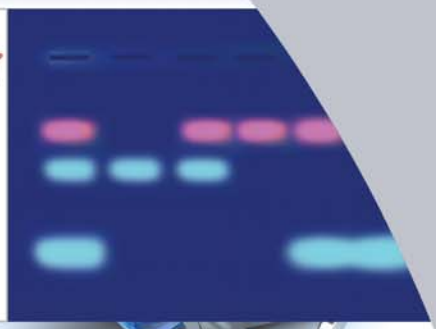
EDVOTEK WORKSHOP

Martian Genetics:
An Electrophoresis Exploration

EDVOTEK®



www.edvotek.com

Introduction

Explore genetics with our "out of this world" workshop! Imagine being the first scientist to explore Mars and discovering extraterrestrials. How would you use biotechnology to learn about the Martians? Discover how DNA technology can be used to explore the relationship between genotype and phenotype. Fluorescent dyes simulate DNA fragments, eliminating post-electrophoresis staining and saving classroom time!

Background Information

Excerpts from Cat. #S-52

PRINCIPLES OF GEL ELECTROPHORESIS

Agarose gel electrophoresis is a common laboratory technique used to separate molecules based upon charge, size and shape. It is particularly useful in separating charged biomolecules such as DNA, RNA and proteins. This technique possesses great resolving power, yet is relatively simple and straightforward to perform.

Agarose, a polysaccharide derived from seaweed, is used to form the separation matrix used for gel electrophoresis. To make a gel, solid agarose powder is added to buffer and melted by boiling. The buffer controls the pH of the solution throughout the electrophoresis process, which influences the charge and stability of biological molecules. Once the solution has cooled to approximately 60° C, it is poured into a gel tray to solidify. A special comb is used to form depressions in the gel called loading wells. Once solidified, the gel is placed in a horizontal electrophoresis chamber and covered with a pH-balanced buffer. Electrodes placed at each end of the electrophoresis chamber generate current when connected to a direct current power supply. The buffer contains ions necessary to conduct the electrical current.

Samples are prepared for electrophoresis by mixing them with glycerol or sucrose, which makes them denser than the electrophoresis buffer. When the samples are loaded into the wells, the dense samples sink through the buffer and remain in the wells. An electrical current is passed through the gel to drive molecules through the gel. Generally, the higher the applied voltage, the faster the samples are separated by electrophoresis. Once the current is applied, the following factors affect the mobility of molecules through a gel:

1. Molecular size: On the molecular level, the gel contains small channels that act as a molecular sieve. Small molecules move through these holes easily, but larger ones have a more difficult time squeezing through the tunnels.

2. Gel concentration: The final concentration of agarose in a gel will change the size of the channels within the gel. Lower percent gels will have larger channels, making it easier to

Background Information

Excerpts from Cat. #S-52

separate large molecules. Higher gel concentrations will have smaller channels, making it easier to separate small molecules.

3. Molecular charge: Given two molecules of similar size and shape, like dyes, the one with the greater amount of charge will migrate faster. Molecules with a net negative charge migrate towards the positive electrode (anode) while those with a net positive charge migrate towards the negative electrode (cathode).

4. Molecular shape: Molecules with a more compact shape, like a sphere, move through the gel more quickly than those with a looser conformation.

Because molecules with different properties travel at different speeds, they become separated and form discrete "bands" within the gel (Figure 1).

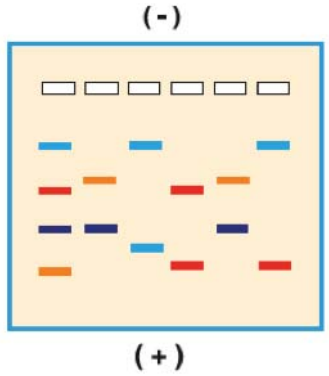


Figure 1: Separation of Dyes by Agarose Gel Elctrophoresis

THE LAWS OF INHERITANCE

In the mid-1800's, Augustine monk Gregor Mendel established basic laws of genetics through careful experimentation using garden peas. Mendel started with several true-breeding varieties of pea plants. Each plant had a different combination of observable characteristics known as traits. He crossed different strains of plants to one another and observed the offspring over several generations. After analyzing the data, Mendel developed a model to describe the inheritance of these characteristics.

Mendel's first law, the law of segregation, stated that alternative forms of the same gene, called alleles, controlled the differences in the pea plants. Each offspring has two copies of the gene, one inherited from each parent. Next, he realized that alleles are dominant or recessive. When a dominant allele is inherited, it will mask the trait coded by the recessive allele. To impart the recessive trait, both alleles must be the recessive type. The second law of inheritance, the law of independent assortment, states that each pair of alleles will segregate separately from one another.

Inheritance of a single gene can be illustrated with a two-by-two grid known as a Punnett Square (Figure 2). The alleles carried by one parent are placed across the top of the grid (columns), and the alleles contributed by the other parent are placed down the side of the grid (rows). By convention, the dominant allele is denoted by an upper-case letter P and the recessive allele by a lower-case letter p. Next, the parental alleles are used to fill in the grid. Each box in the grid is assigned the allele at the head of its column and row. For example, assuming each parent carries one dominant allele and one recessive allele, the Punnett Square predicts that ¼ of the plants will receive two dominant alleles, ½ of the plants will receive one dominant and one recessive allele, and ¼ will receive two recessive alleles. This represents the genetic make-up, or genotype, of the offspring. If an individual has two of the same alleles, either recessive or dominant, they are homozygous for that trait. If an individual has one dominant and one recessive allele, the individual is heterozygous for that trait. The genotypes of the offspring determine their phenotype, or observable traits.

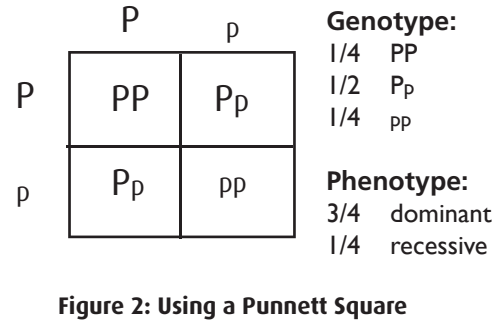


Figure 2: Using a Punnett Square

Background Information

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Mendel's laws apply to more than just plants – today, we know that these basic laws of inheritance apply to humans and other organisms as well! For example, single genes can control the appearance of the fruit fly *Drosophila melanogaster,* including wing shape and size, eye color, and body color. Different alleles of a gene can cause a wide variety of illnesses. In humans, recessive genetic disorders include cystic fibrosis, sickle cell anemia, Phenylketonuria, and many types of cancer. Dominant disorders include Huntington's Disease and Familial Hypercholesterolemia.

Table 1: Human Blood Types

Blood Type	Antigen on Red Blood Cells	Antibody in Serum
A	A	anti-B
B	B	anti-A
AB	both A & B	neither anti-A nor anti-B
O	neither A nor B	both anti-A and anti-B

Scientists have discovered some exceptions to Mendel's basic laws of inheritance. For example, certain genes may have more than two alleles. In humans, an antigen on the surface of red blood cells determines blood types. The gene that codes for this antigen has three different alleles: I^A, I^B, and i. I^A produces the A antigen, I^B produces the B antigen, and i does not produce an antigen. An individual's blood type is dependent upon the combination of these alleles (Table 1). I^A and I^B are codominant, meaning that both traits can be expressed at the same time in a heterozygous person. Both I^A and I^B are dominant to i. This means that a person who presents the A antigen (I^A I^A or I^Ai) on their red blood cells is considered to be Type A. Likewise, a person who presents the B antigen (I^B I^B or I^Bi) on their red blood cells is considered to be Type B. An AB individual (I^A I^B) has both antigens on the surface of their cells, and an O individual (ii) has no antigens on the surface of their cells.

As we learn more about the genes that control different phenotypes, we can analyze various genetic traits using DNA analysis. Specific genes are amplified using the Polymerase Chain Reaction (PCR), a biotechnology technique that allows researchers to quickly create many copies of a specific region of DNA *in vitro*. The samples are then separated using agarose gel electrophoresis. In this electrophoresis experiment, mixtures of UV-reactive dyes represent DNA fragments of different sizes. The results of the experiment will be clearly visible upon exposure to long wave UV light. By using dyes to simulate DNA fragments, we have eliminated post-electrophoresis staining, saving you valuable classroom time. The unique banding patterns will be analyzed using a Martian DNA scenario to simulating genetic testing.

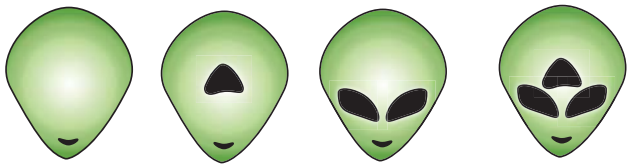
Martian Genetics

Excerpts from Cat. #S-52

In the not-so-distant future, scientists discovered an alien race living on Mars. The scientists noticed that the aliens had different combinations of eyes – eyeless, one, two, or three eyes. In collaboration with the aliens, the scientists were able to decipher the genetic code and to identify the gene responsible for eye number. The trait was linked back to a gene they called "eyeless". Three alleles of eyeless were discovered -- E^1, E^2, and e. Studies show that E^1 is responsible for the one eye phenotype, E^2 for two eyes, and e is for the eyeless phenotype.

As a scientist working on Mars, you have been tasked with analyzing the eyeless gene. Three different alleles of the gene have been identified using PCR. However, you are not sure which band on the electrophoresis gel represents each individual allele. Using the phenotypes below and the results from your electrophoresis experiment to determine which band represents each of the three alleles.

Lane 1 – Control sample -- all three gene forms
Lane 2 – two eyes
Lane 3 – three eyes
Lane 4 – one eye
Lane 5 – one eye
Lane 6 – eyeless



Phenotype	Eyeless	One Eye	Two Eyes	Three Eyes
Possible genotype	ee	E^1e, E^1E^1	E^2e, E^2E^2	E^1E^2

Agarose Gel Electrophoresis

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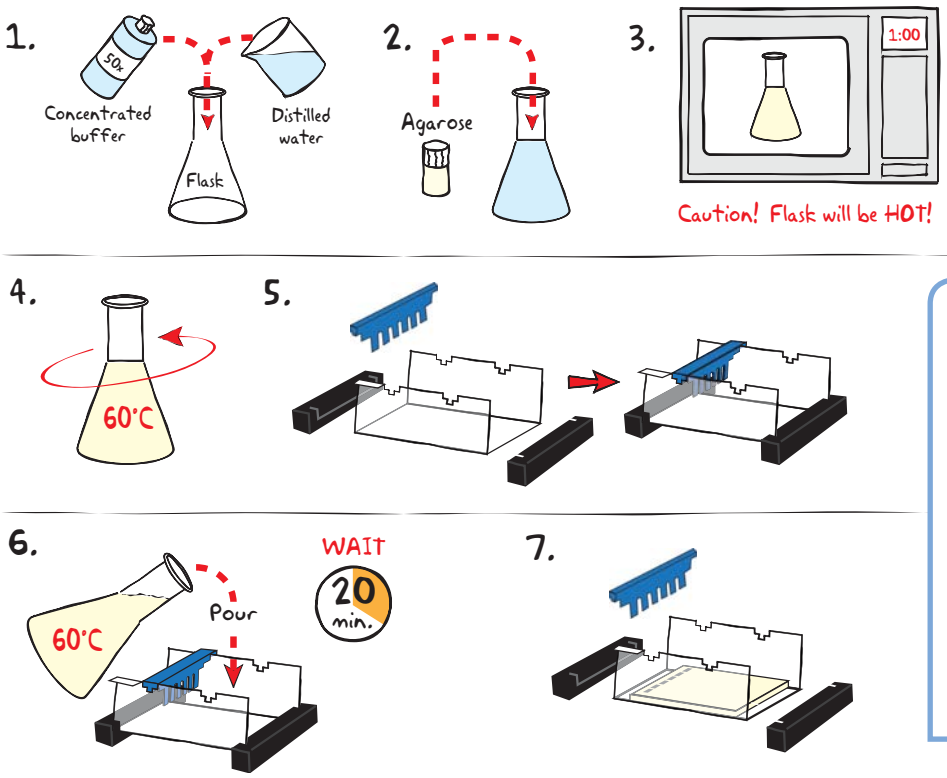

IMPORTANT:

If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at www.edvotek.com

Tech Video

youtube.com/EdvotekInc

Video: Preparing Agarose Gels

CASTING THE AGAROSE GEL

Wear gloves and safety goggles

1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 ml flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — Individual 0.8% UltraSpec-Agarose™ Gel

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 ml	29.4 ml	0.23 g	30 ml
7 x 10 cm	1.0 ml	49.0 ml	0.39 g	50 ml
7 x 14 cm	1.2 ml	58.8 ml	0.46 g	60 ml

Agarose Gel Electrophoresis

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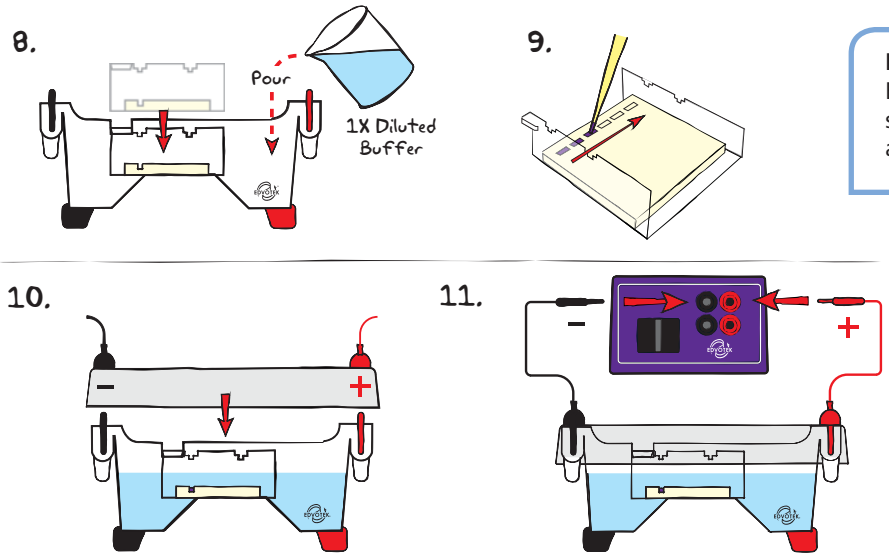


REMINDER:
Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

Tech Video

youtube.com/EdvotekInc

Video: Performing Agarose Gel Electrophoresis

RUNNING THE GEL

Wear gloves and UV safety glasses!

8. **PLACE** the gel (still on the tray) into the electrophoresis chamber. **COVER** the gel with 1X Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35-38 µl) into the well in consecutive order. The identity of each sample is provided in Table 2.
10. **PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). Allow the tracking dye to migrate at least 3.5 cm from the wells.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber. **Visualize the results using a long wave UV light source (black light). Be sure to wear UV safety glasses!**

Table 2: Gel Loading

Lane		
1	Tube A	Standard Dye Marker
2	Tube B	Alien Sample 1
3	Tube C	Alien Sample 2
4	Tube D	Alien Sample 3
5	Tube E	Alien Sample 4
6	Tube F	Alien Sample 5

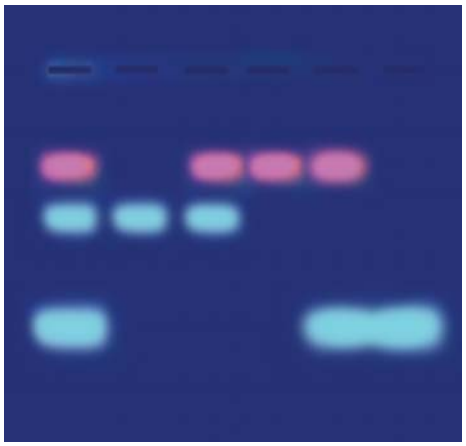
Table B — 1x Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+ & M12 (new)	300 ml	6 ml	294 ml
M12 (classic)	400 ml	8 ml	392 ml
M36	1000 ml	20 ml	980 ml

Table C — Time & Voltage Guidelines (0.8% Agarose Gel)

	Electrophoresis Model		
Volts	M6+ Min. / Max.	M12 (new) Min. / Max.	M12 (classic) & M36 Min. / Max.
150	15/20 min.	20/30 min.	25 / 35 min.
125	20/30 min.	30/35 min.	35 / 45 min.
75	35 / 45 min.	55/70 min.	60 / 90 min.

Experiment Results



Scenario Two: Results Table

Lane	Tube	Sample	Result
1	A	Standard Dye Marker	--------
2	B	Alien 1	genotype: E^2E^3
3	C	Alien 2	genotype: E^1E^2
4	D	Alien 3	genotype: E^1E^1
5	E	Alien 4	genotype: E^1e
6	F	Alien 5	genotype: ee

Related Products

M36 HexaGel™
Electrophoresis Apparatus
Cat. #515 $325

DuoSource™ 150
75/150V Power Supply
Cat. #509 $179

EDVOTEK™ Variable
5-50 µl Micropipet
Cat. #590 $179

Long Wave UV
Mini-Light
Cat. #969 $29

Cat. #S-52

The Secret of the Invisible DNA: A Genetics Exploration

Improved!

Explore genetics with our "out of this world" experiment! In this lesson, we explore how DNA technology can be used to explore the relationship between genotype and phenotype using one of two exciting scenarios (medical diagnostics or alien genetics). Fluorescent dyes simulate DNA fragments, eliminating post-electrophoresis staining and saving you valuable classroom time!

- For 10 Lab Groups
- Complete in 45 min.
- **Cat. #S-52 $65**