

ELECTROPHORESIS OF CHEMICAL DYES

LEARNING OUTCOMES

Students will learn the basic technique of gel electrophoresis, a procedure used in many aspects of genetic research to separate fragments of DNA from one another. Chemical dyes will be used in place of DNA.

QCC STANDARDS

APPLIED BIOLOGY & CHEMISTRY 2:

Process Skills Standards 1 & 3

4.3 Explain how we can distinguish between two individuals by analyzing tissue samples.

10.1 Explain how new technologies can be used to detect defects in genes.

10.2 Describe the basic processes used in genetic engineering.

11.1 Identify the ways genetic engineering can benefit mankind.

11.2 Evaluate the ethical issues surrounding the use of genetic engineering.

BIOLOGY:

Process Skills Standards 1 & 3

10.1 Define important genetic terms.

11.3 List significant contributions of genetic engineering to agricultural and medical practices.

ENVIRONMENTAL SCIENCE:

Process Skills Standards 1 & 3

34.1 Describe the genetic code.

34.2 Differentiate between classical applied genetics and molecular applied genetics.

34.3 Identify the process of gene manipulations.

34.4 Demonstrate an understanding of gene manipulation techniques.

34.7 Describe various applications of biotechnology and the role it will play on future health and environmental clean up.

34.8 Compare the areas of biotechnological applications including gene therapy.

NATIONAL STANDARDS

In all organisms, the instructions for specifying the characteristics of the organism are carried in DNA, a large polymer formed from subunits of four kinds (A, G, C, and T). The chemical and structural properties of DNA explain how the genetic information that underlies heredity is both encoded in genes (as a string of molecular "letters") and replicated (by a templating mechanism). Each DNA molecule in a cell forms a single chromosome.

Scientists in different disciplines ask different questions, use different methods of investigation, and accept different types of evidence to support their explanations. Many scientific investigations require the contributions of individuals from different disciplines, including engineering. New disciplines of science, such as geophysics and biochemistry often emerge at the interface of two older disciplines.

Science often advances with the introduction of new technologies. Solving technological problems often results in new scientific knowledge. New technologies often extend the current levels of scientific understanding and introduce new areas of research.

BACKGROUND & DEFINITIONS

Electrophoresis is a technology in science that allows an individual to separate molecules according to size. The procedure works analogous to a screen, in which larger particles are less likely to move through than small particles. Thus, in electrophoresis, using agarose gel as the "screen", smaller molecules will move farther through than larger particles. When these fragments are seen, the electrophoresis will produce bands of color where fragments have stopped. Ideally, if two samples are placed side by side in the gel, fragments of equal size will move an equal distance with one another, letting an individual identify whether or not similar size fragments are in two different samples. These procedures form the basis of DNA fingerprinting, where electrophoresis separates fragments of DNA. A blood sample from a crime scene can be compared to a blood sample from a suspect. When chemicals are used to cut up pieces of the DNA, both samples will be cut at the same place if the crime scene sample came from our suspect. When these two samples are placed in an electrophoresis device, identical banding patterns should occur, leading us to interpret that the crime scene DNA and the suspect's DNA are the same.

MATERIALS & EQUIPMENT

For Buffer Solution:

Balance
1L Beaker
1000 ml graduated cylinder
Magnet stirrer
Large container
Tris base
Boric acid
EDTA
Distilled water

For 0.8% Agarose:

Microwave
250ml bottle
Balance
Weighing paper
Hot water bath
Agarose powder

For 2% Dye Stock:

6 tubes with caps
10 ml grad. cylinder
Weighing paper
Pyronin Y
Methyl Orange
Safranin O
Xylene Cyanol
Bromphenol Blue
water

For Dye Samples:

10 ml graduated cylinder
25 ml graduated cylinder
Small container with cap (6)
Stirring rod
Glycerol
2% dye stocks
Distilled water

Other Materials:

Genemate yellow 1-200 ul pipette tips
Graduate micropipettes, 5/pack
Gel electrophoresis apparatus
Electrophoresis Power Supply
Microfuge tubes, 0.5 ml

WEB RESOURCES

http://www.rit.edu/~pac8612/electro/E_Sim.html - Virtual electrophoresis

<http://ntri.tamuk.edu/electrophoresis/home.html>

<http://www.sciam.com/askexpert/biology/biology3.html>

<http://www.biology.washington.edu/fingerprint/dnaintro.html>

<http://www.pbs.org/wgbh/nova/sheppard/analyze.html>

SAFETY

Safety goggles and gloves needed while handling chemicals and glass.

DURATION

50-100 minutes depending upon whether teacher or students prepare gel.

PROCEDURE

To make various chemical solutions for activity:

1. TBE Buffer: Add 10.8g Tris base, 5.5g boric acid, and 0.74g EDTA into a 1000ml beaker.
2. Measure 1000 ml of deionized or distilled water and beaker containing the dry chemicals.
3. Using the electric stirrer, mix chemicals until all are dissolved (this is the TBE Buffer). Cover and store at room temperature. Sample can be reused several times before disposal.

1. 0.8% Agarose: Weigh 1g of agarose on weighing paper and add to 250ml bottle.
2. Add 125ml of TBE buffer solution. The container should never be filled more than halfway in order to prevent the solution from boiling over!
3. If a bottle is used, leave the cap loose to release air during boiling. If you use a flask, cover the opening and neck of flask with plastic wrap for the same reason.
4. Swirl to mix. Microwave the solution on high until the powder is completely dissolved and is clear in color.
5. If you plan to use this solution over several class periods, you will want to keep it liquefied. To do this, store the bottle of agarose in a hot water bath. Be sure that the bottle or flask is covered to prevent evaporation. A hot plate with a pot of water can substitute for a laboratory water bath.

1. 2% Dye Stock: Measure 0.2g of one of the dye powders onto folded weighing paper.
2. Add the dye powder into a tube (10-15ml).
3. Add 10 ml distilled water to the dye, and cap the tube. Shake well to dissolve the dye. For some dyes, not all the powder will go into solution. Label the tube.
4. Repeat steps 1-3 for each dye.
5. Store at room temperature.

1. Dye Samples for Electrophoresis: Prepare a 50% glycerol solution by adding 10ml glycerol into a 25 ml graduated cylinder with 10ml distilled water. Mix the glycerol and water by stirring. The glycerol solution can be stored in a small, closed container in the refrigerator.
2. Dilute the dye stock for electrophoresis. Using a pipette, add 2ml of the glycerol solution into a 25ml graduated cylinder, followed by 1ml of a 2% dye solution.
3. Add 17ml distilled water. Stir to mix the ingredients together. This will be used in the procedure. Each group will use 12 ul of the dye sample per lane on the gel. Store unused dye samples in a small closed container in the refrigerator.
4. Repeat steps 2 - 4 for each dye stock.
5. Prepare an unknown dye sample. Mix 2ml glycerol solution with 1ml each of 2% methyl orange, 2% bromophenol blue, and 2% xylene cyanol in a 25ml graduated cylinder. Add 15ml distilled water. Stir the ingredients together. The unknown dye sample is ready to use, and store any unused dye sample in a small closed container in the refrigerator.

1. Practice Dye: Students will need to practice adding a dye to the gel before beginning the "real" procedure. Use the bromophenol blue sample for this. Prepare sample tubes for each student by adding 12 ul (microliters) of the practice dye to a small microcentrifuge tube (0.5 or 0.65 ml size).

1. Prepare individual samples for groups. Label small microtubes (0.5 or 0.65 ml size) with numbers 1- 6 for each of the dyes to be tested. Prepare enough tubes for all the lab stations. Place 12ul of each sample into individual tubes. Make sure labels match the dye. Store in refrigerator.

Doing the Lab

1. Making the gel: Set up black casting dams and combs in electrophoresis unit. Place one comb in the slot near the red (+) electrode and the other comb in the middle slot.
2. Pour agarose into the gel deck. Pour close to the top of the gel deck, but do not overfill.
3. Practice using the micropipette and unmarked samples while the gel is solidifying.
4. When solid, remove the black casting dams, add TBE buffer to electrophoresis unit, and remove the combs.
5. Loading the dye: Practice loading unmarked practice samples in the wells near the red electrode. Load the numbered dye samples in the wells in the middle of the gel.
6. Connect electrophoresis unit to power supply.
7. Turn on the power supply, and set voltage to ~100 V. The dyes will start resolving toward both the negative and positive poles.
8. Electrophorese samples for about 10 minutes. Turn off the power supply, disconnect power cords from the chamber, and remove top of electrophoresis chamber.

1. Analyzing the results: Carefully remove casting tray with gel. Place gel in large weigh tray.
2. Draw a diagram of the banding pattern of the samples.
3. Compare known dyes with the unknown dye sample.

Students should compare the banding pattern of their unknown sample with the known samples. Have them make a conclusion as to the composition of the unknown and provide evidence for their decision. Tie this procedure in to DNA fingerprinting in analyzing unknowns by comparing them to knowns.

Also, you may want to tie this in to physical science concepts by asking why the chemicals move in specific direction (they have charges and are "attracted" to certain charges induced by the electricity).

EXTENSION

Have students research the history of forensic sciences leading up to DNA fingerprinting. Topics might include specific court cases in which fingerprinting has been used, how this technology evolved, and other applications in which electrophoresis is used in genetic studies. Assessment could include formal reports or poster presentations. Technology could be included to develop Power Point presentations.

Procedure and chemical "recipes" taken from <http://biotech.biology.arizona.edu>.

Student Sheet

OVERVIEW

Electrophoresis is a technology in science that allows an individual to separate molecules according to size. The procedure works analogous to a screen, in which larger particles are less likely to move through than small particles. Thus, in electrophoresis, using agarose gel as the "screen", smaller molecules will move farther through than larger particles. When these fragments are seen, the electrophoresis will produce bands of color where fragments have stopped. Ideally, if two samples are placed side by side in the gel, fragments of equal size will move an equal distance with one another, letting an individual identify whether or not similar size fragments are in two different samples. These procedures form the basis of DNA fingerprinting, where electrophoresis separates fragments of DNA. A blood sample from a crime scene can be compared to a blood sample from a suspect. When chemicals are used to cut up pieces of the DNA, both samples will be cut at the same place if the crime scene sample came from our suspect. When these two samples are placed in an electrophoresis device, identical banding patterns should occur, leading us to interpret that the crime scene DNA and the suspect's DNA are the same.

PROCEDURE

Chemicals will be prepared for you before class.

1. Making the gel: Set up black casting dams and combs in electrophoresis unit. Place one comb in the slot near the red (+) electrode and the other comb in the middle slot.
 2. Pour agarose into the gel deck. Pour close to the top of the gel deck, but do not overflow.
 3. Practice using the micropipette and unmarked samples while the gel is solidifying.
 4. When solid, remove the black casting dams, add TBE buffer to electrophoresis unit, and remove the combs.
 5. Loading the dye: Practice loading unmarked practice samples in the wells near the red electrode. When you feel that you are successful in loading the dyes, load the numbered dye samples in the wells in the middle of the gel.
 6. Connect electrophoresis unit to the power supply.
 7. Turn on the power supply, and set voltage to ~100 V. The dyes will start resolving toward both the negative and positive poles.
 8. Electrophorese samples for about 10 minutes. Turn off the power supply, disconnect power cords from the chamber, and remove top of electrophoresis chamber.
1. Analyzing the results: Carefully remove casting tray with gel. Place the gel in a large weigh tray.
 2. Draw a diagram of the banding pattern of the samples.

QUESTIONS

1. Describe what happened to the dyes when the power was turned on.
2. Why do you think the dyes moved as they did?
3. Describe the similarities in banding patterns between the dyes.
4. Provide an argument, with evidence, as to the composition of the unknown sample. Compare your conclusion with others in class.
5. How can this technique be used in crimes?