Uncovering "actors" in Arabidopsis Through Blinding Its Light Sensing Capabilities

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Academy of Environmental Science

ABSTRACT:

Genetic engineering of plants can be simply used to accelerate plant improvement, a process that has been going on for generations. Over the last ten-thousand years, individual plant lines with elite characteristics have been selected and bred. Today, gene technology can transfer genes from one species to another resulting in a much greater number of potential phenotypes. While crop plants actually feed people, much can be learned about plant biology from study of laboratory-friendly models, like *Arabidopsis thaliana*. In a controlled lab environment, using two separate Arabidopsis plants (wild type and some genetically altered to not respond to light; in this case mutants with defects in blue or red light sensoring will be used (*See reference Dr. Kevin Folta, *Responding to the Rainbow* Project). The variation in macromolecules, especially proteins and nucleic acids, can be studied using electrophoresis; protein extraction/separation/and staining; protein in-gel digestion; mass spectrometry; and peptide mass fingerprinting (PMF). Throughout this series of experiments, students will view the proteins as "the actors" and the genes as "the script" while identifying and matching proteins within the separate plant samples. Genetic comparisons will be made between the two plants, as well as, possible peptide differences based on the light-sensing mutations, comparing the mutant Arabidopsis plant to the unadulterated Arabidopsis plant.

MISSION:

The mission is to discover proteins that play important roles in sensing blue and red light using model plant Arabidopsis thaliana.

DESCRIPTION:

This unit will include a week- long series of labs related to research in the areas of: biology, genetics, biotechnology, proteomics, and bioinformatics. Students will develop the skills and conceptual understanding needed to understand the procedures used to determine a reasonable conclusion based on data collected from each consecutive lab. Working in teams, students will use scientific methodology, follow aseptic conditions, and gather both quantitative and qualitative data to determine outcomes.

This action proposal was inspired by the UF/HHMI/ICORE *2010 Emerging Pathogens Summer Institute* in Gainesville, Florida. Due to outstanding presentations, numerous laboratory experiences, and professional relationships, I developed a better understanding of the research and direct application of skills that led to the development of this action proposal.

I. BACKGROUND.

Arabidopsis thaliana, also known as the mouse-ear cress or thale cress (a genus of the mustard family), is widely used for genetic mapping and sequencing due to the fact that it has one of the smallest plant genomes (157 million base pairs and five chromosomes). According to the Arabidopsis Information Resource (TAIR) the plant has 27,000 genes and 35,000 encoded proteins. This plant is used often in the lab and is easily accessible.

Electrophoresis is often used in genetics to separate out different size DNA molecules on an agar plate in a buffer solution. This is done by pipetting the DNA samples into wells that are formed in the agar plate, then placing the gel in a chamber filled with buffer solution where electrical currents pull the negatively charged DNA molecules to the positive pole in the chamber. Bands or dark lines are "formed" on the agar gel, showing the smaller molecules moving more rapidly than the larger molecules. The bands closest to the starting wells represent the larger molecules, where conversely, the smaller molecules constitute the bands furthest from the starting wells. It is like the difference between two people trying to make their way through a very crowded street quickly. The larger man cannot get through the crowd as quickly as a much smaller boy, therefore the child goes much further in the same amount of time.

Electrophoresis is used in just the same way to form bands of proteins and nucleic acids. Smaller molecules are more effective at moving through the gel. This technique is exactly how we separate out the proteins and nucleic acids. The proteins in the cells from the Arabidopsis plant are extracted, run through the process of electrophoresis, and then stained. You can see patterns and determine a smaller number of proteins present in relatively large quantities. Unknown proteins can be identified using Peptide Mass Fingerprinting (PMF) technology involving mass spectrometry and protein database searching

II. IMPLEMENTATION.

This lab is intended to be used for the Genetics class and/or Advanced Biology students. Because the Academy's block system is 185 minutes twice a week and 75 minutes once a week, the labs should be completed within a one week period. Students will have both pretest and posttests in the areas of expected outcomes, and write a series of daily lab journal entries and Formal Lab Reports. Other forms of assessments will include: teacher observations during student group work and discussions, performance- based assessments, and student self assessments. Safety procedures and aseptic conditions will be maintained throughout the activities and labs. The mutagenized Arabidopsis thaliana plants will be supplied by Dr. Kevin Folta, Associate Professor, University of Florida, Gainesville, Horticultural Sciences Department and the Graduate Program in Plant Molecular and Cellular Biology. Students will be supplied equipment from UF-CPET-ICORE in order to proceed with labwork. Dr. Sixue Chen, Associate Professor, University of Florida, Gainesville, Department of Biology will assist in more advanced equipment and procedures near the end of the lab procedures in a culminating visit to his lab at the University of Florida. Digital photographs will document all aspects of the labs and careful records will be kept

concerning all data found. Strict protocol will be followed throughout all procedures and data collection.

III. EXPECTED OUTCOMES

After completing this activity students are expected to be capable of demonstrating competence in the following skills and concepts:

A. Measure solutions and transfer liquids using a micropipette

B. Describe the SDS-gel electrophoresis process (SDS-Sodium Dodecyl Sulfate)

C. Observe and analyze patterns of isolated protein bands

D. Identify differential gel bands between the two samples

E. Measure and plot the molecular weight of the markers

F. Measure the mobility of proteins of interest and calculate weights of proteins using standard curve

G. Develop basic understanding for Mass Spectrometry procedure

H. Analyze the acquired spectrum for samples from Mass Spectrometer

I. Use accumulated spectrum (3 spectra) to evaluate protein identification quality with "FBI" Fingerprint Database Searching-Protein Identification PMF (Peptide Mass Fingerprinting)

EXPERTISE:

Kristen Russell is currently a science teacher at the Academy of Environmental Science (AES), Citrus County's only public charter school. Ms. Russell teaches Science Research, Executive Internship, Biology, Genetics, Chemistry, Anatomy/Physiology, Physical Science, and Ecology, grades 9-12. During this time, she assisted in or led in the following: Coordinator; Citrus County Regional Science and Engineering Fair 2006-2010; Coordinator; Florida Regional Envirothon; Academy of Environmental Science Charter School Federal Dissemination Grant 2003-2004. Curriculum in the core courses taught are updated and reviewed bi-annually to incorporate the latest research.

LITERATURE:

Folta, Dr.Kevin (2008). *"Responding to the Rainbow"*

http://www.respondingtotherainbow.com

Folta, Dr. Kevin. Folta Lab Publications University of Florida;

http://www.arabidopsisthaliana.com

"TAIR- Genome Annotation:"

http:// www.arabidopsis.org/portals/genAnnotation/gene_structural_annotation/annotation_data.jsp.

Chen, Dr. Sixue . Protein Fingerprint Acquisition/NCBI database: http://www.moleculardetective.org University of Florida

Chen, Dr. Sixue. http://www.biology.ufl.edu/People/faculty/cv/schen.pdf

University of Florida Center For Precollegiate Education and Training: http://www.cpet.ufl.edu

Science For Life Website: HHMI Program UF-ICORE: http://www.hhmi.org/coolscience/resources

Howell, Stephen H., *Molecular Genetics of Plant Development*; Cambridge University Press, 1998

BUDGET AND BUDGET JUSTIFICATION: The following list summarizes the supplies needed for the activity including the approximate costs and sources of supplies.

Item	Quantity	Measurement	item#	Dealer	UNIT COST	TOTAL COST
Mini-PROTEAN Tetra Cell	1	2-gel	165-8005EDU	Bio-Rad	299.25	299.25
10% Resolving Precast Gels	2	10/pk	456-1033EDU	Bio-Rad	72.00	144.00
					GRAND TOTAL $	443.20

Uncovering “actors” in Arabidopsis through Blinding Its Light Sensing Capabilities: Proteomics-Protein Extraction, Separation and Identification

Grade Span: 11-12th Grade

Content Emphasis: Genetics and/or Advanced Biology

Florida Science Standards:

Nature of Science Body of Knowledge (Grades 9-12)

SC.912.N.3.5 Describe the function of models in science, and identify the wide range of models used in science.

Physical Science Body of Knowledge (Grades 9-12)

SC.912.P.8.2. Differentiate between physical/chemical properties and physical/chemical changes

SC.912.P.8.6. Distinguish between bonding forces holding compounds together and other attractive forces, including hydrogen bonding and van der Waals forces.

Life Science Body of Knowledge (Grades 9-12)

SC.912.L.15.6 Discuss/Distinguish characteristics of the domains and kingdoms of living organisms.

SC.912.L.16.10 Evaluate the impact of biotechnology on the individual, society, and the environment, including medical and ethical issues.

SC.912.P.17.20 Predict the impact of individuals on environmental systems and examine how human lifestyles affect sustainability.

SC.912.P.18.1 Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.

SC.912.P.18.4 Describe the structure of proteins and amino acids. Explain the functions of proteins in living organisms. Identify some reactions that amino acids undergo. Relate the structure and functions of enzymes.

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Learning Goals:

After completing this activity students are expected to be capable of demonstrating competence in the following skills and concepts:

A. Measure solutions and transfer liquids using a micropipette
B. Describe the SDS-gel electrophoresis process (SDS-Sodium Dodecyl Sulfate)
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This series of lessons is to be performed with the assistance of Dr. Sixue Chen, Associate Professor, University of Florida, Gainesville, and Department of Biology, where more advanced equipment and procedures will be necessary to carry out this culminating activity in his lab at the University of Florida.

UF/CPET/ ICORE labs will also be utilized for the activities and CPET staff will also be facilitators throughout the lab sessions.

Dr. Kevin Folta, Associate Professor, University of Florida, Gainesville, Horticultural Sciences Department and the Graduate Program in Plant Molecular and Cellular Biology, will also lend assistance for these lab experiments.

Estimated time:

The total time for the three experiments and "FBI" Fingerprint Database Searching-Protein Identification PMF is approximately 10 hours. Experiment #1 (parts 1 and 2) can be considered a stand-alone lesson. Time needed: 2hours 45 minutes.

Materials/Resources:

15 Arabidopsis plants (mutants)

15 Arabidopsis plants (unadulterated)

Digital scales (calibrated)

Eppendorf tubes (30)

Blue pestels

Incubator

Liquid nitrogen (NOT to be used by students- Dr. Sixue Chen will use in lab prep)

Centrifuge

SDS gels (30) - 10% Resolving Precast Gels (polyacrylamide gels)

15 Mini-PROTEAN Tetra Cells

*SDS gel buffer (enough to cover all precast gels in tetra cell chambers) Prepared by staff

Comassie Blue (SimplyBlue or BioSafe)

Distilled water

Pipettes and tips

Micropipettes

Forceps

Gloves

*SDS gel sample buffer:

100mM Tris, pH 6.8, 2% SDS, 5% B-mercaptoethanol, 15% glycerol, 0.02% bromophenol blue

Teacher Preparation:

The labs will be performed at the University of Florida Campus through UF/CPET. Buffer solutions, stains, and other solutions needed will be prepared in advance. If a teacher wants to implement an experiment or several experiments at the high school laboratory, items to do these labs can be ordered through Bio-Rad.

Students should be familiar with the process of micropipetting, and have a clear understanding of lab safety procedures and aseptic conditions in the laboratory prior to starting this series of experiments. A formal introduction will be held in the form of a short lecture (with students writing notes in journals) and clear stated objectives showing how observations, comparisons, and data will be collected and analyzed.

Students should have a clear understanding of the process of electrophoresis, the solutions used, and the formation of agarose plates to run molecules (whether it is DNA or proteins). *Implementation of a DNA electrophoresis lab experiment is highly recommended to promote student understanding prior to the experiments used in this plan.

Lab Procedures: [Adapted from Dr. Sixue Chen, Associate Professor, University of Florida, Gainesville, Florida, Department of Biology]

Introduction:

One of the most common ways of studying macromolecules- especially proteins and nucleic acids- is by electrophoresis. Electrophoresis can be thought of as a form of chromatography, in which the molecules move through a supporting matrix in response to an applied electric field. Examples of a suitable matrix include polyacrylamide and agarose, which form gels similar in texture to gelatin (Jell-O). Polyacrylamide and agarose gels are set up in such a way that proteins and nucleic acids migrate through them at a rate that is proportional to their size.

Electrophoresis is often used in genetics to separate out different size DNA molecules on an agar plate in a buffer solution. This is done by pipetting the DNA samples into wells that are formed in the agar plate, then placing the gel in a chamber filled with buffer solution where electrical currents pull the negatively charged DNA molecules to the positive pole in the chamber. Bands or dark lines are “formed” on the agar gel, showing the smaller molecules moving more rapidly than the larger molecules. The bands closest to the starting wells

represent the larger molecules, where conversely, the smaller molecules constitute the bands furthest from the starting wells. It is like the difference between two people trying to make their way through a very crowded street quickly. The larger man cannot get through the crowd as quickly as a much smaller boy; therefore the child goes much further in the same amount of time.

Electrophoresis is used in just the same way to form bands of proteins and nucleic acids. Smaller molecules are more effective at moving through the gel. This technique is exactly how we separate out the proteins and nucleic acids. The proteins in the cells from the Arabidopsis plant are extracted, run through the process of electrophoresis, and then stained. You can see patterns and determine a smaller number of proteins present in relatively large quantities. Unknown proteins can be identified using Peptide Mass Fingerprinting (PMF) technology involving mass spectrometry and protein database searching.

Arabidopsis thaliana, also known as the mouse-ear cress or thale cress (a genus of the mustard family), is widely used for genetic mapping and sequencing due to the fact that it has one of the smallest plant genomes (157 million base pairs and five chromosomes). According to the Arabidopsis Information Resource (TAIR) the plant has 27,000 genes and 35,000 encoded proteins. This plant is used often in the lab and is easily accessible. The Arabidopsis plant will be used in this series of experiments.

Objectives:

In this experiment, students will extract proteins from both a control and mutant Arabidopsis leaves. The leaves will then be analyzed through the process of electrophoresis in a polyacrylamide gel. Students will compare the protein patterns between the mutant Arabidopsis plant and the unadulterated Arabidopsis plant. Some protein bands can be excised and digested with a specific proteolytic enzyme, (e.g. trypsin) and then identified by PMF technology involving mass spectrometry and protein database searching.

Expected Outcomes:

After completing this activity students are expected to be capable of demonstrating competence in the following skills and concepts:

A. Measure solutions and transfer liquids using a micropipette
B. Describe the SDS-gel electrophoresis process (SDS-Sodium Dodecyl Sulfate)
C. Observe and analyze patterns of isolated protein bands
D. Identify different gels bands between the two samples
E. Measure and plot the molecular weight of the markers

F. Measure the mobility of proteins of interest and calculate weights of proteins using standard curve
G. Develop basic understanding for Mass Spectrometry procedure
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DAY 1:

Arabidopsis leaves with and without light-sensing mutations (provided by Dr. Kevin Folta)

Lecture 1. Introduction to Proteomics (Dr. Sixue Chen)

Experiment 1. Part 1. - Protein Extraction from Arabidopsis Leaves

Please note that steps 1-3 have been done for you, and you will begin with step 4.

1. Weigh leaves from control and mutant plants (~0.1 g fresh weight) and put in labeled eppendorf tube
2. Quick freeze in liquid nitrogen
3. Grind control and treated samples into fine powder, dipping in liquid nitrogen every few seconds so the leaves do not defrost
4. Add ~150 ul (1.5 ul per mg fresh weight) SDS gel ample buffer
5. Continue grinding for a couple minutes with blue pestles
6. Put the tubes at 95 degrees C to denature the protein for 10 minutes
7. Centrifuge at the highest speed at room temperature for 10 minutes
8. Load 25 ul supernatant of each control and mutated sample on a SDS gel with a molecular weight marker standard; load 5ul of marker

SDS gel sample buffer:

100 mM Tris, pH 6.8, 2% SDS, 5% B-mercaptoethanol, 15% glycerol, 0.02% bromophenol blue

Experiment 1. Part 1. – Protein Separation and Staining

SDS-gel electrophoresis: Load the samples on a polyacrylamide gel, together with molecular weight markers and samples from other groups. Pre-stained molecular weight markers (Cat. No. 161-0305) will be loaded so that you can see how they move under the influence of the

electric field. Run the gel at 100 volt until the blue dye front is a couple of millimeters from the bottom of the gel (approx. 45 minutes). The marker you loaded is a composite of six proteins with known molecular weights listed below.

MOLE WEIGHT MARKERS	M.W. (kD)
Phosphorylase	113
Bovine Serum Albumin	92
Ovalbumin	53
Carbonic Anhydrase	35
Soybean Trypsin Inhibitor	29
Lysozyme	22

Gel Staining: Comassie Blue (Simply Blue or BioSafe) Staining

1. Add distilled water to cover your gel and leave it on a shaker for 5 minutes (students can gently agitate the gels in a flat tray if a mechanical shaker is not available).
2. Repeat step 1 twice. Discard and replace the distilled water before agitating for a second time.
3. Add 20 ml stain solution and leave it for more than an hour.
4. Transfer the gel to distilled water to destain (This step will be done for you if time does not permit). Gels can be stored in 2% NaCl solution (or 0.01% NaN_3) in refrigerator for several weeks.

Experiment 2. – Protein In-Gel Digestion

Have students analyze the results of the gels from the protein samples that were isolated. Observe the pattern of protein bands. Identify differential gel bands between the two samples.) Optional: measure the distance migrated by each of the molecular weight markers. On semi-logarithmic paper, plot the molecular weight of the markers (dependent variable) as a function of the distance each moved from the top of the gel (independent variable). The graph should result in a straight-line. This is a MW standard curve. Measure the mobility of your proteins of interest and calculate the molecular weights of the proteins in your samples using the standard curve).

**The following activity will be prepared and guided by graduate students, faculty, and/or professors at the University of Florida.

Procedures:

1. Cut out one band of your interest from gel (cut it carefully into a 1mm square) and place into
   1.5 ml microcentrifuge tube (labeled TUBE #1).
2. Add 200 ul of 100% ACN. Vortex for 5 minutes to dehydrate gel.
3. Remove any liquid from tube. There should be a hard whitish piece of gel remaining in the tube.
4. Remove all the liquid using a pipette with a small tip attached to a big tip.
5. Add 35 ul of trypsin solution to dried gel. Allow gel pieces to swell on bench for 5 minutes.
6. Place gel (in tube) in the microwave digestion apparatus at 50 Watts power at 55 degrees C for 10 minutes.
7. Add 40 ul of 80% acetonitrile/0.1% formic acid. Vortex for 5 minutes
8. Spin down briefly and remove all liquid and transfer gel into a new 1.5 ml microcentrifuge tube (labeled TUBE #2).
9. Repeat steps #7 and #8 and combine liquid in TUBE #2.
10. Place sample into speed-vac and evaporate for 30 minutes. Samples are ready for mass spectrometry MALDI-TOF MS or ESI-MS.
11. For MALDI-TOF MS, dissolve in 1ul MATRIX solution and spot on MALDI plate. Let dry.

Following this lab, students can form two groups and visit 1) ICBR Proteomics for Peptide Mass Fingerprinting (PMF) and database searching, and 2) the University of Florida Chemistry department for mass spectrometry demo.

Dr. Sixue Chen, University of Florida has shared his experiences in protein identification by Peptide Mass Fingerprinting (PMF). He has toured groups through his laboratory, doing a demonstration of the MALDI-TOF MS calibration procedure. Students would be able to locate and place their sample from the previous experiment in a well that would be placed in the MALDI-TOF MS Peptide Fingerprint Acquisition apparatus. This information can be saved and burned to a disk for the students.

Assessment:

Student assessments will include: Individual student scientific journals; Teacher observations and questioning prior to, during and after experiments; a TRUE/FALSE pretest/posttest; and an ADVANCED ANALYSIS QUESTION sheet (see next three pages).

Name: _____ Date: _____

Part 1: Is the Answer True or False? Circle one- Pretest/Posttest

1. Peptide sequences can be determined by fragmenting the peptides in the mass spectrometer. _____
2. Proteins are micromolecules with a molecular mass smaller than a simple sugar like sucrose. _____
3. Acrylamide is usually chosen for analyzing protein or small nucleic acids while agarose is usually chosen for analyzing large nucleic acids. _____
4. PMF stands for Protein Manipulation Forensics. _____
5. Smaller proteins or nucleic acids move through the gels more rapidly than do larger molecules. _____
6. Arabidopsis thaliana is a plant with a large genome. _____
7. A milliliter is larger than a micro liter. _____
8. Electrophoresis is used to form bands of DNA, proteins, and carbohydrates. _____
9. We can evaluate protein identification quality with “FBI” Fingerprint Database Searching-Protein Identification PMF. _____
10. Proteomics is an area of scientific study. _____

Part 2-ADVANCED ANALYSIS QUESTIONS:

1. Can all proteins be confidently identified using PMF technology? What are the factors that affect protein identification by PMF?
2. Practice PMF with your own data or use PMF data from www.moleculardetective.org website. What are the identities of the proteins after protein database searching using the mass spectra acquired? What are the functions of the proteins you identified? Do they make sense?
3. Compare your protein profiles from your unadulterated Arabidopsis plant and your mutant Arabidopsis plant leaves. Using your standard curve, calculate the mass of two highly abundant protein bands found in the unadulterated plant and three abundant protein bands found in the mutant sample.
4. Plot of log of the MW as a function of mobility. Theoretically, this should result in a straight-line relationship. Does it?

Teacher Answers For True-False Pretest/Posttest:

1. True
2. False macromolecules, larger
3. True
4. False peptide mass fingerprinting
5. True
6. False widely used because it has one of the smallest plant genomes
7. True
8. False DNA, proteins, and NUCLEIC ACIDS (Not carbohydrates)
9. True
10. True

Mission Biotech

Preparing for the Biotechnology Field

Mission Biotech Collaboration Pilot

- In the spring of 2011, 20 sophomore students at the State University of New York, School of Environmental Science and Forestry (ESF) are participating in a 4-week program using Mission Biotech (MBt) as part of their educational curriculum.

BTC 497

- Research Problem Design & Professional Development is a course designed to prepare students in the biotechnology program for their upcoming internship and independent research problems classes.

Project Plan

- Completion of Pre and Post Test assessments/ Survey
- Completion of all four levels of the simulation activity
- Maintain a Virtual Lab Notebook
- 2-page summary report and assessment of MBt

Written Survey

- To explore how students learn about biotechnology and biotechnology careers
- Survey student ideals about technology and science including:

a. Learning styles
b. Careers
c. Biotechnology

Game Level Objectives

Successful completion of:

- Level 1- DNA extraction
- Level 2- PCR techniques
- Level 3- reverse transcription
- Level 4- screening and testing for unknown virus

Expectations

- Understanding of core concepts in biotechnology
- Awareness of careers in biotechnology
- Communicate with others in a virtual learning environment
- Identify and follow common protocols used in a biotech laboratory

Learning Objectives

- Understanding of core concepts in biotechnology, including cellular biology, DNA, RNA, and viruses
- Determine the appropriate equipment needed for a given task
- Analyze real-time PCR
- Extract DNA
- Understand Reverse Transcription protocol

Support and Troubleshooting

- Internet access
- DVD
- Play “locally” or login?
- http://missionbiotech.com