EDVOTEK®

THE BIOTECHNOLOGY EDUCATION COMPANY®

Edvo-Kit #

S-50

Edvo-Kit #S-50

Why Do People Look Different?

Experiment Objective:

In this experiment, students explore basic concepts in Mendelian inheritance, including that hereditary information is contained within genes. This information is then used to analyze a simulated genetic test.

See page 3 for storage instructions.

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Safety Data Sheets can be found on our website: **www.edvotek.com/Safety-Data-Sheets**

Experiment Components

READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS

Store QuickStrip™ samples in the refrigerator immediately upon receipt.
All other components can be stored at room temperature.

Components (in QuickStrip™ format)		Check (√)
A	Mother's Simulated DNA Sample	❑
B	Father's Simulated DNA Sample	❑
C	Child #1 Simulated DNA Sample	❑
D	Child #2 Simulated DNA Sample	❑
E	Child #3 Simulated DNA Sample	❑
F	Child #4 Simulated DNA Sample	❑

REAGENTS & SUPPLIES

- UltraSpec-Agarose™ ❑
- Electrophoresis Buffer (50x) ❑
- Practice Gel Loading Solution ❑
- Microtipped Transfer Pipets ❑

Experiment #S-50 is designed for 10 gels.

Store QuickStrip™ samples in the refrigerator immediately upon receipt. All other components can be stored at room temperature.

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Heat Source
- 500 ml Beaker or Flask
- Hot gloves
- Distilled or deionized water
- Balance
- Automatic micropipets with tips (optional)

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

Background Information

In the mid-1800's, Augustine monk Gregor Mendel established basic laws of genetics through careful experimentation using garden peas. To perform these experiments, Mendel started with several true-breeding varieties of pea plants. Each plant had a different combination of observable characteristics known as traits. He crossed a true-breeding plant with purple flowers to one with white flowers and then observed the offspring (Figure 1). Each plant in the first generation (or F1) had purple flowers. Mendel then crossed the plants from the F1 generation to each other. To his surprise, the second generation (second filial, or F2) showed a ratio of three purple flowered plants for every one with white flowers. From this data, Mendel hypothesized that the factors that made flowers white are hidden by those responsible for making flowers purple.

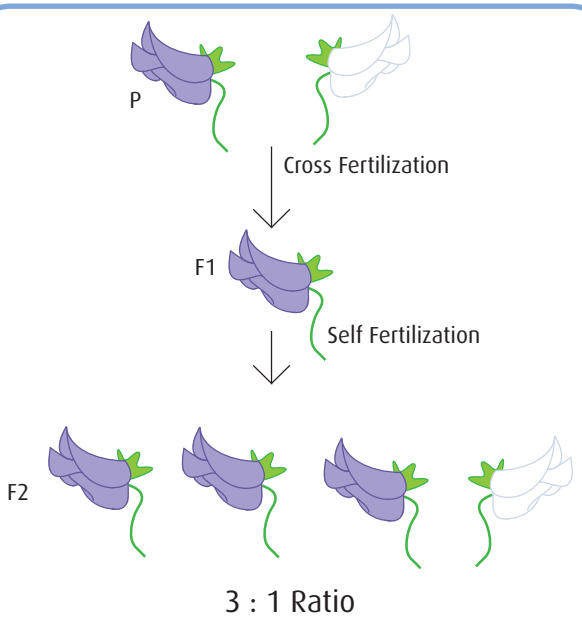


Figure 1: Testcross between two true-breeding pea plants

Mendel performed the same analysis using pea plants with different traits, including pigmentation, plant height, seed coat color, and seed texture. After analyzing the data, he noticed the same 3:1 ratio between the different traits. From this data, he developed a model to describe how these characteristics were inherited. First, he realized that alternative forms of the same gene, called alleles, were responsible for the differences in the pea plants. With the flower color gene, one allele produces the purple color, and the other produces white. Each plant 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, causing each plant in the F1 generation to be purple. To impart the recessive trait (white flowers), both alleles must be the recessive type.

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 (the purple gene) is denoted by an upper-case letter P and the recessive allele (the white gene) 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 parents carries one dominant allele and one recessive allele, the Punnett Square predict 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 makeup, or genotype,

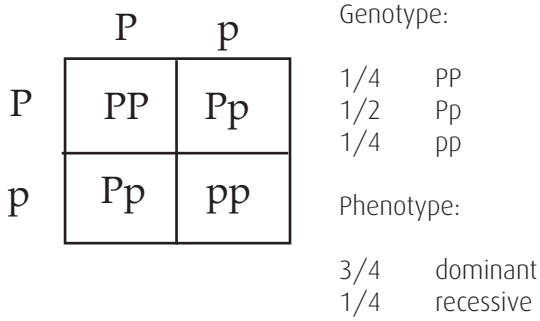


Figure 2: Using a Punnett Square

Background Information, continued

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 observable characteristics, or phenotype. Since 75% of the offspring should have at least one copy of the dominant allele, the flowers will appear purple. The remaining 25% of the plants should have the recessive white-flower phenotype. However, these numbers are only estimates of what is expected. Actual observations may differ from predictions, especially when analyzing small numbers of offspring. When analyzing many offspring, as with insects or plants, actual observations come close to the estimations.

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! Certain traits are easy to observe in offspring. For example, single genes can control the appearance of the fruit fly *Drosophila melanogaster*, including wing shape and size, eye color, and body color. In dogs, coat color is influenced by an allele that codes for black or brown pigment. Easily observable physical characteristics in humans include the widow's peak and attached earlobes. Different alleles of a gene can also cause disease. In humans, single genes are responsible for a wide variety of illnesses. Recessive disorders include cystic fibrosis, sickle cell anemia, Phenylketonuria, and many types of cancer. Dominant disorders include Huntington's Disease and Familial Hypercholesterolemia.

Traditionally, traits have been traced through populations through interviewing people and creating complex family trees called pedigrees. As we learn more about the genes that control these 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 copied DNA is digested with special enzymes called restriction endonucleases, which act like molecular scissors to cut DNA at specific sites. Depending on the distances between recognition sites, digestion of DNA by a restriction enzyme will produce DNA fragments of varying lengths.

The sample is then analyzed by agarose gel electrophoresis, a technique that separates DNA molecules by size. 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 stay in the wells. An electrical current is passed through the gel to drive molecules through the gel. Smaller fragments pass through the gel faster than the larger fragments. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. After electrophoresis, the samples are visualized using a special stain that sticks to DNA.

Each genetic test produces unique DNA fragments that allow researchers to distinguish between different genotypes at the molecular level. In this experiment, we will be exploring the relationship between genotype and phenotype by exploring a family's eye colors. Students use DNA analysis to figure out the genotypes of two brown-eyed parents and their four children. For this simulation, we will use the convention that brown will be dominant and blue will be recessive. (In reality, the genetics of eye colors is quite complicated.) One should remember that the two different alleles presented in this simulation could represent alleles for other phenotypes, including recessive genetic diseases. In this experiment, the DNA fragments produced by restriction digest are represented by various dyes. The small orange band represents the brown allele, while the larger blue band represents the blue allele. By using brightly colored dyes to simulate DNA fragments, we have eliminated post-electrophoresis staining, saving you valuable classroom time.

Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students explore basic concepts in Mendelian inheritance, including that hereditary information is contained within genes. This information is then used to analyze a simulated genetic test

WORKING HYPOTHESIS

If a child receives one of the two alleles from each parent, then a DNA fingerprint analysis of blood from mother, father, and child should prove this.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Wear gloves and safety goggles

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

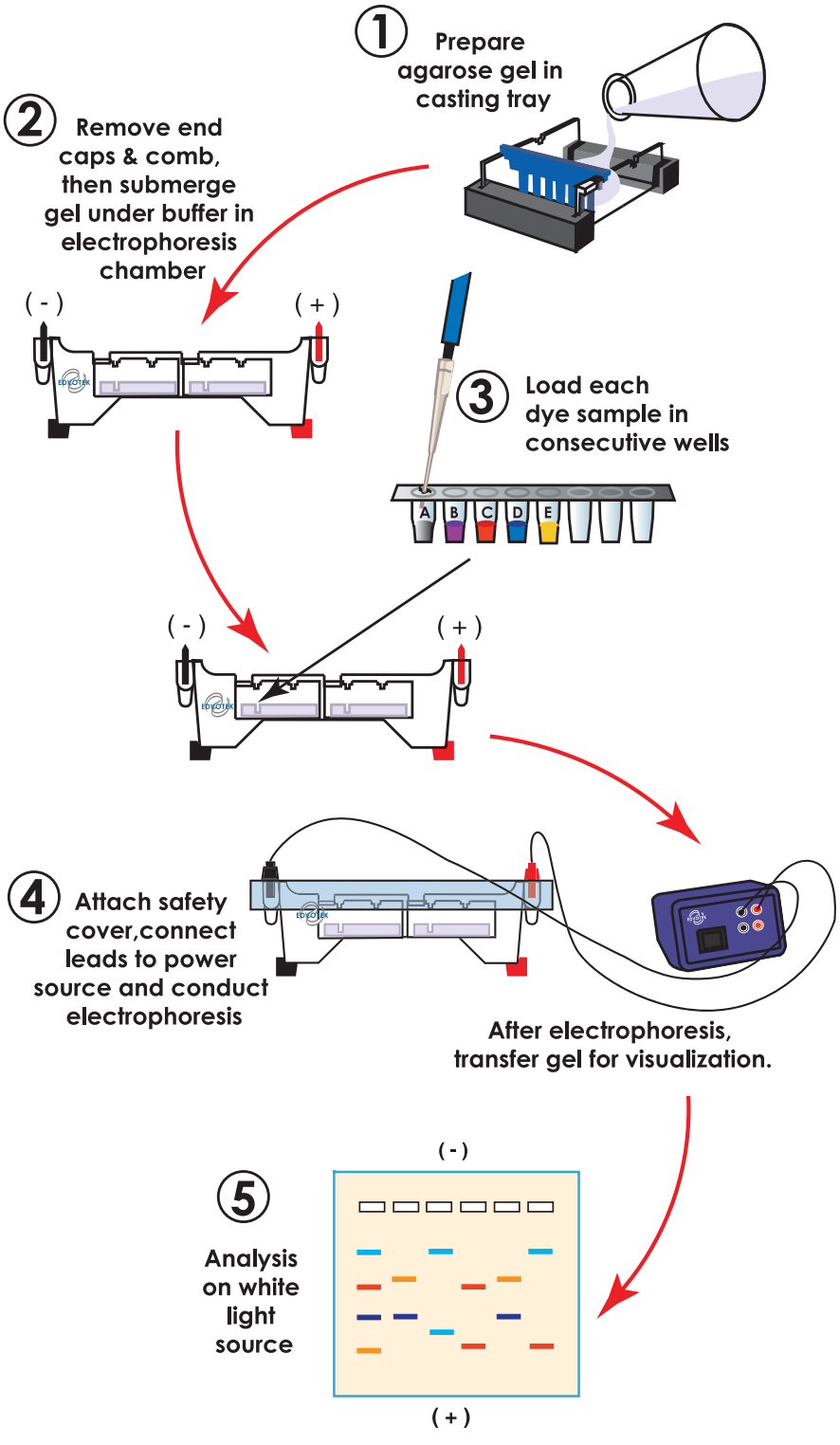
During the Experiment:

- Record your observations.

After the Experiment:

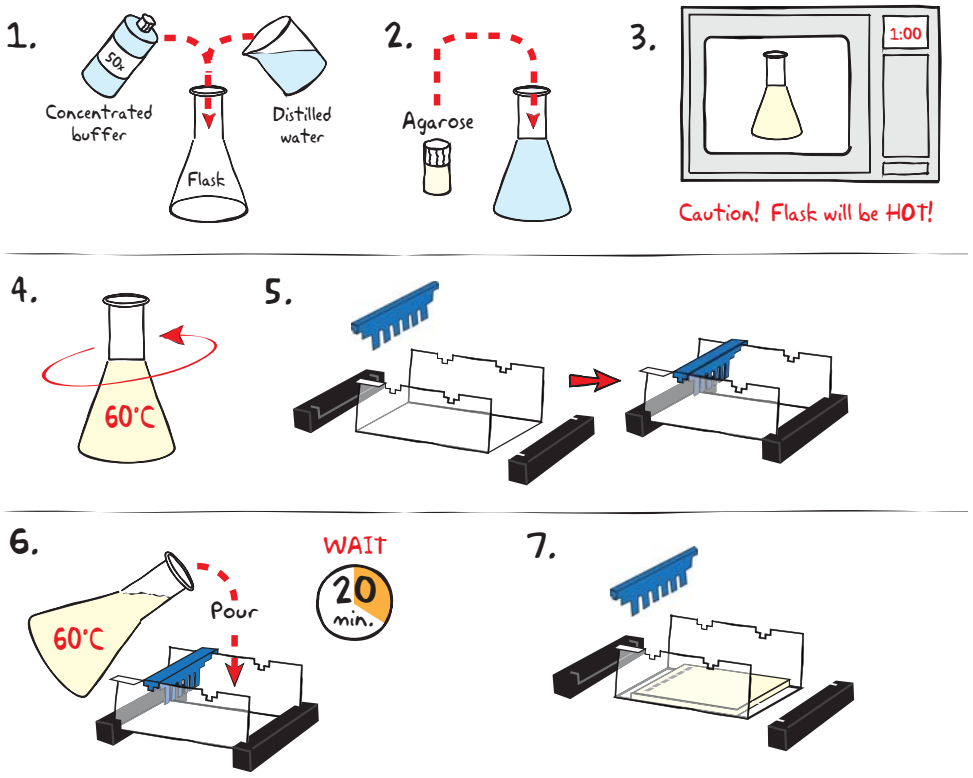
- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Experiment Overview



Gel pattern will vary depending upon the experiment.

Agarose Gel Electrophoresis



IMPORTANT:

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

Wear gloves and safety goggles

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 ml flask (see 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

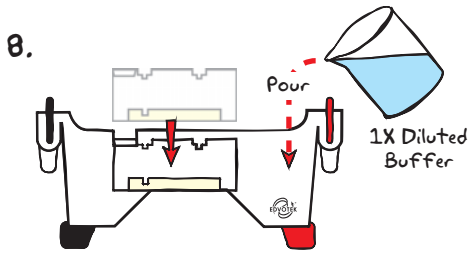
Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 ml	29.4 ml	0.23 g	30 ml
7 x 10 cm	1.0 ml	49.0 ml	0.39 g	50 ml
7 x 14 cm	1.2 ml	58.8 ml	0.46 g	60 ml

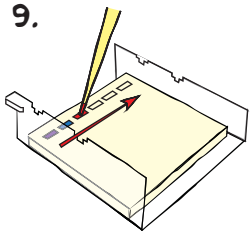
Agarose Gel Electrophoresis, continued

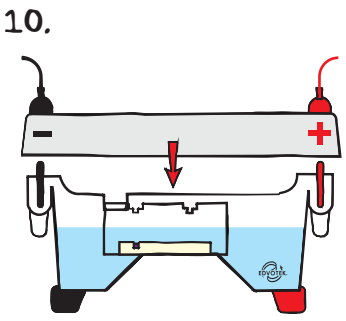
Reminders:

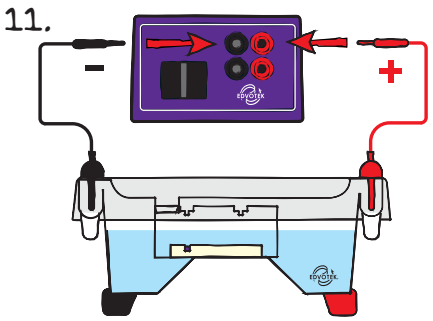
If unfamiliar with gel loading, consider performing the optional activity in Appendix C, Practice Gel Loading, prior to performing the experiment.

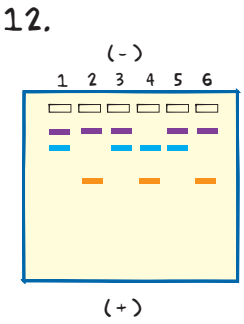
Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.











8. **PLACE** gel (on the tray) into 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 1.
10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the dye 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).
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and **VISUALIZE** the results. No staining is necessary.

Table 1: Gel Loading

Lane		
1	Tube A	Mother's DNA Sample
2	Tube B	Father's DNA Sample
3	Tube C	Child 1 DNA Sample
4	Tube D	Child 2 DNA Sample
5	Tube E	Child 3 DNA Sample
6	Tube F	Child 4 DNA Sample

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 and Voltage Guidelines (0.8% Agarose Gel)

Electrophoresis of Dyes	
Volts	Recommended Time
125	20 min.
70	45 min.
50	90 min.

Study Questions

1. Define the following: genotype, phenotype, homozygous, and heterozygous.

2. Using B for the brown allele and b for the blue allele, what is the genotype of the parents? Using the genotypes, create a 2 X 2 matrix to predict the phenotypes of the children.

3. For all four children, what are their genotypes?

4. From the gel, can you determine the eye color of the grandparents?

5. If two blue-eyed people had a child, what would you predict the eye color of the child to be?

Instructor's Guide

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATION:

This section outlines the recommended prelab preparations and approximate time requirement to complete each prelab activity.

What to do:	When:	Time Required:
Prepare QuickStrips™	Up to one day before performing the experiment.	40 min.
Prepare diluted Electrophoresis Buffer		
Prepare molten agarose and pour gel		

Pre-Lab Preparations:

SEPARATION OF PCR PRODUCTS BY AGAROSE GEL ELECTROPHORESIS

This experiment requires a 0.8% agarose gel per student group. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

Individual Gel Preparation:

Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See the Student's Experimental Procedure. Students will need Electrophoresis Buffer (50x), distilled water and agarose powder.

Batch Gel Preparation:

To save time, a larger quantity of agarose solution can be prepared for sharing by the class. Electrophoresis buffer can also be prepared in bulk. See Appendix B.

Preparing Gels in Advance:

Gels may be prepared ahead and stored for later use. Solidified gels can be stored under buffer in the refrigerator for up to 2 weeks.

Do not freeze gels at -20° C as freezing will destroy the gels.

Gels that have been removed from their trays for storage should be "anchored" back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

NOTE:
Accurate pipetting is critical for maximizing successful experiment results.

If students are unfamiliar with using micropipets, we recommend performing the optional activity found in Appendix C, Practice Gel Loading, prior to conducting the experiment.

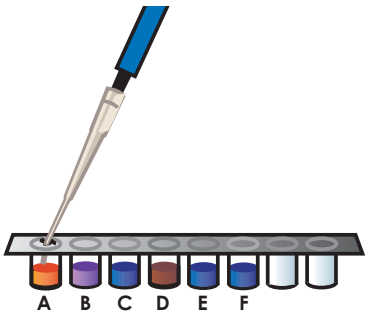
Each Student Group should receive:
- Electrophoresis Buffer (50x)
- Distilled Water
- UltraSpec-Agarose™
- Ready-to-Load™ Samples

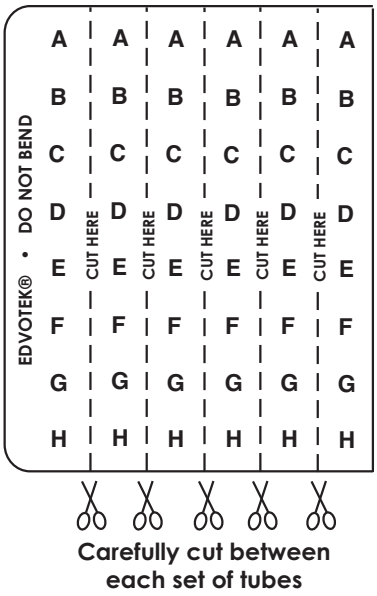
SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

QuickStrip™ tubes consist of a microtiter block covered with a protective overlay. Each well contains pre-aliquoted dyes.

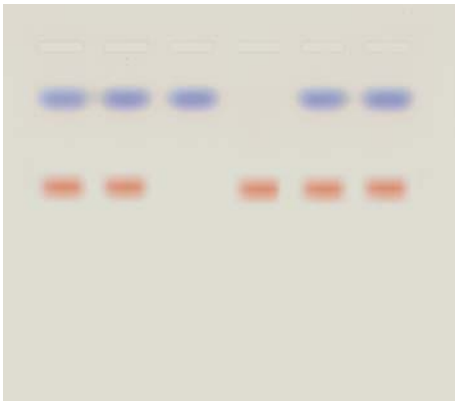
Using sharp scissors, carefully divide the block of tubes into individual strips by cutting between the rows (see diagram at right). Take care not to damage the protective overlay while separating the samples.

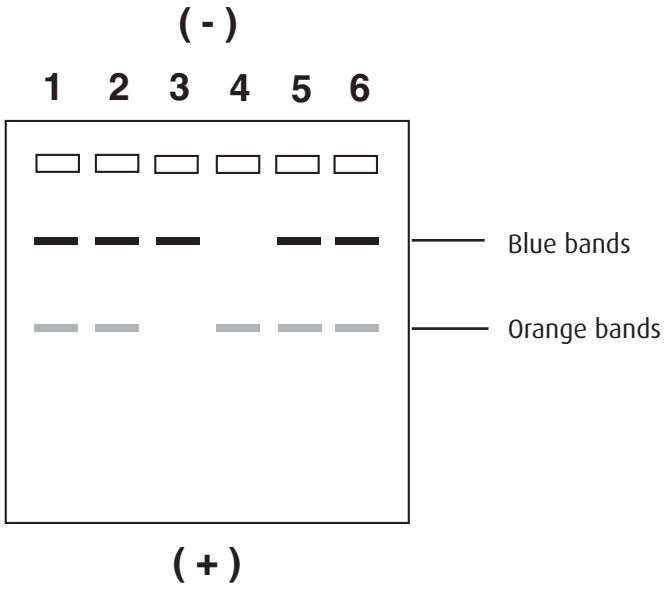
Each lab group will receive one set of tubes. Before loading the gel, remind students to tap the tubes to collect the sample at the bottom of the tube.





Experiment Results and Analysis





Lane	Tube	
1	A	Mother's DNA
2	B	Father's DNA
3	C	Child #1 DNA
4	D	Child #2 DNA
5	E	Child #3 DNA
6	F	Child #4 DNA

Actual results will yield bands of varying intensities. The idealized schematic shows the relative positions of the bands, but are not depicted to scale.

Lane	Tube	Sample	Genotype	Phenotype
1	Tube A	Mother's DNA Sample	Bb	Brown eyes
2	Tube B	Father's DNA Sample	Bb	Brown eyes
3	Tube C	Child 1 DNA Sample	bb	Blue eyes
4	Tube D	Child 2 DNA Sample	BB	Brown eyes
5	Tube E	Child 3 DNA Sample	Bb	Brown eyes
6	Tube F	Child 4 DNA Sample	Bb	Brown eyes

Please refer to the kit insert for the Answers to Study Questions

Appendices

A EDVOTEK® Troubleshooting Guide
B Bulk Preparation of Agarose Gels
C Practice Gel Loading

Safety Data Sheets can be found on our website: **www.edvotek.com/Safety-Data-Sheets**

Appendix A
EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
Bands not visible on the gel	The electrophoresis buffer was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
	The dyes ran off of the gel because the polarity of the leads was reversed.	Ensure that leads are attached in the correct orientation.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
Very light colored band seen after electrophoresis	Pipetting error.	Make sure students pipet 35 µl of dye sample per well.
Poor separation of bands	Gel was not prepared properly.	Make sure to prepare a 0.8% gel.
Dye bands disappear when the gels are kept at 4° C.	The dye molecules are small and will diffuse out of the gel.	The results must be analyzed upon the completion of electrophoresis

Appendix B
Bulk Preparation of Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities for sharing by the class. Unused diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

Bulk Electrophoresis Buffer

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in Table D.

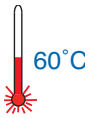
Table D — Bulk Preparation of Electrophoresis Buffer

50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 ml		2,940 ml	3000 ml (3 L)

Batch Agarose Gels (0.8%)

For quantity (batch) preparation of 0.8% agarose gels, see Table E.

1. Use a 500 ml flask to prepare the diluted gel buffer
2. Pour 3.0 grams of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Dispense the required volume of cooled agarose solution for casting each gel. The volume required is dependent upon the size of the gel bed and DNA staining method which will be used. Refer to Appendix A or B for guidelines.
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis.

Note:
The UltraSpec-Agarose™ kit component is usually labeled with the amount it contains. Please read the label carefully. If the amount of agarose is not specified or if the bottle's plastic seal has been broken, weigh the agarose to ensure you are using the correct amount.

Table E — Batch Prep of 0.8% UltraSpec-Agarose™

Amt of Agarose (g)	+	Concentrated Buffer (50X) (ml)	+	Distilled Water (ml)	Total Volume (ml)
3.0		7.5		382.5	390

Appendix C
Practice Gel Loading

Accurate sample delivery technique ensures the best possible gel results. Pipetting mistakes can cause the sample to become diluted with buffer, or cause damage to the wells with the pipet tip while loading the gel.

If you are unfamiliar with loading samples in agarose gels, it is recommended that you practice sample delivery techniques before conducting the actual experiment. EDVOTEK electrophoresis experiments contain a tube of practice gel loading solution for this purpose. Casting of a separate practice gel is highly recommended. One suggested activity is outlined below:

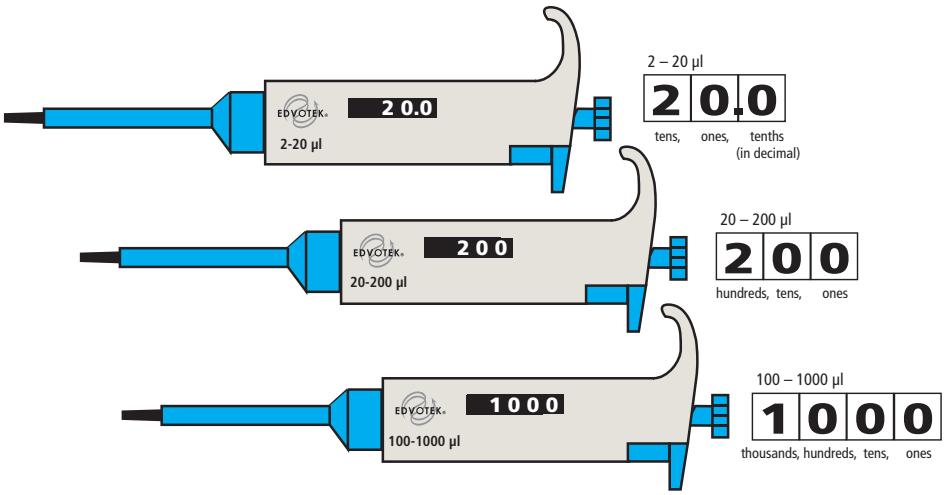
1. Cast a gel with the maximum number of wells possible.
2. After the gel solidifies, place it under buffer in an electrophoresis apparatus chamber.

   Alternatively, your teacher may have cut the gel in sections between the rows of wells. Place a gel section with wells into a small, shallow tray and submerge it under buffer or water.
3. Practice delivering the practice gel loading solution to the sample wells. Take care not to damage or puncture the wells with the pipet tip.
   - For electrophoresis of dyes, load the sample well with 35-38 microliters of sample.
   - If using transfer pipets for sample delivery, load each sample well until it is full.
4. If you need more practice, remove the practice gel loading solution by squirting buffer into the wells with a transfer pipet.
5. Replace the practice gel with a fresh gel for the actual experiment.

Note:
The agarose gel is sometimes called a "submarine gel" because it is submerged under buffer for sample loading and electrophoretic separation.

Note: If practicing gel loading in the electrophoresis chamber, the practice gel loading solution will become diluted in the buffer in the apparatus. It will not interfere with the experiment, so it is not necessary to prepare fresh buffer.

Appendix C
Practice Gel Loading



Wear gloves and safety goggles

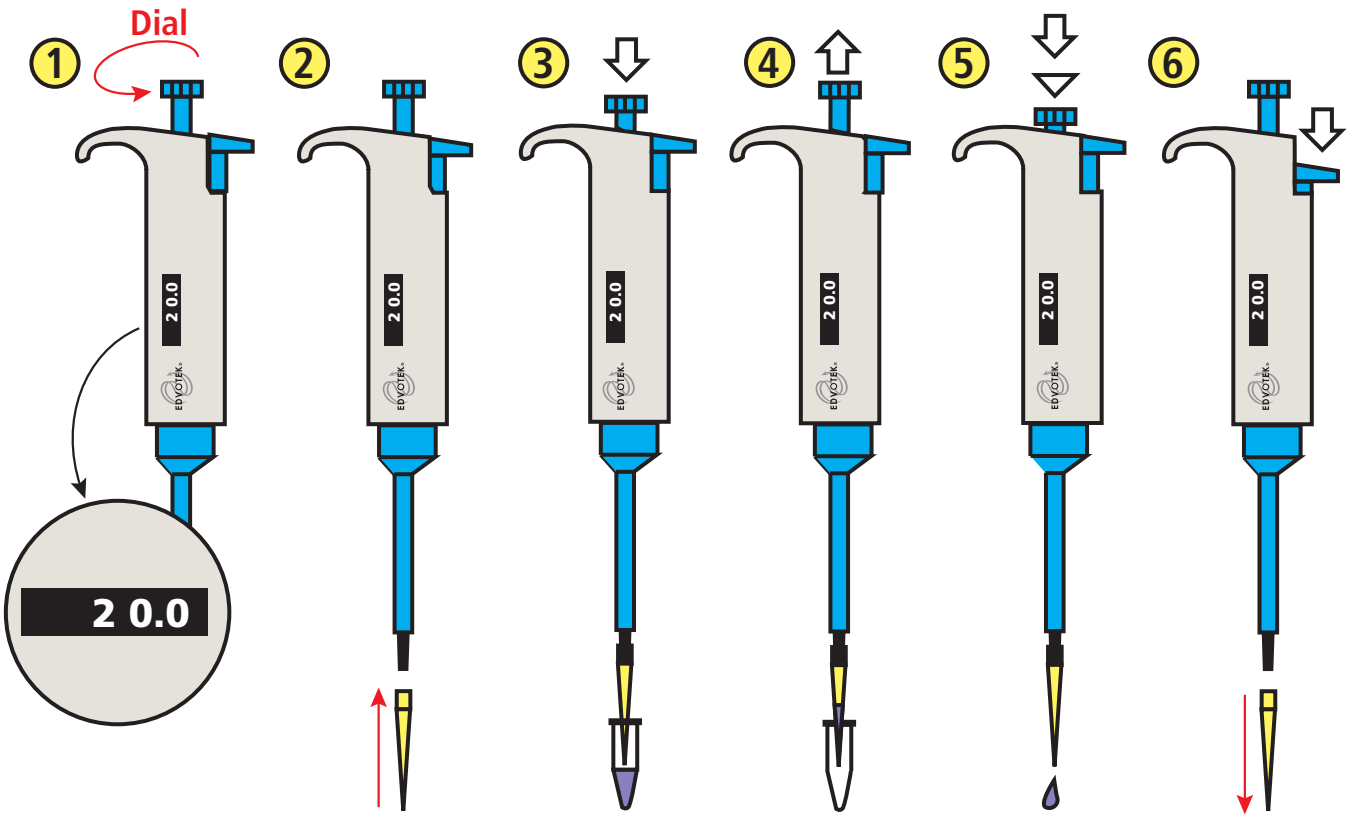
SETTING THE VOLUME OF AN ADJUSTABLE VOLUME MICROPIPET

1. **CHOOSE** the correct micropipet for the volume you are measuring. Make sure that the volume to be measured **DOES NOT EXCEED** the upper or lower volume setting of the micropipet.

2. **DETERMINE** the units measured by the micropipet by looking at the volume setting. The setting will appear in the window on the side of the micropipet. Note that the different micropipets use different scales for their measurements. Some micropipets are accurate to a tenth of a microliter, while others are accurate to one microliter.

3. **SET** the volume by twisting the top of the plunger. In general, twisting the plunger clockwise reduces the volume, and twisting the plunger counter clockwise increases the volume.

Appendix C
Practice Gel Loading



MEASURING LIQUIDS WITH A MICROPIPET


Wear gloves and safety goggles

1. **SET** the micropipet to the appropriate volume by adjusting the dial.
2. **PLACE** a clean tip on the micropipet.
3. **PRESS** the plunger down to the first stop. **HOLD** the plunger down while placing the tip beneath the surface of the liquid.
4. Slowly **RELEASE** the plunger to draw sample into the pipette tip. Position the pipet tip over the well. Be careful not to puncture or damage the well with the pipet tip.
5. **DELIVER** the sample by slowly pressing the plunger to the first stop. Depress the plunger to the second stop to expel any remaining sample. **DO NOT RELEASE** the plunger until the tip is out of the buffer.
6. **DISCARD** the tip by pressing the ejector button. Use a new clean tip for the next sample.