Stolen Soybeans!!!

Introduction

Lesson Introduction

Genetically modified, or Bt crops, have been in the spotlight over the last few years. The range of acceptance to these Bt crops can vary in their acceptance. But one thing is for sure – they are a hot commodity in the agriculture world. In this lab your students will use electrophoresis techniques to solve a mystery of stolen soy beans.

Grade Level: 9-12
Time Needed: 90 minutes

Learning Objectives

After completing this lesson, students will be able to:

1. Set up, run, and interpret the results of a gel electrophoresis lab
2. Describe how scientists work in the lab
3. Describe the difference between natural and Bt crops/food
4. Describe the benefits of Bt foods

Next Generation Science Standards (NGSS)

As a result of activities in grades 9 - 12, all students should develop:

Topic

- **LS3:** Inheritance & Variation

Performance Expectation

- **HS-LS3-3:** Apply Concepts of statistics and probability to explain the variation and distribution of expressed traits in a population

Dimension

Practices:

- Asking Questions
- Carrying out Investigations
- Analyzing & Interpreting Data
- Constructing Explanations
- Engaging in Argument from Evidence

Disciplinary Core Ideas:

- LS3.B: Variation of Traits

Cross-Cutting Concepts:

- Patterns

Materials

- ☐ **Power Supply /Gel Boxes**
  - For a classroom of 24 students, it is best to have **4 power supplies and 8 gel boxes:**
  - Gel electrophoresis boxes Horizon 58 (Labrepco) $400 each
  - Power Supplies Model 250 VWR 27370-265 $500 each

  Option 2: Carolina Biological set (http://www.carolina.com/product/213620.do)

  Perfect for use in AP® Biology and the ABC CORD curriculum. Package I contains enough equipment for electrophoresis of 12 gels at one time. The laboratory manual, *DNA Science: A First Course,* gives complete instructions on molecular techniques for educators trained in science, but who lack a background in molecular biology.

  Option 3: Build Your Own

  (See attachment http://learn.genetics.utah.edu/content/labs/gel/build_gel_box.pdf)
- ☐ **Micropippettes**

  16 adjustable volume micropipettes: (Rainin.com)

  8 each PR-20 (2-20ul) $150 each educational discount

  8 each PR-200 (20-200ul) $150 each educational discount
- ☐ **50 cc test tubes** 1 per station
- ☐ **Distilled water** (150 ml per station)
- ☐ **1X TAE (Tris Acetate EDTA buffer)**

  To make 50X TAE Buffer
  - 242.0g Tri Base
  - 57.1 ml glacial Acetic Acid
  - 100 ml 0.5M EDTA (18.612 g EDTA in 100 ml dH20, pH to 8.0)
  - QS to 1 liter with dH20
- ☐ **NaCl Solution**. 1M

  Each station will need **1 ml** pre-aliquoted in a 1.8 ml eppendorf tube OR provide each station with a bottle of 1M NaCl solution (approx 25 ml) and provide them with fixed 1000 ul micropipettes
- ☐ **Agarose (**0.2 g per student team per gel)
- ☐ **Dye samples**

  Pre-aliquot 1ml in 1.8ml microcentrifuge tubes of six samples including one sample that is a mix of two dyes of the same charge, but different size/weight.

  *For this lab, the **agarose gel must be made in 1X TAE buffer**, but you can use a dilute salt solution (1 ml of a 1M NaCl solution in 125 ml distilled water) in place of buffer when running the gels.*

Procedure

1. **Make a 0.8% agarose gel:**
   - Transfer the 0.2 grams of agarose into your 125ml Erlenmeyer flask.
   - Measure **25 ml** 1X TAE Buffer (bottle with green tape) using the 50 cc centrifuge tube and add to the agarose in the Erlenmeyer flask. Swirl gently to mix.
   - Microwave for 20 seconds. Check to see if all the particles are dissolved and the solution is very hot. If not, heat again for no more than ten seconds.
   - Bring to your station and let cool until it is lukewarm to the touch. To help the agarose cool, swirl it gently in the flask. If the agarose is too hot, it will leak out the ends of the red striped gel tray.
   - While the gel is cooling, place the black dams in the gel box on both ends of the gel tray insert. Be sure the flat side of the dams are against the gel tray insert. The tops of the dams should be perfectly flat if they are put in correctly.
   - Insert the **8-well comb** in the red striped tray in the middle of the tray.
   - When the agarose has cooled to the point that you can hold the bottom of the Erlenmeyer flask comfortably, pour the 25 ml of agarose into the gel tray.
   - Let the gel harden for at least 10 minutes. Do not move the tray while setting.
   - Once the agarose is hardened, remove the two black dams.
   - Add 125 ml of a dilute salt solution (1 ml 1M NaCl diluted in 125 ml distilled water) – an amount sufficient to just cover the gel.
   - Carefully "wiggle" the comb back and forth to separate the comb from the gel and then lift *slowly and gently* out of the gel.

2. **Add the dye samples to the wells in the agarose gel:**
   - The DNA from 5 unknown plants as well as a known sample have been extracted and stained so that the DNA is visible.
   - Carefully pipette 10 ul of each of the samples into separate wells. Change pipette tips with each dye.
   - The known DNA sample needs to be in well #1. This will act as your control.
   - One of the most common mistakes in using a pipette is not bracing arms or hands. This may result in pushing the pipette through the bottom of the well, resulting in loss of the dye under the agarose, rather than in the well.
   - Very small amounts of dyed DNA will often "float" out of the wells. These small amounts of dyed DNA will be diluted in the buffer and will not affect the running of the dyes that remain in the wells.
   - Remember to write down the order in which you pipette the DNA dyes.

Procedure (continued)

3. Run the dyed samples

- After all dyed samples are pipetted into the gel wells, close the lid, attach the power cords, and set the voltage range switch to 100 volts.
- Be sure you connect the wires so the black wires are closest to the power supply "run to red"
- Run the gel for approximately 10 minutes or until you get good separation of all the dyes.

4. Record Your Results

- Turn the electrophoresis unit off and observe your results. Record your results on the back of this page by drawing where each of the dyes are in the gel.
    - Record the distance each of the dyes traveled to determine which of the dye DNA samples match the known Pioneer DNA sample.
    - Determine whether or not the seeds were stolen.
- You can also have a permanent record of your results by doing a "blot" on a paper towel. Place the agarose gel onto several folded paper towels. Cover with several more paper towels and apply gentle pressure to the gel-paper towel stack. This will allow the dye to transfer out of the gel onto the paper towels, giving you a record of your results.

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Background

Agarose gel electrophoresis is a powerful and widely used method that separates molecules on the basis of electrical charge, size, and shape. The method is particularly useful in separating charged biologically important molecules such as DNA (deoxyribonucleic acids), RNA (ribonucleic acids), and proteins.

Agarose gel electrophoresis possesses great separating power, yet is very simple to perform. An agarose gel is made by boiling agarose powder in an acid/base controlled (buffered) solution. The solution is then cooled and poured into a mold where it becomes solid, like jell-o. A comb-shaped mold with square teeth is placed in the gel so that rectangular holes (wells) can be made when the agarose cools and gets solid. The gel is submerged in a buffered (acid-base controlled) chamber (gel box) containing two electrical contacts ("+" and "-" electrodes).

Samples are prepared for electrophoresis by mixing them with a thick sugar solution. This makes the samples heavier than the buffer solution in the gel box. These samples are carefully loaded into the rectangular wells using a very expensive pipette (a micropitetter or transfer pipette). The heavier samples sink through the buffer solution and settle into the rectangular wells – one sample in each well.

A power supply is connected to the electrophoresis gel box and direct current is applied to the samples. The buffer solution completes the circuit between the positive and negative electrodes. Charged molecules in the samples enter the agarose gel through the sides of the wells and move between the agarose molecules. Molecules with a negative charge (anions) move toward the red positive (+) electrode (the anode). Molecules with a positive charge (cations) move toward the negative black negative electrode (cathode). The higher voltage used, the faster the molecules travel. The buffer serves to make the water a better conductor of electricity and to control acid-base (pH) extremes. The pH is important to the charge and stability of many types of molecules.

Agarose is a very large sugar molecule found in certain kinds of marine algae. The agarose gel contains molecule sized pores, acting like molecular strainers. The pores in the gel control the speed that molecules can move. Smaller molecules move through the pores more easily than larger ones. Molecules can have the same molecular size (weight) and electrical charge, + or -, but different shapes. Molecules having a compact shape (eg a baseball versus a beach ball) can move more easily through the pores. Given two molecules of equal size (weight) and shape, the one with the greater electrical charge will move faster. Conditions of charge, size, and shape interact with one another depending on the structure and composition of the molecules, buffer conditions, gel thickness, and voltage.

Pre-Lab:

Go to the DNAi website **www.dnai.org > Manipulation > Techniques > sorting and sequencing.**
View the Gel Electrophoresis 2-D animation, and answer the following questions.

1. How does the process of gel electrophoresis separate DNA fragments?
2. What is the purpose of the agarose gel?
3. What is the purpose of adding blue "tracking" dye to the DNA samples?
4. Explain why DNA has an overall negative charge.
5. Why is the fact that DNA has a negative charge so important in the gel electrophoresis process?
6. Explain how an agarose gel can separate DNA fragments of different lengths.
7. What is a restriction map?

Go to the WHO site http://www.who.int/foodsafety/areas_work/food-technology/faq-genetically-modified-food/en/ and answer questions (8 & 9)

8. What are genetically modified / biotechnology organisms? Ho long has this technology been around?
9. Identify at least 5 biotechnology crops.

IDENTIFICATION OF STOLEN SOY BEAN SPECIES
AGAROSE GEL ELECTROPHORESIS

INTRODUCTION:

You are working as a laboratory technician for Pioneer Hi-Bred, a DuPont business. This is a science based company out of Johnson, Iowa that breeds and produces hi-bred seeds. Their products are shipped to over 90 countries. They produce products from corn to soybeans. Many of their lines are genetically modified. Pioneer scientists have been using state of the art technology to increase productivity and maximize profit. It takes many years and a multitude of thousands of dollars to produce such quality seeds. So – farmers will pay more for theses seeds and abide by a set of rigorous guidelines. Because of their superior quality of the seeds, people have begun stealing them. The company recently got word about a local farmer who allegedly stole seed from one of Pioneer's long time customers. Officials have collected samples of several of the crops from both the alleged farmer and from the Pioneer customer. You have been given six samples to test using agarose gel electrophoresis to find out if the alleged farmer is using stolen seed by comparing the patterns created in the gels.

MATERIALS:

- ☐ Electrophoresis apparatus and power supply
- ☐ Agarose (0.2 gram in 2 ml Eppendorf tube – pre-measured)
- ☐ 125 ml Erlenmeyer flask
- ☐ 50 cc centrifuge tube for measuring the TAE buffer
- ☐ 1X TAE buffer
- ☐ 1 ml of a 1M solution of NaCl to be diluted in 125 ml distilled water.
- ☐ micropipette (0-20 ul)
- ☐ micropipette tips

METHOD:

1. To make a 0.8% agarose gel:

- Transfer the 0.2 grams of agarose into your 125ml Erlenmeyer flask.
- Measure **25 ml** 1X TAE Buffer (bottle with green tape) using the 50 cc centrifuge tube and add to the agarose in the Erlenmeyer flask. Swirl gently to mix.
- Microwave for 20 seconds. Check to see if all the particles are dissolved and the solution is very hot. If not, heat again for no more than ten seconds.
- Bring to your station and let cool until it is lukewarm to the touch. To help the agarose cool, swirl it gently in the flask. If the agarose is too hot, it will leak out the ends of the red striped gel tray.
- While the gel is cooling, place the black dams in the gel box on both ends of the gel tray insert. Be sure the flat side of the dams are against the gel tray insert. The tops of the dams should be perfectly flat if they are put in correctly.
- Insert the **8-well comb** in the red striped tray in the middle of the tray.
- When the agarose has cooled to the point that you can hold the bottom of the Erlenmeyer flask comfortably, pour the 25 ml of agarose into the gel tray.
- Let the gel harden for at least 10 minutes. Do not move the tray while setting.
- Once the agarose is hardened, remove the two black dams.
- **Add 125 ml of a dilute salt solution (1 ml 1M NaCl diluted in 125 ml distilled water) – an amount sufficient to just cover the gel.**

- Carefully "wiggle" the comb back and forth to separate the comb from the gel and then lift *slowly and gently* out of the gel.

Adding the dye samples to the wells in the agarose gel:

- The DNA from 5 unknown plants as well as a known sample have been extracted and stained so tht the DNA is visable.
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Running the dyed samples

- After all dyed samples are pipetted into the gel wells, close the lid, attach the power cords, and set the voltage range switch to 100 volts.
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Recording the Results

- Turn the electrophoresis unit off and observe your results. Record your results on the back of this page by drawing where each of the dyes are in the gel.
    - Record the distance each of the dyes traveled to determine which of the dye DNA samples match the known Pioneer DNA sample.
    - Determine whether or not the seeds were stolen.
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SOY LEAF DNA SAMPLES:

Well #1 – Known DNA Sample

Well #2 - Farmer #1

Well # 3 – Farmer #2

Well # 4 – Farmer #3

Well #5 – Farmer #4

Well # 6 – Farmer #5

CATHODE (-) **ANODE (+)**

1. Known sample sample(s)_____.
2. Positive match sample (s)_____.
3. Negative match sample (s) _____.
4. Dye sample to be sent for further investigation _____.
5. Why did you add a dilute salt solution rather than distilled water to cover the gel?
6. What are your possible sources of error in this lab?
7. What was the purpose of the control in this lab?
8. What do you think should be the punishment of those who steal BT seeds?
9. What are the benefits of using BT seeds in agriculture?
10. Please identify three other ways that electrophoresis technology can be used.
11. Compare and contrast the benefits of using biotechnology.
12. How is genetic engineering like computer programming?

Data Presentation

Using the data collected, make a case for or against the alleged farmer. Be sure to use the information you learned about gel electrophoresis in your presentation.

Follow Up Questions

A. Why do we use Electrophoresis

B. What are the main concerns of using biotechnology

C. How does the process work?

D. How could biotechnology affect your privacy?

E. Would you buy genetically modified foods?
Why or Why not?