EDVOTEK®

THE **BIOTECHNOLOGY**
EDUCATION COMPANY®

Edvo-Kit #
S-49

Edvo-Kit #S-49

In Search of My Father

Experiment Objective:

Students will learn how agarose gel electrophoresis separates different sizes of dye molecules that represent DNA fragments. They will learn how these fragments form unique DNA patterns for each person, which is the basis for solving maternity and paternity identity.

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	Standard dyes with assigned base pair equivalents	❑
B	Mother 1 DNA	❑
C	Mother 2 DNA	❑
D	Boy 1 DNA	❑
E	Boy 2 DNA	❑
F	Father (surviving, married to Mother 1)	❑

REAGENTS & SUPPLIES

- Practice Gel Loading Solution ❑
- UltraSpec-Agarose™ ❑
- Electrophoresis Buffer ❑
- 1 ml pipet ❑
- 100 ml graduated cylinder (packaging for samples) ❑
- Microtipped Transfer Pipets ❑

Experiment #S-49 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
- Automatic micropipets with tips (optional)
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Visualization system (white light box)
- Distilled or deionized water

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 a war torn country, two young boys were separated from their respective parents who had been imprisoned by the regime. The children were cousins who were born two months apart and looked strikingly similar. Their mothers were half sisters who shared a common mother (Figure 1). After several years, the regime was overthrown and replaced by a new government that released all political prisoners from prison. The prisoners were reinstated in their respective communities and all charges against them were dropped.

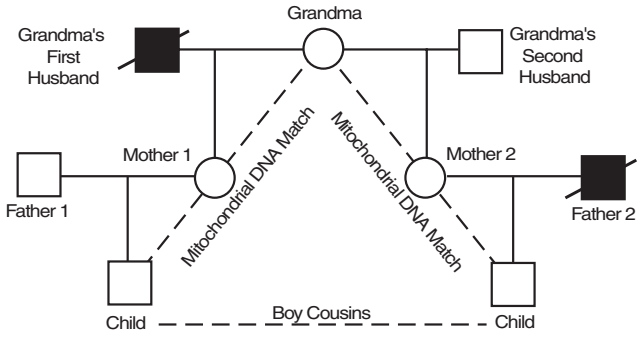


Figure 1: Pedigree of the Boys in Question

During this period, the two boys had not been separated from each other, and had been adopted by a high-ranking military officer who had no children of his own. Both the officer and his wife died in a liberation uprising and for a short period of time the boys were placed in an orphanage. On their 18th birthday, the boys were released from the orphanage and they immediately began to search for information regarding their lost parents.

The brothers contacted a number of elders from their village. The elders informed them that after their biological parents were released from prison, one of the fathers was assassinated by individuals who were sympathetic to the fallen regime. The second biological father was found in the village, suffering from amnesia. Two women, who were thought to be their biological mothers, were in a rehabilitation facility that housed approximately 200 women. Upon arrival to the center it was very obvious that the patients had not received proper medical attention. Based on age and appearance, about ten women fit the profile of the two mothers.

DETERMINATION OF PARENTAGE USING DNA FINGERPRINTING

First, the boys sought to determine which of the ten women were their mothers by performing mitochondrial DNA fingerprinting. Mitochondria, the "powerhouse" of the cell, are unique organelles in that they contain a small DNA genome. This genome is useful for identifying maternity because mitochondria are inherited through the female line. Before conception, a human egg contains a large number of mitochondria. In contrast, human sperm contains very few mitochondria. Upon fertilization of the human egg by a sperm, the developing zygote contains mitochondria obtained from the mother's egg.

Mitochondrial DNA fingerprinting tests can be used as an initial screening technique because they are less expensive than chromosomal DNA testing and results are available in a shorter period of time. In this case, since the boys were cousins (their mothers were half-sisters who shared the same mother), the mitochondrial testing results would identical for the two boys. The tests identified two women with mitochondrial DNA fingerprinting patterns that matched that of the boys.

Background Information

In order to match the boys with the correct parents, chromosomal DNA fingerprinting tests were ordered for the boys, the mothers, and the surviving father. Chromosomal DNA, which is present in the nucleus of every living cell, is the genetic material that acts as a blueprint for all of the proteins synthesized by that cell. Unlike mitochondrial DNA, chromosomal DNA is an equal composite of both parents. In each chromosome pair, one is inherited from the father and the second from the mother. Although most of this DNA is identical between individuals, small sequence differences, or "polymorphisms", occur at specific locations throughout the genome. These polymorphisms include single base pair changes and repetitive DNA elements. By examining several of these polymorphic regions, we can generate a unique "DNA fingerprint" for that person.

DNA fingerprints can allow us to distinguish one individual from another. Because polymorphisms are inherited, DNA fingerprints can also be used to determine paternity/maternity (and other familial relationships). To determine parentage, the DNA fingerprints of the boys are compared with the DNA fingerprints from the surviving father and the two mothers. Since chromosomal DNA is inherited from both parents, the DNA fingerprint of a child will contain a mixture of polymorphisms from each parent. The pattern is easily recognizable as matching visible DNA bands on the gel and can be experimentally demonstrated in this simulation experiment.

Experiment Overview

EXPERIMENT OBJECTIVE:

Students will learn how agarose gel electrophoresis separates different sizes of dye molecules that represent DNA fragments. They will learn how these fragments form unique DNA patterns for each person, which is the basis for solving maternity and paternity identity.

WORKING HYPOTHESIS

If DNA samples collected from different mothers and fathers are examined at variable polymorphic sites, then one should be able to match the children with their real mother and father by the DNA fingerprinting method.

Wear gloves and safety goggles

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.

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.

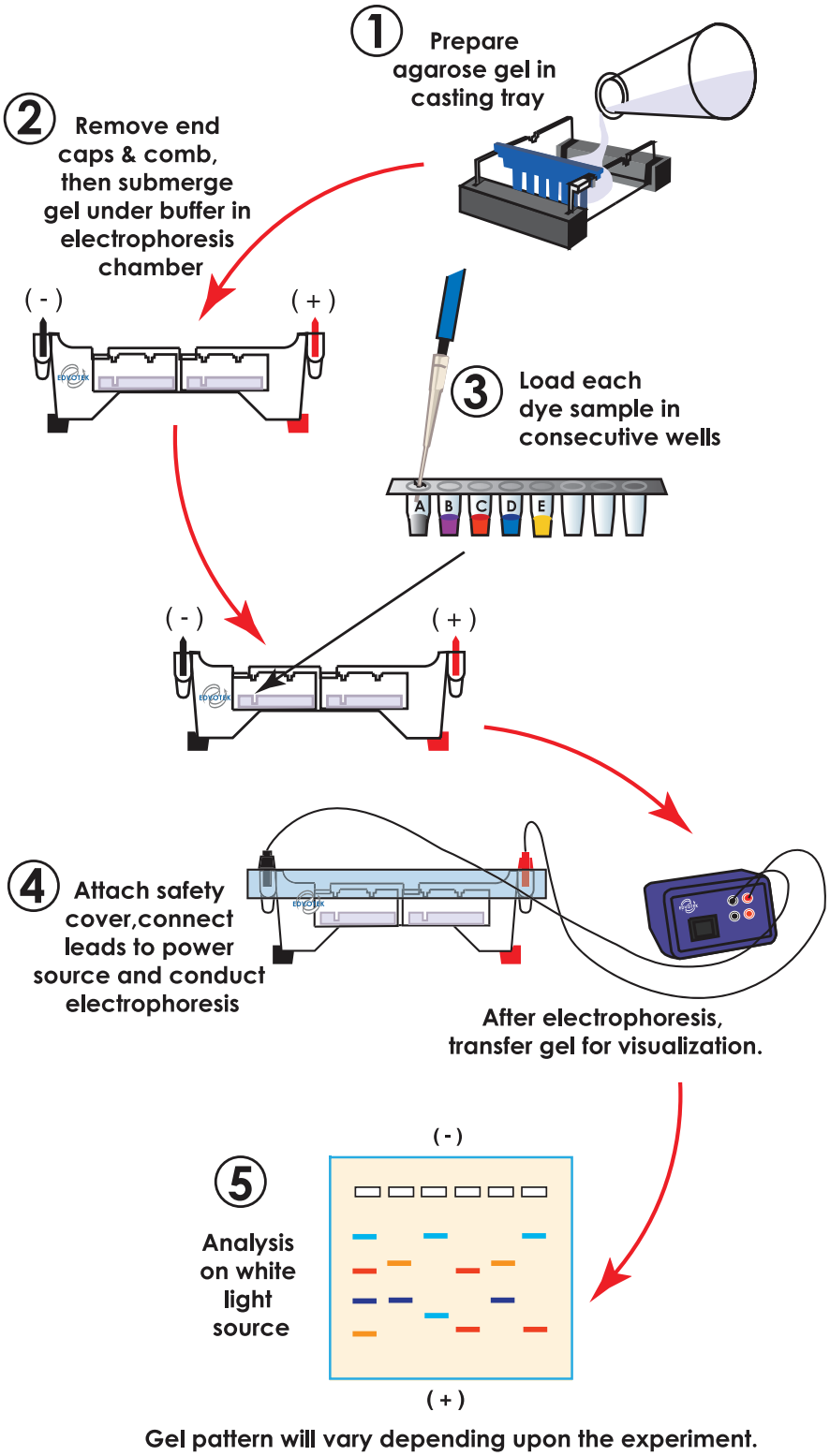
During the Experiment:

- Record your observations.

After the Experiment:

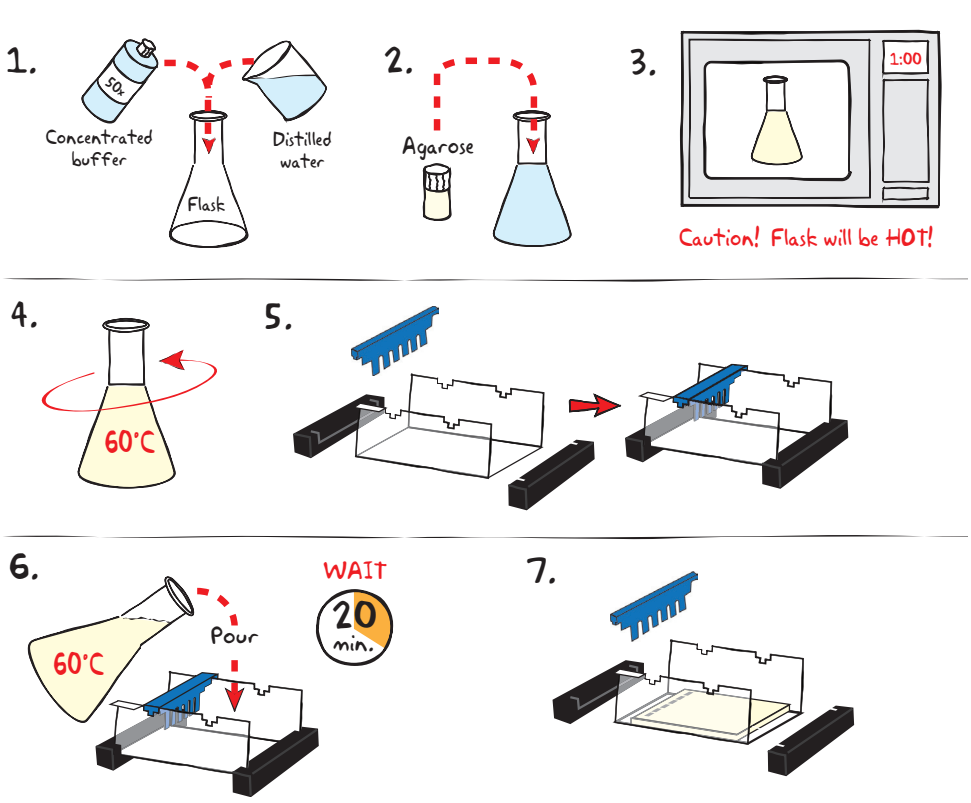
- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

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**

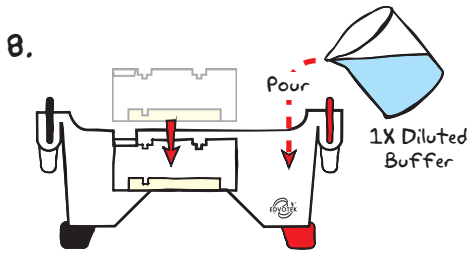
Wear gloves and safety goggles

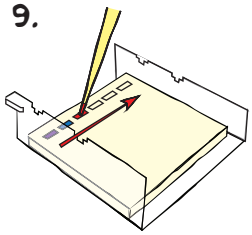
1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 ml flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask 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	0.6ml	29.4 ml	0.24 g	30 ml
7 x 10 cm	1.2 ml	58.8 ml	0.48 g	50 ml

Agarose Gel Electrophoresis

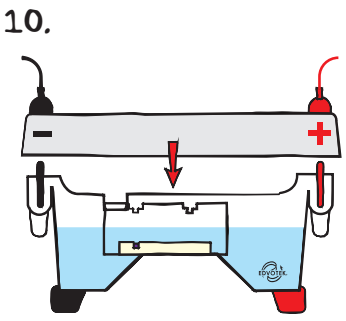


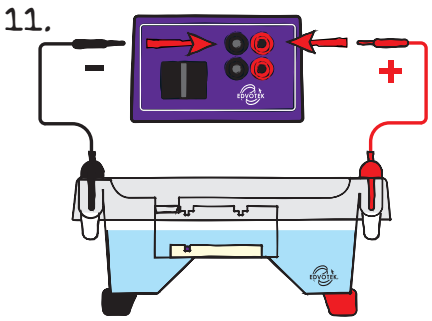


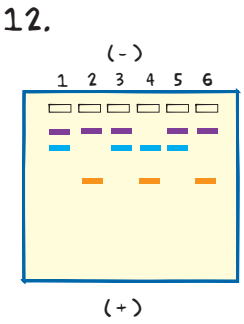
Reminders:

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 negatively charged 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	Standard Dye Markers
2	Tube B	Mother 1
3	Tube C	Mother 2
4	Tube D	Boy 1
5	Tube E	Boy 2
6	Tube F	Father

Staining is not required for Experiment #S-49, but results must be analyzed upon completion of the electrophoretic separation. Because dye molecules are extremely small they will diffuse out of the gel. Thus, the gel cannot be saved.

Table B: 1x Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+	300 ml	6 ml	294 ml
M12	400 ml	8 ml	392 ml
M36 (blue)	500 ml	10 ml	490 ml
M36 (clear)	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.

Critical Thinking and Hypothesis Development

1. What is the variable in this experiment?
2. What is the control in this experiment?
3. What could one change in the experiment if this experiment was repeated?
4. Write a hypothesis that would reflect a change.
5. Based on the evidence obtained from the analysis of the gel, which child, mother and father are reunited? Explain.

Study Questions

1. What do the different dye bands that were separated by electrophoresis represent?
2. Why do different individuals, such as siblings, have different fingerprints?
3. What is the basis of mitochondrial DNA fingerprint analysis?
4. What is the difference between mitochondrial and cell DNA-based fingerprinting analysis.

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) concentrated buffer, 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.

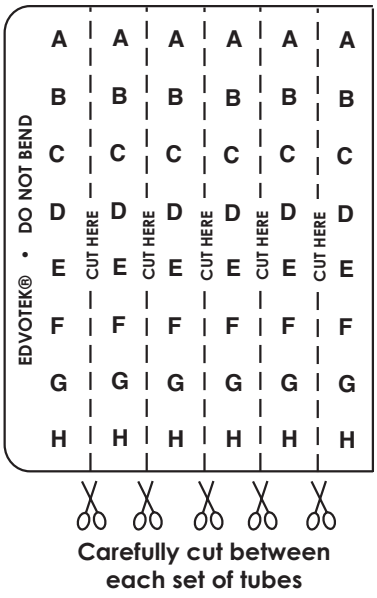
Each Student Group should receive:
- Electrophoresis Buffer (50x)
- Distilled Water
- UltraSpec-Agarose™
- Ready-to-Load™ Samples

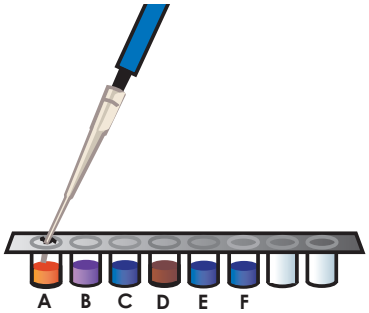
SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

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.

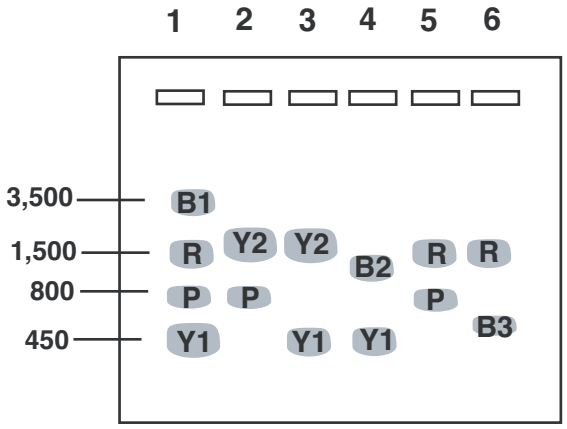
Each lab group will receive one set of tubes. Before loading the gel, remind students to tap the tubes to collect the sample at the bottom of the tube.





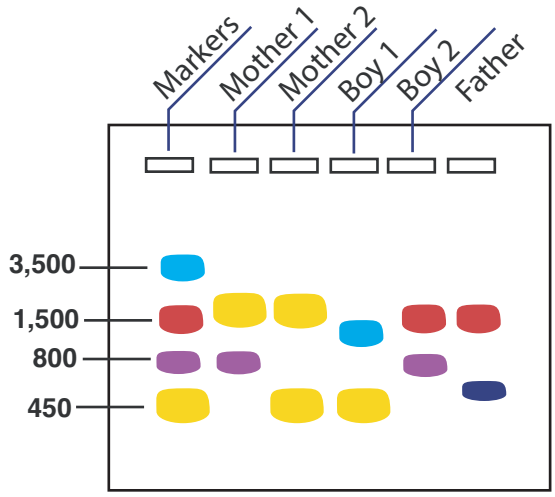
Experiment Results and Analysis

S-49 gel result photo



Color legend

B1	Blue 1
B2	Blue 2
B3	Blue 3
P	Purple 1
R	Red
Y1	Yellow 1
Y2	Yellow 2



In the two idealized schematics, the relative positions of dye molecules are shown but are not depicted to scale.

Lane	Tube	Sample
1	Tube A	Standard Dye Markers
2	Tube B	Mother 1
3	Tube C	Mother 2
4	Tube D	Boy 1
5	Tube E	Boy 2
6	Tube F	Father

The purple band of Boy 2 matches the purple band of Mother 1. The red band of Boy 2 matches the red band of the father. Therefore, Boy 2 is the child of Mother 1 and the father in this scenario.

The lower yellow band from Boy 1 matches the lower yellow band from Mother 2. Neither of the bands from Boy 1 match the father. Therefore, Boy 1 is the child of Mom 2 and the deceased father.

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.

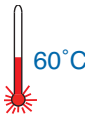
Table D — Bulk Preparation of Electrophoresis Buffer

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:

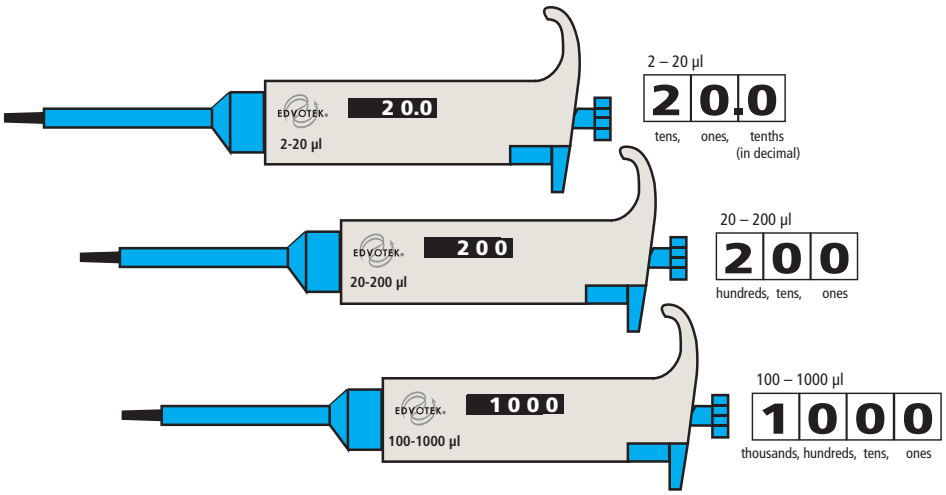
1. Cast a gel with the maximum number of wells possible.
2. After the gel solidifies, place it under buffer in an electrophoresis apparatus chamber.

   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

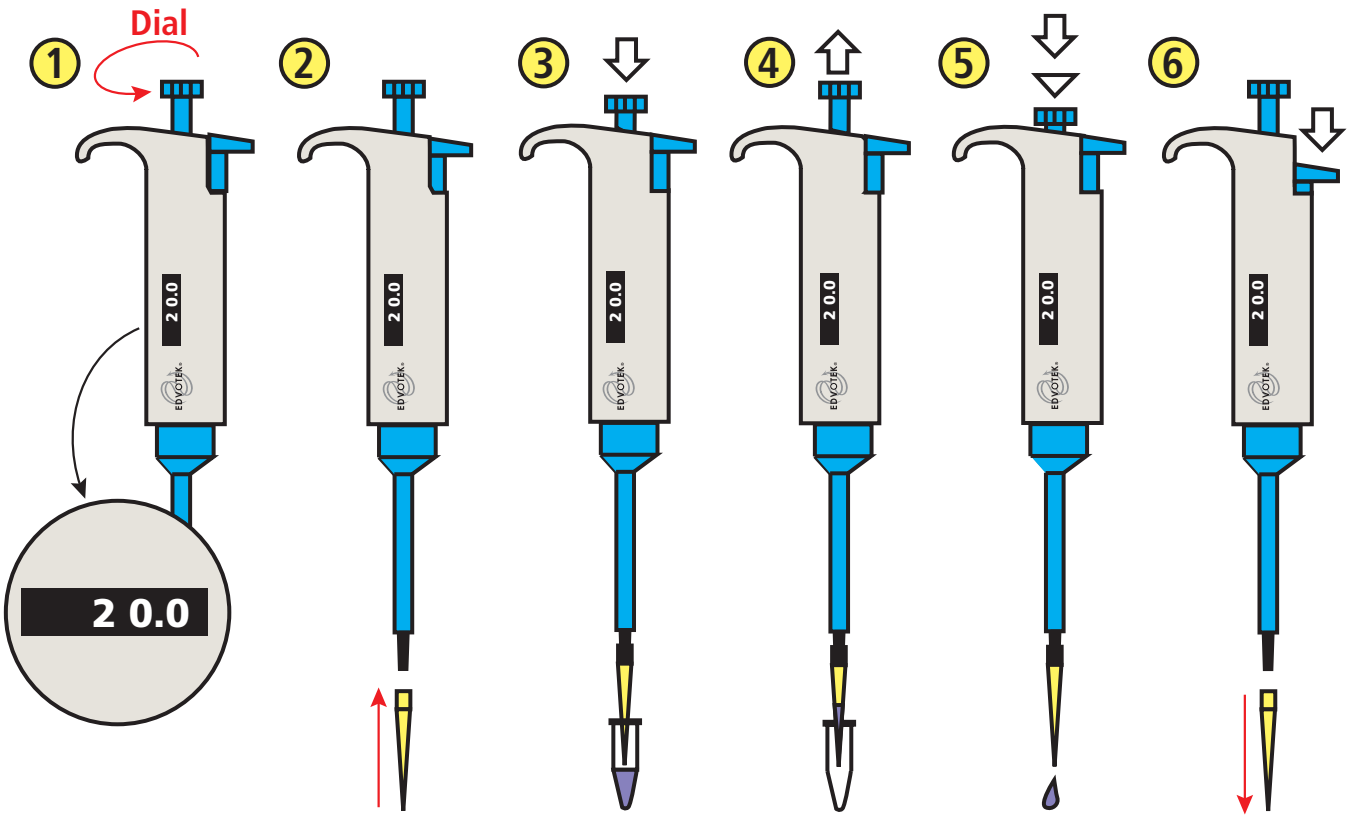
SETTING THE VOLUME OF AN ADJUSTABLE VOLUME MICROPIPET

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.