INQUIRY & INVESTIGATION

Proteomics in the Classroom: An Investigative Study of Proteins in Microorganisms

RECOMMENDED FOR *AP Biology*

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Abstract

As advances in biotechnology and molecular biology rapidly expand in research settings, it is vital that we continue to prepare high school students to enter and thrive in those modern laboratories. This multistep, inquiry-based lab describes highly adaptable methods to teach students not only current molecular techniques and technologies, but also about proteomics and microorganisms. Students participate in protein extraction, gel electrophoresis, mass spectrometry, and data analysis to identify proteins present in microorganisms.

Key Words: *Proteomics; genetic diversity; mass spectrometry; gel electrophoresis; microorganisms; online database.*

Laboratory experiences have long been accepted as valuable learning tools for students (Downing, 1917). Nevertheless, for various reasons, teachers have increasingly turned to "cookbook" laboratories. Although many students in high school science courses often have to complete mandatory laboratories, it is clear that these expository "laboratories in a box" are not necessarily beneficial to student learning (Johnstone & Al-Shuaili, 2001). Instead, the activities need to have aspects of planning and ownership by the student to foster better understanding of concepts (Vance, 1952). Our proteomics project takes this into account and allows students to use new technologies (proteomics and bioinformatics) to "make discoveries." Students involved in this active exploration of course content are more engaged in science (American Association for the Advancement of Science, 1990; National Research Council, 1996).

Laboratory experiences have long been accepted as valuable learning tools for students.

The basis of scientific research is investigation of the unknown, sometimes with surprising results. The activity described here can incorporate slight modifications that allow students to conduct an investigation based on their own choices. Students follow guidelines as a framework to work within; but ultimately, open-ended questions and scenarios that provide choice should be posed in a way that allow true student discovery. Questions that can be asked during this activity include

- What are the specific roles of proteins in a given microorganism?
- What impact do environmental changes have on the proteins present in an organism?
- What are the noticeable molecular differences between organisms within the same domain?

There are many correct answers to these questions, and it is the goal of this activity to let students reach one of them. Lower-level questions posed throughout the procedure ensure that students understand why the protocol is followed in order to arrive at a conclusion to the higher-order questions posed.

This laboratory activity has been created to emphasize state, national, and AP science standards (Table 1), including the following concepts: organic molecules in organisms, prokaryotic cells, molecular genetics, diversity of organisms, and the use of technology to study DNA and proteins. Further, the techniques and technologies that are incorporated include micropipetting, protein extraction, gel electrophoresis, protein digestion, mass spectrometry, and bioinformatics. See Box 1 for student learning outcomes. At the time this activity was completed, no formal post-assessment was administered because the learning outcomes had been assessed earlier in the year. Instead, a qualitative, summative assessment via good laboratory results and student presentations was chosen because of the nature of the project and the time of the year. Students showed competency in all areas.

Although there is a broad scope to this activity, knowledge is most usable when ideas are linked into large conceptual networks (Marx et al., 1997). It is suggested that students who complete this project can form conceptual networks of not only what gel electrophoresis or mass spectrometry does, but why scientists use it. Additionally, student ownership can be incorporated as follows:

- Let students choose the bacterial species.
- Let students alter the environmental conditions in which the organism is living.
- Let students choose what protein band(s) to excise on the gel (if completing protein digestion and MALDI-TOF MS).

Students actively construct ideas when working on authentic tasks that require them to apply those ideas (Resnick, 1987; Collins et al.,

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Table 1. Standards and learning outcomes emphasized.

Standard	AP Biology "Essential Knowledge" (2012–2013 Curriculum Framework)	AP Biology "Science Practices" (2012–2013 Curriculum Framework)	National Science Education Content Standard (Grades 9–12)
Organic molecules in organisms	3.A.1(d), 4.A.1, 4.C.1(a)		C, The Cell
Structure of cells	2.B.3(c)		C, The Cell
Molecular genetics	3.B.1(b,d)		C, Molecular basis of Heredity
Genetic diversity of organisms	1.B.1, 3.C.1		C, Biological Evolution
Use of technology to study organic molecules	3.A.1(e)		E
Environmental impacts on organisms	1.A.1(d,e), 2.C.2, 2.D.1, 2.D.3, 4.A.3(c), 4.C.2		C, Behavior of Organisms
Engagement in scientific questioning		Practice 3	
Data collection strategies		Practice 4	
Data analysis		Practice 5	
Students work with scientific theories		Practice 6	
Relate knowledge across domains		Practice 7	

Sources: College Board, 2010, 2011; National Research Council, 1996.

Box 1. Student Learning Outcomes.

At the end of this lesson, students should be able to

- Accurately use micropipettes
- Describe the basics of protein extraction
- Explain why protein expression differs in different organisms
- Describe how environmental conditions can affect protein expression
- Set up and run sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)
- Digest proteins and describe the process of protein digestion
- Analyze matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) data, and explain how it works and why it is used
- Identify the roles of specific proteins in microorganisms

1989; Newman et al., 1989). This activity allows students to work on an authentic proteomics project, yet still fosters new conceptual frameworks in the students.

○ Description of Teaching Unit

For the initial completion of this activity, STEM Academy AP Biology students investigated proteins of their own choosing and their specific roles in the microorganism. This activity is recommended for student in upper-level biology courses such as AP/IB/AICE Biology, biotechnology, or health science. It is recommended that students first complete a majority of one of these courses because of the concepts that are integrated into this project. For the exact methodology and materials that students should use, refer to Box 2 and Tables 2 and 3.

Many species of prokaryotes are available through common scientific supply retailers and can be purchased as pre-inoculated bacterial cultures. Providing students with several choices of common bacterial species is encouraged because it incorporates student choice into the project with minimal cost. *Serratia marcescens* was chosen for this initial activity because of its immediate availability, bright red color, and fast growth. Nevertheless, other species that could be used may include high school strains of *Escherichia coli*, *Rhodospirillum rubrum*, and *Bacillus stearothermophilus*.

The first time this activity was conducted, it took small groups of students roughly 5 hours to complete. These 5 hours were broken down into the recommended 1-hour blocks over the course of several days (see Figure 1 and Box 2). The amount of time will increase from 5 hours if students manipulate an environmental variable for the initial growth of their bacteria. Because of limited time, students did not manipulate this variable in the initial activity. However, as a proof of concept, this activity was completed a second time but the temperature was manipulated for the initial growth of the *S. marcescens*. An environmental variable (e.g., different growth temperatures, presence/intensity of light, different growth mediums) should be included in the activity because it costs little and incorporates student choice. The previously mentioned species of bacteria allow for easy flexibility of environmental variables (*S. marcescens* and *B. stearothermophilus* for temperature, *R. rubrum* for light, and *E. coli* for aerobic/anaerobic conditions), but students should be given the freedom to explore other growth conditions as well.

Once the bacteria were successfully cultured, samples were pelleted in a microcentrifuge tube and given to each student group. Students then extracted the proteins from their organisms by using a bacterial protein extraction reagent. These reagents are extremely simple to use and usually require only the addition of the reagent

Box 2. "Quick Guide" – Student instructions and sample questions.

Question: What is/are the specific role(s) of some of the proteins in the organism *Serratia marcescens*?

A. Protein extraction (1 hour).

1. Add 200 µL of "A" per 0.1 g of cell pellet.Pipette the suspension up and down until it is homogeneous.
2. Incubate 10–15 minutes at room temperature.
3. Centrifuge lysate at 15,000 × *g* for 5 minutes to separate soluble and insoluble proteins.
4. Remove 10 µL of supernatant and put into new microcentrifuge tube.
5. Add 10 µL of "B" to the supernatant in the new microcentrifuge tube created in A4.
6. Vortex (1 minute).
7. Put the tubes at 95°C to denature the protein for 5 minutes.
8. If out of time, freeze samples

Student Questions:

a. What is the purpose of lysing the cells?
b. Why did you denature the proteins at 95°C and not room temperature? Why not at 100°C?

B. SDS-PAGE (1 hour).

1. Centrifuge samples from Part A at the highest speed at room temperature for 10 minutes.
2. Load 10 µL of supernatant into a well on the gel.
3. (Select groups) Load 5 µL molecular weight marker into first well.
4. Run gel electrophoresis (200 v) following manufacturer guidelines until complete.
5. (Steps 5–7 to be done outside of class)
6. Remove gels and rinse with distilled water, repeat.
7. Stain with 20 mL coomassie blue.
8. Destain with distilled water.

Student Questions:

a. What is the purpose of including the molecular weight standard?
b. What do the different bands represent, and why are they in different locations in the gel?

C. In-gel protein digestion (1 hour).

1. Rinse new 1.5-mL microcentrifuge tube with 200 µL of "C" and then dispose of liquid.
2. Cut a band of interest from the gel into 1-mm squares.
3. Place into cleaned 1.5-mL microcentrifuge tube from step C1.
4. Add 400 µL of "E." Vortex for 8 minutes.
5. Remove (and dispose of) the fluid and repeat above step to destain gel.
6. Remove all liquid from the tube (and dispose of liquid).
7. Chill gel in ice.
8. Add "D" to cover the gel. Let sit for 10 minutes.
9. Overlay the gel with 50 µL of "F."
10. Incubate overnight at 37°C.

Student Questions:

a. Why is it important to use only a 1-mm square and not a larger piece of the gel?
b. What process is taking place in the overnight incubation?

Box 2. (Continued)

D. Peptide Extraction (1 hour).

1. Remove supernatant from incubated tube to new 1.5-mL microcentrifuge tube
2. Add 160 µL "G" to tube with gel still in it, then vortex for 10 minutes.
3. Spin briefly, then remove all liquid to the collected supernatant tube created in step D1.
4. Add an additional 40 µL "G" to the tube with the gel still in it, then vortex for 5 minutes.
5. Spin briefly, then remove all liquid to the collected supernatant tube created in step D1.

Student Questions:

a. Why are we saving the supernatant and not the gel pieces?
b. What levels of structure remain in the peptide fragments?

E. Send samples to University of Florida (UF), Proteomics Department for MALDI-TOF MS. Allow for 1 week to process.

1. Go to http://www.moleculardetective.org for information and mailing address to the UF, Proteomics Department. (Optional: contact UF for information on how to bring students into the MALDI-TOF MS laboratory to see how the process is completed).

Student Questions:

a. What is the purpose of MALDI-TOF MS?
b. What data will be returned to you after MALDI-TOF MS is completed?

F. Analysis of results from MALDI-TOF MS. Allow for 1 hour of class time and then additional group/individual student research outside of classroom.

1. Students go to http://www.moleculardetective.org and click on "Peptide Search Engine."
2. Insert data obtained from UF into "Query" and chose the correct taxonomy from the drop-down menu (bacteria).
3. Analyze data in order to find the most probable protein.
4. Research proteins.

Student Questions:

a. What is the identity (identities) of your proteins?
b. What role do these proteins play in your organism?
c. Can all the proteins be confidently identified? If not, why?

to the bacterial pellet and a short incubation. Once the students had extracted the proteins and allowed for a 5-minute incubation with sodium dodecyl sulfate (SDS) gel sample buffer (see Table 2 for constituents of buffer), the proteins were loaded into a gel and run through SDS-PAGE following the protocols provided with the chamber. Additionally, a broad-range molecular weight marker was loaded by students into the first lane of each gel. After the gels had run to completion, students carefully removed the gels and proceeded to add a Coomassie blue stain. It is important to note that although the staining process is simple, it is also time-consuming. It is recommended that the staining procedure be done outside of classroom hours and that the stained gel be allowed to sit overnight in purified water.

After the gels were destained, students compared and contrasted the banding patterns on the gels. It is recommended that students take pictures of their gels. These images can allow for future analysis

Table 2. Items given to students in microcentrifuge tubes. Letter abbreviation indicates how the tube was labeled.

Letter Abbreviation	Item	Constituents	Purpose(s)
A	Bacterial protein extraction reagent	B-PER	Lyses cells
B	Sodium dodecyl sulfate (SDS) – gel sample buffer	62.5 mMTris-HCl 25% glycerol 2% SDS 0.01 bromophenol blue DTT – dithiothreitol	pH buffer Eases gel loading Destroys secondary and tertiary structures Tracks the progress of the gel run Breaks disulfide bonds
C	Acetonitrile (ACN)	ACN	Digests gel and dries it out
D	Trypsin (sequencing grade)	Trypsin	Digestive enzyme
E	ACN/ammonium bicarbonate (ABC)	50% ACN 50% ABC	Digests gel and dries it out pH buffer
F	ABC	ABC	pH buffer
G	ACN/$H_2$0/formic acid	80% ACN 20% H_2O 0.1% formic acid	Digests gel and dries it out Helps remove peptides from gel
S	Bacterial sample	*Serratia marcescens*	Organism lysed

Table 3. Other items used in this activity.

Item	Comments
Molecular weight marker	Use in lane 1 of all gels. Peptides with known molecular weights. Broad range is recommended.
Coomassie blue stain	Gel stain
Gel electrophoresis buffer	Liquid to conduct charge in the chambers, stable pH
Mini/microcentrifuge	8,000 or 15,000 × g
SDS-PAGE chamber with appropriate power supply	
Precast gels	Use Tris-HCl 12% gel that meet chamber specifications
Micropipettes of varying sizes with appropriate tips	
Microcentrifuge tubes	
Hot water bath (95°C)	

of banding patterns. Students then chose a visible protein band on the gel and excised it. Protein digestion was completed by the students (an overnight process), which required the use of the enzyme trypsin (sequencing grade). The trypsin was placed directly with the excised gel and allowed to incubate overnight at 37°C. The protein extraction after the digestion included the removal of the supernatant from the microcentrifuge tube and then the application of a weak formic acid solution (see Table 2 for solution constituents). Samples were then sent to the University of Florida (UF) for peptide fingerprinting through MALDI-TOF MS (a free service through http://www.moleculardetective.org). The instructor can decide whether details of how MALDI-TOF MS actually works are included in the lesson, but general information should be presented to the students. This should include a description of MALDI-TOF MS (i.e., an ionization process in which the mass of organic molecules can be determined) and the type of data that it gives the user (mass/intensity of molecules/peptides in the sample). The data are returned from UF in a text file that can be copied and pasted directly into the Matrix Science MASCOT Peptide Mass Fingerprint (also available at http://www.moleculardetective.org). When students arrived at the website, they clicked on "Protein Search Engine" on the home page and pasted the data directly into the "Query" box. The taxonomy of the organism used was also selected from the drop-down menu (choosing "bacteria" seemed to be sufficient).

Once the most likely protein was determined (proteins are given a "score" in the MASCOT results, signifying statistical significance), student groups researched the overall role of that protein in the microorganism. The use of online resources is not only invaluable to help in the identification of peptides, but is also suggested to supplement the education of the student by appealing to many learning modes (Owston, 1997). Students submitted a lab report and presented their results in a short meeting in which questions were asked about how their group came to their conclusions. This ensured that students had to think critically and problem-solve in response to questioning, communicate in writing with the lab report, and collaborate with peers in their group. This varied data-collection method has been linked to student success (Uchida, 1996).

○ Extensions

Parts of this activity, such as running the gel, can be done in classrooms where protein structures and functions have not been covered or where students are using biotechnology to answer other biological questions. For instance, multiple species can be lysed and run in separate lanes of the gel, and then differences and similarities can be compared to observe the genetic biodiversity of microorganisms.

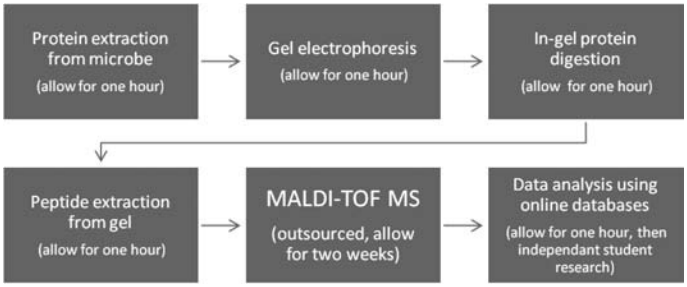

Figure 1. Work flow of proposed proteomics project with recommended times.

In the future, a bioinformatics lab can be designed to specifically analyze protein functions *in silico*. It is also possible to use online resources to look up three-dimensional models of some of the proteins found in the samples (e.g., RCSB Protein Data Bank, http://www.pdb.org/; Jmol, http://www.jmol.org/). These online models could then be used to create tangible models of the proteins (e.g., 3D Molecular Designs, http://3dmoleculardesigns.com/). Levels of protein structure can also be explored if tangible models are to be created.

○ Analysis of Results & Suggestions

A simple protein extraction provided a gel with sufficient banding patterns that students could visualize (Figure 2). Although the bacterial cells could have been frozen before lysis, this step was not completed. However, some species of bacteria may require freezing before lysis. It is also vital to make sure that the gel sample buffer runs to the end of the gel to ensure maximum separation of proteins. Additionally, students should be informed that there is a limit of detection and that only the most abundant proteins (minimum 10 ng) will actually show up on the gel.

After UF researchers analyzed the samples, we easily observed peptide fingerprints. Figure 3 shows student MALDI-TOF MS results, which identified two proteins present in the gel band (the peaks represent mass of the most common peptides found in the sample). Because of the large amount of proteins in the microorganisms and the use of a smaller gel, we found multiple proteins in a single band. This led students to identify multiple proteins per gel band and then choose the most statistically significant results. The low resolution obtained led to discussion of two-dimensional gel electrophoresis.

As mentioned above, as a proof of concept, *S. marcescens* was grown at two different temperatures (24°C and 37°C) in order to see whether minor environmental changes caused different banding patterns. Figure 4 shows differences in banding patterns between the different environmental conditions. Two of the noticeably different protein bands found in the 37°C lane were digested, extracted, and run through MALDI-TOF MS. Analysis showed that these proteins were most likely heat shock proteins that appear to have been up-regulated in the sample. These results indicate that small differences in environmental conditions can change the banding patterns on the gel.

Students need to be aware that they are working with living organisms, potentially harmful chemicals, and other hazardous

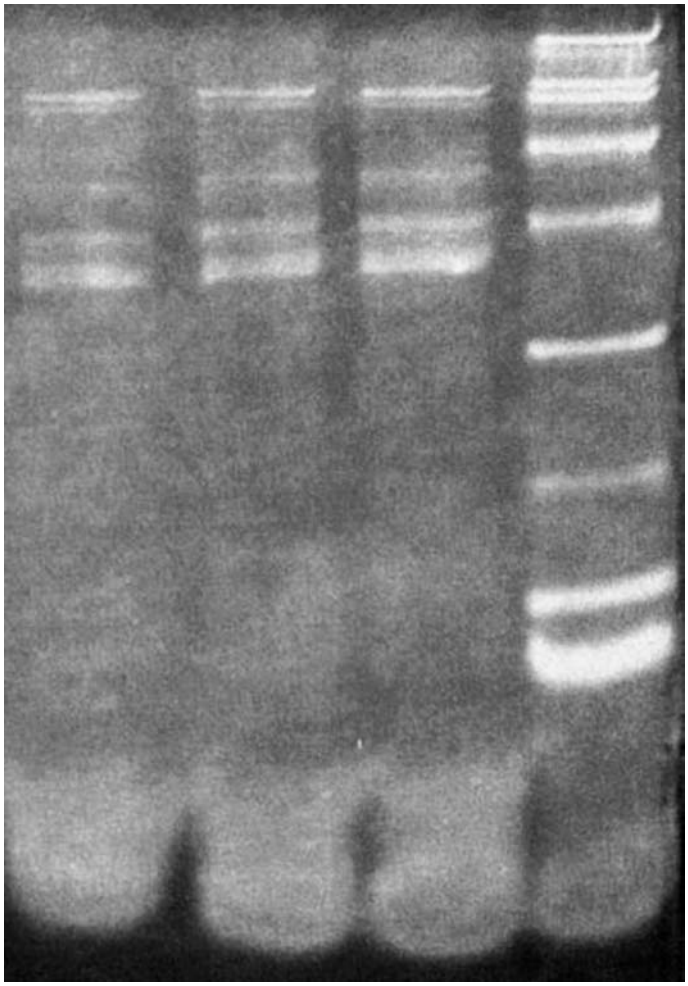

Figure 2. Student SDS PAGE gel results (false color) at the beginning stages of destaining. Right lane is protein standard; all other lanes are *S. marcescens*.

material. Appropriate safety techniques must be strictly implemented. Teachers should begin the initial setup of this lab early so that enough time is given to obtain needed chemicals and other supplies, some of which are not common in high schools. The setup of this activity is time-consuming and will require several hours of work the day before the commencement of the project. It is our opinion that the setup time is justified by the fact that the entire activity will span 5 days and is not much more involved than setting up a normal gel electrophoresis run.

Certain steps require centrifugation or incubation for up to 10 minutes. Students can use this time to answer the questions provided (Box 2) or the teacher can check for understanding by asking other questions related to the procedure or concepts. Additionally, allow at least 1 week to get mass spectrometry results from UF.

It is recognized that not all high schools will be able to use these activities because of unavailability of materials and funding. However, with the increase of STEM programs and biotechnology academies, this project can be successfully implemented in many high schools for a similar cost as other laboratory activities that emphasize biotechnology. Only small amounts of reagents are used, and the quantity ordered can be kept to a minimum. Additionally, some reagents (such as the gel electrophoresis buffer) can be reused,

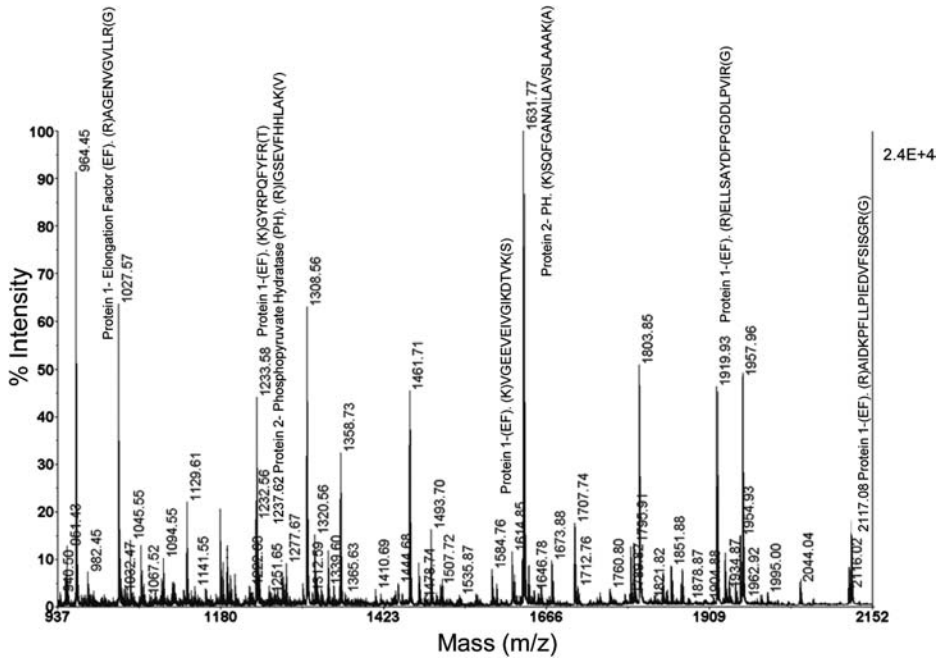

Figure 3. Student sample results from MALDI-TOF MS. Two dominant proteins were identified: protein 1, an elongation factor; and protein 2, phosphopyruvatehydratase.

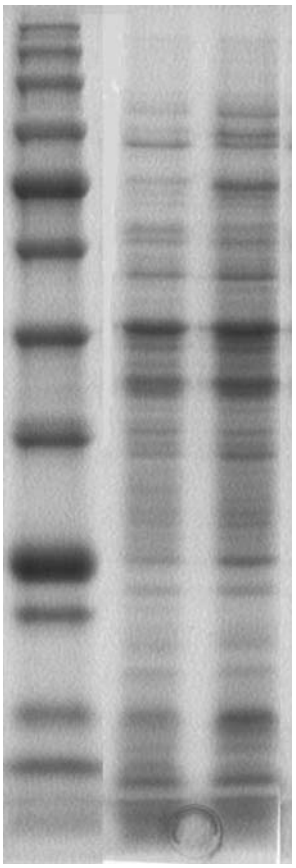

Figure 4. Environmental manipulation of *S. marcescens*. Left lane is protein standard, middle lane is *S. marcescens* grown at 24°C; right lane is *S. marcescens* grown at 37°C. Minor, but present, differences can be noticed.

though there may be a slight reduction in resolution with used buffer.

○ Conclusion

Proteomics is going to become more and more important in the field of biology. The result of this collaboration is an authentic proteomics activity that can be completed in a high school laboratory. This lab is the same type of work that is completed in proteomics laboratories and allows students to form a conceptual network of ideas that links many learning standards together in one activity. The investigation of these proteins requires inquiry on the students' part and can lead to possible future projects in both secondary and postsecondary education.

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