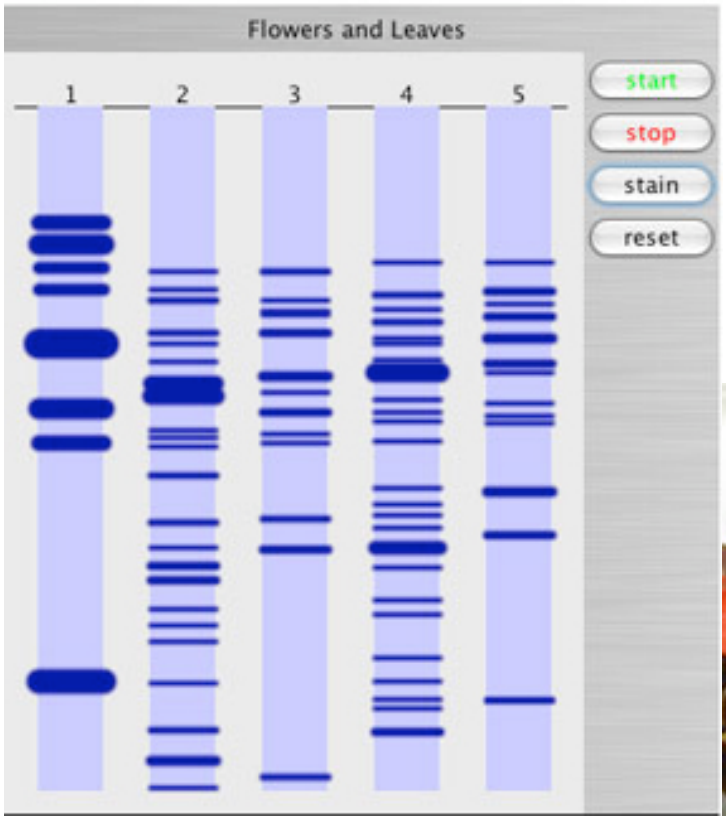

Figure 5 : Animation of banding patterns obtained when approximately 5 grams of material is ground in buffer from flowers (lane 2 and 4) and leaves (lane 3 and 5) of two varieties of begonia.
Lane 1 contains the standards described in Figure 4.

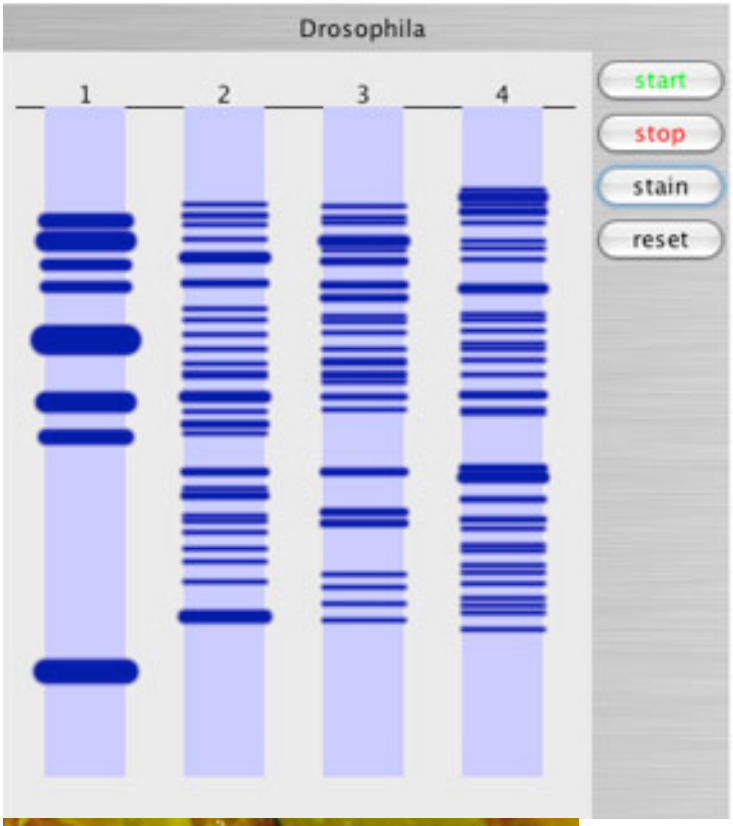

Figure 6: Animation of banding patterns obtained from 12 late instar larvae (lane 2), 20 early pupae (lane 3) and 12 adults (lane 4). Lane 1 contains the standards described in Figure 4. In the photograph below, a larva can be observed left center, a early pupa right center.

We consider these animations to be high resolution, since Java compiles a 1000 X 1000 matrix to display banding patterns. We developed these animations from photographs taken of magnified images of gels on a light box. We decided to reproduce up to 40 of the major bands per lane (dependent on material used), which is more than students would normally be able to resolve in their photographs of gels. Bands were allowed to vary in thickness as they do on a gel. A start button signals the start of the run. In the full version of the animation, students only see tracking dye moving down the gel. If they stop the run after the tracking dye disappears (moves off the end of the lane), they may lose bands just as they would in a real run. Stopping the run too soon leads to bands not being separated as well as they would be if students allowed the tracking dye to move to near the end of the gel. Students then must stain their bands before they can visualize them.

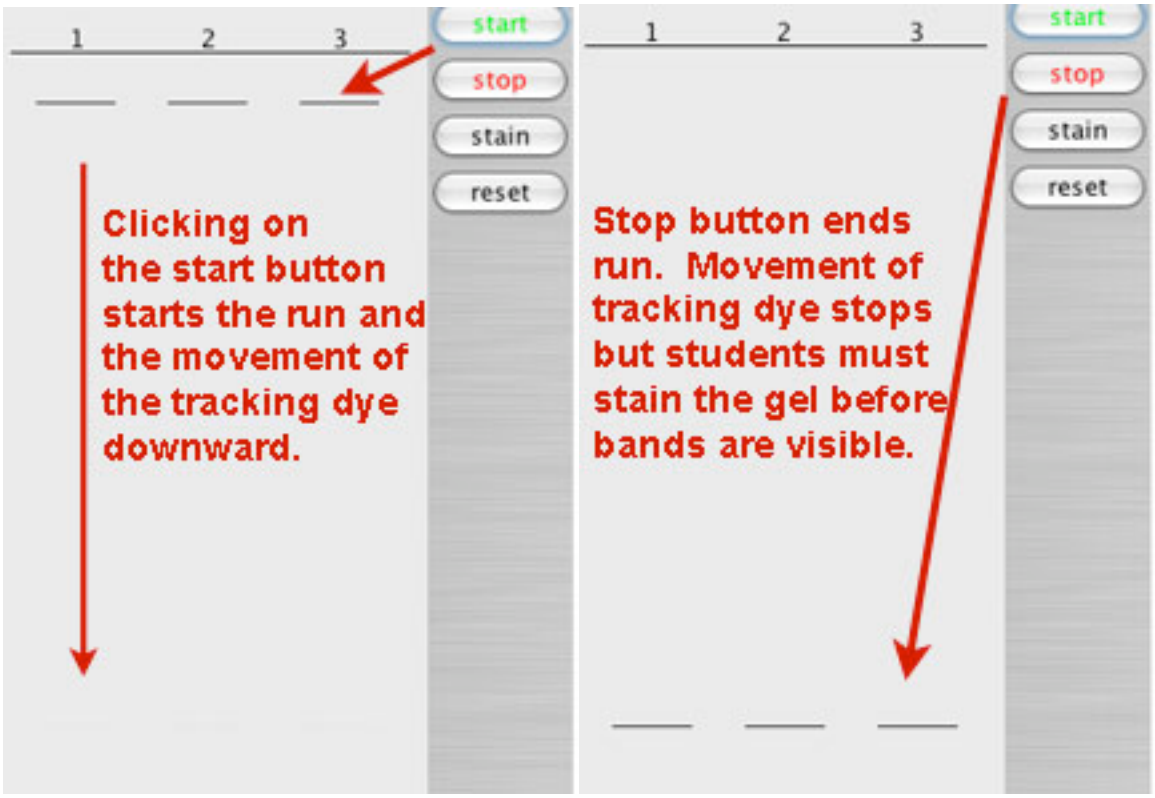

Figure 7: Students start and stop the run using the movement of tracking dye to indicate the relative movement of the denatured proteins as they form bands. Proteins must be stained before bands are visualized.

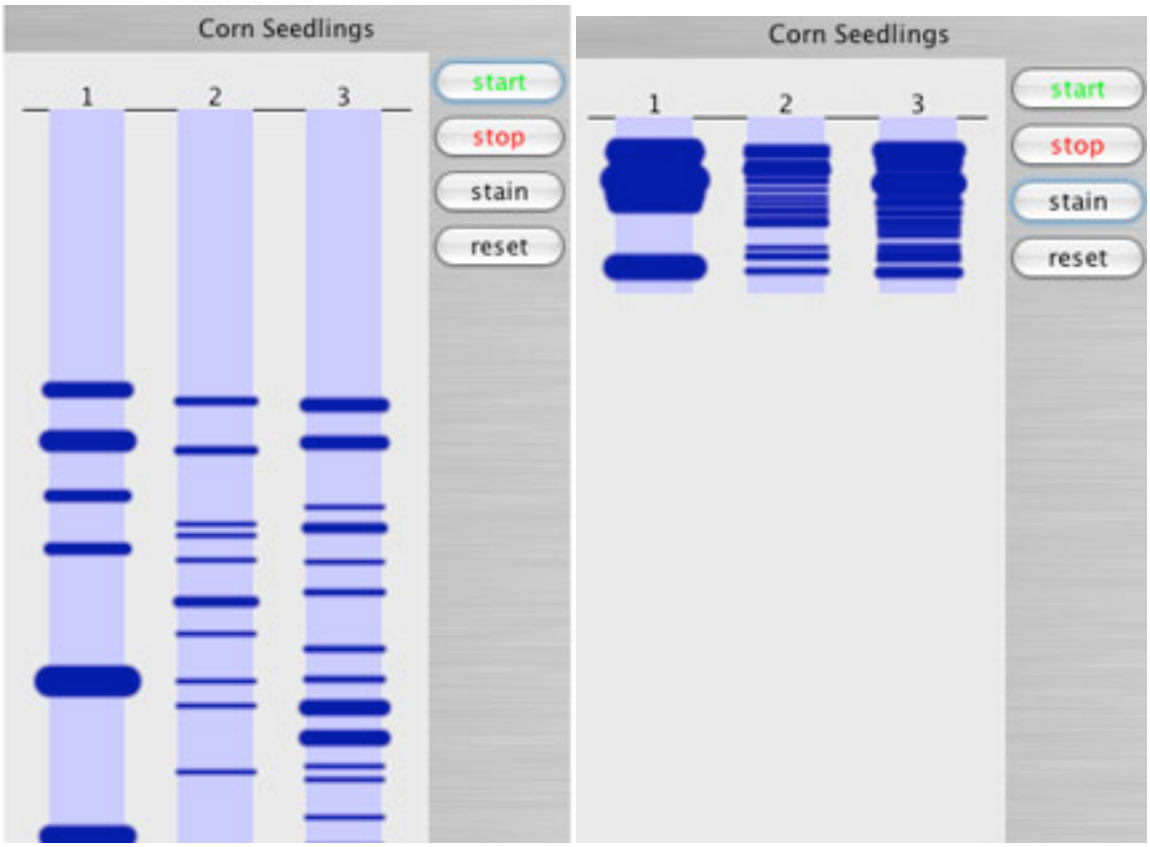

Figure 8: If students do not stop the run in time, bands may run off gels. Students can also stop the run before bands have moved far enough apart to be resolvable.

Animation availability

The animations are delivered as a Zip package on this CD. In 2009, they may be downloaded from the following web site: **http://www.ncsu.edu/project/interactivebiology .** The Zip package contains three folders and the usual read-me-first text file that describes the contents of the package. The sample folder will contain files and the java for the animations described above.

The animation package:

The sample folder contains html pages that can be linked to any web page. When opened, these pages will show animations for white and green corn seedlings, plant parts, and three stages in the *Drosophila* life cycle (larvae, pupae, adults), and life stages of insect from planet Y.

You must have the html page for a particular gel and the protein. jar file in the same folder for the gel animations to work. So if you wish to show the animations from a web site, be sure you place both the html pages of interest and the protein.jar file in a folder on your website. For example, if I wished to show the corn seedling animation to students from the web, I would duplicate the protein.jar file and the html file for the corn seedlings and place them into a new folder. Make sure you remove the term copy from the new protein.jar file. Place the new folder on your web site. Link to the html page in that folder and you are done. Of course, you can also copy the

folder on to your computer hard drive or show the animations directly from the CD by opening the desired html page in a web browser.

Customizing the samples provided

The banding patterns for the sample animations are already programmed and you will probably mess up the animations if you try to change the patterns. However, there are parameters that you can change in these animations. The gel animations can show only the tracker dye and/or buttons or the banding patterns simply develop as soon as the animation is accessed.

To change these parameters, you must first open the html page for a particular gel in an application that will allow you to view and change the source code. There are programs (html editors such as Dreamweaver) that are designed to edit html documents. However you can also open html documents in many text editors (such as Microsoft word) and view the source code. Simply select source code html and you will see something like the following.

<!DOCTYPE HTML PUBLIC "-//W3C//DTD HTML 4.01 Transitional//EN">
<html>
<head>
<title>Protein Gel</title>
<meta http-equiv="Content-Type" content="text/html; charset=iso-8859-1">
</head>

The instructions for changing the gel parameters will indicate how to change the source code to change duration (how fast the proteins move down the gel), whether you see stop and start buttons, and even band color if you wish. In the corn-diff-versions folders are three html files that exhibit some of these modifications. You can view the source code of these files after reading the instructions and see how it differs from the source code for the full version for the corn seedlings in the sample file. Then make a copy of the full corn html page and practice making any modifications you desire. You can change the name of this page or keep copy in the title; make sure, however, that you never modify the name of the protein.jar file or your html page will not be able to find this file and your animation will not work. Also, never modify the name of the sample in the html page itself (in the source code) or it will not know which banding pattern to call up. Mistakes can easily be trashed and the logic of the code should be understandable after a few trials**.**

When you have completed your changes, simply save them if you are using an html editor. If you are using a text editor, choose to save the file as a .txt file. Once the file is saved, change the extension back to .htm and open in a browser. The animations should run in most web browsers: Firefox and Safari on Macs and Firefox and Explorer on Pcs have been tested.

The Custom Folder

We have provided a low-resolution page gel generator for those wishing to generate their own animations. The matrix here is only 40 by 40 to make it easily modifiable, but you should be able to generate about 20 discernable bands per lane. You can add as many lanes as you wish. Bands are created by replacing a 0 in the code with a band thickness (1-9) . So below in position I have placed a band of thickness 1 in the first position, a band of thickness 2 in the second position and so on. Lanes 2 and 3 have no bands in them.

<param name="lane1"
value="1,2,3,4,5,6,7,8,9,0,1,2,3,4,5,6,7,8,9,0,1,2,3,4,5,6,7,8,9,0,1,2,3,4,5,6,7,8,9,0">
<param name="lane2"
value="0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0">
<param name="lane3"
value="0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0">

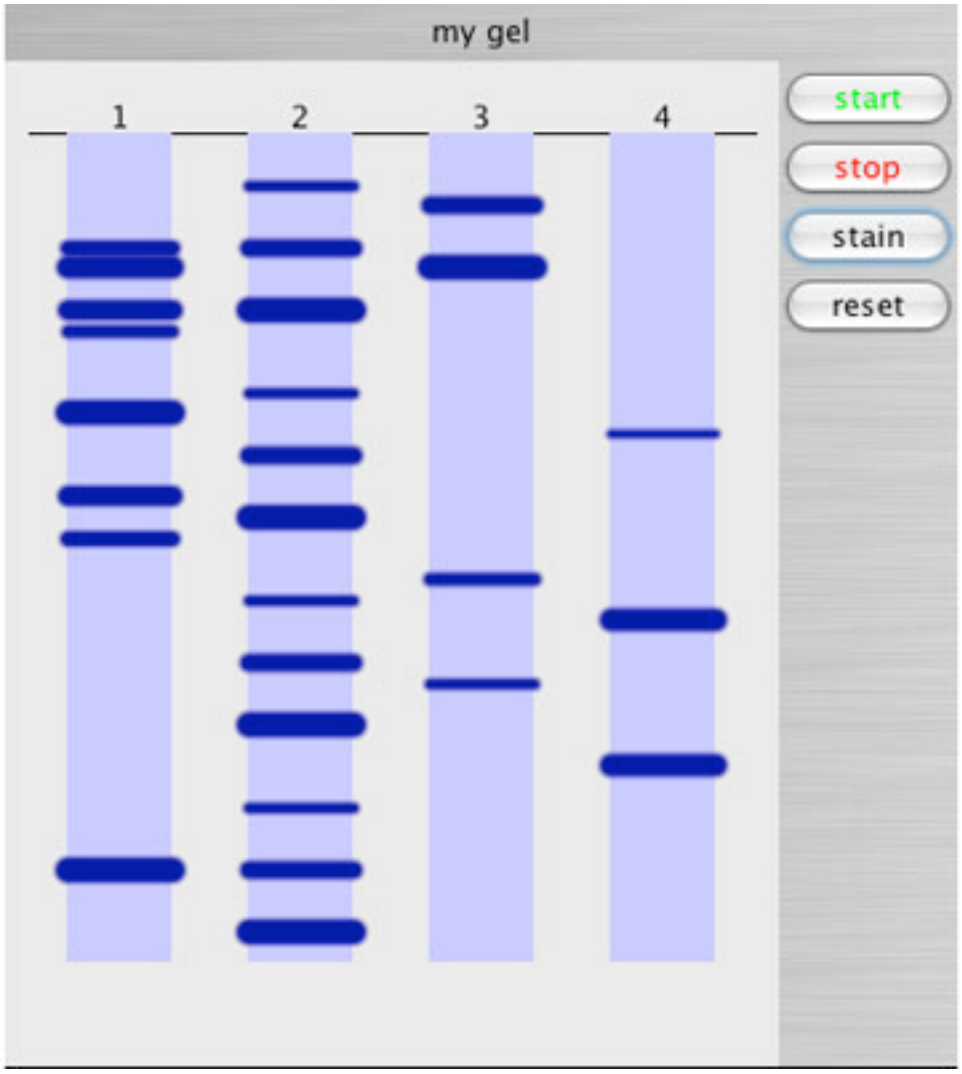

Figure 9. The gel pictured or mygel was generated by changing (with changes highlighted in red) the source code to
<applet code="protein.BandsApplet.class" archive="proteinall.jar" width="400"
height="500">
<param name="title" value="my gel" />
<param name="scenario" value="custom" />

<param name="lane1"
value="0,0,0,0,0,5,8,0,7,4,0,0,0,9,0,0,0,7,0,5,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,9,0,0,0,0" />
<param name="lane2"
value="0,0,3,0,0,6,0,0,9,0,0,0,3,0,0,6,0,0,9,0,0,0,3,0,0,6,0,0,9,0,0,0,3,0,0,6,0,0,9,0" />
<param name="lane3"
value="0,0,0,6,0,0,9,0,0,0,0,0,0,0,0,0,0,0,0,0,0,4,0,0,0,0,3,0,0,0,0,0,0,0,0,0,0,0,0,0">
<param name="lane4"
value="0,0,0,0,0,0,0,0,0,0,0,0,0,2,0,0,0,0,0,0,0,0,8,0,0,0,0,0,0,8,0,0,0,0,0,0,0,0,0">
<!-- add as many lanes as desired -->

As with the changes described previously, you can use a text or html editor to make any of the desired changes. Guidelines for those more familiar with editing html documents are always available after the code in source view (look for GUIDELINES FOR APPLET PARAMS after the code for that page).

We sincerely hope that these resources are of value to you and are willing to help in any way if you encounter problems. Please contact M. Niedzlek-Feaver, mnfeaver@unity.ncsu.edu for any additional information on the animations or a for copy of the wet laboratory exercises.

Acknowledgements

We wish to thank Ms. P. Aune for helping us assemble the materials needed to recreate the wet lab. Ms. P. Aune is the lab coordinator for the Biological Sciences program and can be contacted for advice on supplies and protocol for the former wet laboratory.

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We also wish to thank Mr. Jason Wingate, our graduate student assistant, who played a major role in producing the gels on which the animations are based. Mr. Jerome Brewster, another graduate assistant, and Jason also helped photograph the gels on which the animations were based.