The objective of this experiment is to develop a basic understanding of electrophoretic theory, and to gain “hands-on” familiarity with the procedures involved in horizontal gel electrophoresis to separate different molecules.
Principles and Practice of Agarose Gel Electrophoresis

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</table>

All 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.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA. None of the experiment components are derived from human sources.

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Experiment Components

Dye samples are stable at room temperature. However, if the experiment will not be conducted within one month of receipt, it is recommended that the dye samples be stored in the refrigerator.

Dye samples do not require heating prior to gel loading.

READY-TO-LOAD™ DYE SAMPLES FOR ELECTROPHORESIS

A  Orange
B  Purple
C  Red
D  Blue 1
E  Dye Mixture
F  Blue Dye Mixture (Blue 1 + Blue 2)

REAGENTS & SUPPLIES

- UltraSpec-Agarose™ powder
- Concentrated electrophoresis buffer
- Practice Gel Loading Solution
- 1 ml pipet
- Microtipped Transfer Pipets

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- Flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Visualization system (white light)
- Distilled or deionized water
Agarose gel electrophoresis is widely used to separate molecules based upon charge, size and shape. It is particularly useful in separating charged biomolecules such as DNA, RNA and proteins.

Agarose gel electrophoresis possesses great resolving power, yet is relatively simple and straightforward to perform. The gel is made by dissolving agarose powder in boiling buffer solution. The solution is then cooled to approximately 55°C and poured into a gel tray where it solidifies. The tray is submerged in a buffer-filled electrophoresis apparatus which contains electrodes.

Samples are prepared for electrophoresis by mixing them with components that will give the mixture density, such as glycerol or sucrose. This makes the samples denser than the electrophoresis buffer. These samples can then be loaded with a micropipet or transfer pipet into wells that were created in the gel by a template during casting. The dense samples sink through the buffer and remain in the wells.

A direct current power supply is connected to the electrophoresis apparatus and current is applied. Charged molecules in the sample enter the gel through the walls of the wells. Molecules having a net negative charge migrate towards the positive electrode (anode) while net positively charged molecules migrate towards the negative electrode (cathode). Within a range, the higher the applied voltage, the faster the samples migrate. The buffer serves as a conductor of electricity and to control the pH. The pH is important to the charge and stability of biological molecules.

Agarose is a polysaccharide derivative of agar. In this experiment, UltraSpec Agarose™ is used. This material is a mixture of agarose and hydrocolloids which renders the gel to be both clear and resilient. The gel contains microscopic pores which act as a molecular sieve. The sieving properties of the gel influences the rate at which a molecule migrates. Smaller molecules move through the pores faster than larger ones. Molecules can have the same molecular weight and charge but different shapes. Molecules having a more compact shape (a sphere is more compact than a rod) can move faster through the pores.

Factors such as charge, size and shape, together with buffer conditions, gel concentrations and voltage, affects the mobility of molecules in gels. Given two molecules of the same molecular weight and shape, the one with the greater amount of charge will migrate faster. In addition, different molecules can interact with agarose to varying degrees. Molecules that bind more strongly to agarose will migrate more slowly.

In this experiment, several different dye samples will be applied to an agarose gel electrophoresis and their rate and direction of migration will be observed. Dyes A, B, C and D are all negatively charged at neutral pHs. However, these molecules differ with respect to their structure, chemical composition and the amount of charge they carry. Dye F has a net positive charge and therefore will migrate in the opposite direction of the other dyes. This experiment will also demonstrate the ability of agarose gel electrophoresis to separate the mixture of dyes into their individual components by the application of a combination of dyes to the same sample well.
Experiment Overview and General Instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment is to develop a basic understanding of electrophoretic theory, and to gain "hands-on" familiarity with the procedures involved in agarose gel electrophoresis to separate different molecules.

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 NOTEBOOK RECORDINGS:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:

- Record (draw) your observations, or photograph the results.

Following the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.
**Experiment Procedure**

1. Prepare agarose gel in casting tray
2. Remove end blocks & comb, then submerge gel under buffer in electrophoresis chamber
3. Load each sample in consecutive wells
4. Attach safety cover, connect leads to power source and conduct electrophoresis
5. Analysis on white light source

**Experiment Overview: Flow Chart**
Experiment 101

Principles and Practice of Agarose Gel Electrophoresis

Agarose Gel Electrophoresis

Prepare the Gel

1. Prepare an agarose gel with specifications summarized below.

- Agarose gel concentration required: 0.8%
- Recommended gel size: 7 x 10 cm or 7 x 14 cm
- Number of sample wells required: 6
- Placement of well-former template: Middle set of notches (7 x 10 cm)
  Middle set of notches (7 x 14 cm)

Load the Samples

2. Load 35 - 38 µl dye samples in tubes A - F into the wells in consecutive order.

<table>
<thead>
<tr>
<th>Lane</th>
<th>Tube</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>Orange</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>Purple</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>Red</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>Blue 1</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>Dye Mixture</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>Blue Dye Mixture (Blue 1 + Blue 2)</td>
</tr>
</tbody>
</table>

Run the Gel

3. After dye samples are loaded, connect the apparatus to the direct current (D.C.) power source and set the power source at the required voltage.

4. Check that current is flowing properly - you should see bubbles forming on the two platinum electrodes. Conduct electrophoresis for the length of time specified by your instructor.

5. After electrophoresis is completed, transfer the gel to a white light box for visualization.

6. Document the results of the gel by photodocumentation.

   Alternatively, place transparency film on the gel and trace it with a permanent marking pen. Remember to include the outline of the gel and the sample wells in addition to the migration pattern of the bands.

* Note dyes do not require staining - Analyze and document results immediately following gel electrophoresis (dyes will diffuse and will eventually fade from the gel).

Reminders:

During electrophoresis, the dye samples migrate through the agarose gel towards the positive electrode. Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

Step-by-step guidelines for agarose gel preparation are summarized in Appendix C.
Study Questions

1. On what basis does agarose gel electrophoresis separate molecules?

2. Explain migration according to charge.

3. What conclusion can be drawn from the results of sample F?

4. Why is glycerol added to the sample solutions before they are loaded into the wells?

5. What would happen if distilled water were substituted for buffer in either the chamber solution or the gel solution?
Notes to the Instructor & Pre-Lab Preparations

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in planning and implementing this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances. If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site. Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

EDUCATIONAL RESOURCES, NATIONAL CONTENT AND SKILL STANDARDS

By performing this experiment, students will learn to load samples and run agarose gel electrophoresis. Experiment analysis will provide students the means to transform an abstract concept into a concrete explanation.

EDVOTEK Ready-to-Load Electrophoresis Experiments are easy to perform and are designed for maximum success in the classroom setting. However, even the most experienced students and teachers occasionally encounter experimental problems or difficulties. EDVOTEK web site resources provide suggestions and valuable hints for conducting electrophoresis, as well as answers to frequently asked electrophoresis questions.

Laboratory Extensions and Supplemental Activities

Laboratory extensions are easy to perform using EDVOTEK experiment kits. For example, a dye sizing determination activity can be performed on any electrophoresis gel result if dye markers are run in parallel with other dye samples. For dye sizing instructions, please visit our website. For a laboratory extension to this experiment, we suggest Cat. #S-45.

Visit the EDVOTEK web site often for continuously updated information.
Notes to the Instructor & Pre-Lab Preparations

**APPRECIATE TIME REQUIREMENTS**

1. **Gel preparation:**
   Whether you choose to prepare the gel(s) in advance or have the students prepare their own, allow approximately 30 minutes for this procedure. Generally, 20 minutes of this time is required for gel solidification.

2. **Micropipeting and Gel Loading:**
   If your students are unfamiliar with using micropipets and sample loading techniques, a micropipetting or practice gel loading activity is suggested prior to conducting the experiment. Two suggested activities are:
   - EDVOTEK Expt. # S-44, Micropipetting Basics, focuses exclusively on using micropipets. Students learn pipeting techniques by preparing and delivering various dye mixtures to a special Pipet Card™.
   - Practice Gel Loading: EDVOTEK Series 100 electrophoresis experiments contain a tube of practice gel loading solution for this purpose. It is highly recommended that a separate agarose gel be cast for practice sample delivery. This activity can require anywhere from 10 minutes to an entire laboratory session, depending upon the skill level of your students.

3. **Conducting Electrophoresis:**
   The approximate time for electrophoresis will vary from approximately 15 minutes to 2 hours. Different models of electrophoresis units will separate DNA at different rates depending upon its design configuration. Generally, the higher the voltage applied the faster the samples migrate. However, maximum voltage should not exceed the indicated recommendations. The Table C example at left shows Time and Voltage recommendations. Refer to Table C in Appendices A or B for specific experiment guidelines.

<table>
<thead>
<tr>
<th>Volts</th>
<th>M6+ Minimum / Maximum</th>
<th>M12 &amp; M36 Minimum / Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>15 / 20 min</td>
<td>20 / 30 min</td>
</tr>
<tr>
<td>125</td>
<td>20 / 25 min</td>
<td>30 / 40 min</td>
</tr>
<tr>
<td>70</td>
<td>30 / 40 min</td>
<td>50 / 80 min</td>
</tr>
<tr>
<td>50</td>
<td>45 / 60 min</td>
<td>75 / 120 min</td>
</tr>
</tbody>
</table>

**PREPARING AGAROSE GELS FOR ELECTROPHORESIS**

There are several options for preparing agarose gels for the electrophoresis experiments:

1. **Individual Gel Casting:** Each student lab group can be responsible for casting their own individual gel prior to conducting the experiment.

2. **Batch Gel Preparation:** A batch of agarose gel can be prepared for sharing by the class. To save time, a larger quantity of UltraSpec-Agarose can be prepared for sharing by the class. See instructions for "Batch Gel Preparation".

3. **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 store gels at -20°C. Freezing will destroy the gels.
**Notes to the Instructor & Pre-Lab Preparations**

**USING AGAROSE GELS THAT HAVE BEEN PREPARED IN ADVANCE**

If gels have been removed from their trays for storage, they should be "anchored" back to the tray with a few drops of hot, molten agarose before placing the gels onto the electrophoresis tray for electrophoresis. This will prevent the gel from sliding around in the tray and/or floating around in the electrophoresis chamber.

**AGAROSE GEL CONCENTRATION AND VOLUME**

Gel concentration is one of many factors which affect the mobility of molecules during electrophoresis. Higher percentage gels are sturdier and easier to handle. However, the mobility of molecules and staining will take longer because of the tighter matrix of the gel.

This experiment requires a 0.8% gel. It is a common agarose gel concentration for separating dyes or DNA fragments in EDVOTEK experiments.

- Specifications for preparing a 0.8% gel can be found in Appendix A.

Tables A-1 and A-2 below are examples of tables from Appendix A. The first (left) table shows reagent volumes using concentrated (50x) buffer. The second (right) table shows reagent volumes using diluted (1x) buffer.

<table>
<thead>
<tr>
<th>Table A.1 Individual 0.8% UltraSpec-Agarose™ Gel</th>
<th>Table A.2 Individual 0.8% UltraSpec-Agarose™ Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of Gel (cm)</td>
<td>Amt of Agarose (g) + Concentrated Buffer (50x) (ml) + Distilled Water (ml) = Total Volume (ml)</td>
</tr>
<tr>
<td>7 x 7</td>
<td>0.23 + 0.6 = 29.4</td>
</tr>
<tr>
<td>7 x 10</td>
<td>0.39 + 1.0 = 49.0</td>
</tr>
<tr>
<td>7 x 14</td>
<td>0.46 + 1.2 = 58.8</td>
</tr>
</tbody>
</table>

* 0.77 UltraSpec-Agarose™ gel percentage rounded up to 0.8%
Notes to the Instructor & Pre-Lab Preparations

READY-TO-LOAD SAMPLES FOR ELECTROPHORESIS

No heating required before gel loading.

EDVOTEK offers the widest selection of electrophoresis experiments which minimize expensive equipment requirements and save valuable time for integrating important biotechnology concepts in the teaching laboratory. Series 100 experiments feature dye or DNA samples which are predigested with restriction enzymes and are stable at room temperature. Samples are ready for immediate delivery onto agarose gels for electrophoretic separation and do not require pre-heating in a waterbath.

Electrophoresis samples and reagents in EDVOTEK experiments are packaged in various formats. The samples in Series 100 and S-series electrophoresis experiments are packaged in one of the following ways:

1) Pre- aliquoted Quickstrip™ connected sample tubes

OR

2) Individual 1.5 ml (or 0.5 ml) microtest sample tubes

SAMPLES FORMAT: PRE-ALIQUOTED QUICKSTRIP™ CONNECTED TUBES

Convenient QuickStrip™ connected sample tubes contain pre-aliquoted ready-to-load samples. The samples are packaged in a microtiter block of tubes covered with a protective overlay. Separate the microtiter block of tubes into strips for a complete set of samples for one gel.

1. Use sharp scissors to separate the block of samples into individual strips as shown in the diagram at right.

   Each row of samples (strip) constitutes a complete set of samples for each gel. The number of samples per set will vary depending on the experiment. Some tubes may be empty.

2. Cut carefully between the rows of samples. Do not cut or puncture the protective overlay directly covering the sample tubes.

3. Each gel will require one strip of samples.

4. Remind students to tap the tubes before gel loading to ensure that all of the sample is at the bottom of the tube.
Experiment Results and Analysis

In the idealized schematic, the relative positions of dye fragments are shown but are not depicted to scale.

Lane | Tube
--- | ---
1 A Orange
2 B Purple
3 C Red
4 D Blue 1
5 E Dye Mixture
6 F Blue Dye Mixture (Blue 1 + Blue 2)
Study Questions and Answers

1. On what basis does agarose gel electrophoresis separate molecules?
   
   Agarose gel electrophoresis separates molecules based on size, charge and shape.

2. Explain migration according to charge.
   
   Molecules having a negative charge migrate toward the positive electrode; positively charged molecules migrate toward the negative electrode.

3. What conclusion can be drawn from the results of sample F?
   
   The color blue has no relationship to charge. Blue 2 has a positive charge; Blue 1 has a negative charge.

4. Why is glycerol added to the sample solutions before they are loaded into the wells?
   
   Glycerol adds density to the samples so they sink through the buffer and into the wells.

5. What would happen if distilled water were substituted for buffer in either the chamber solution or the gel solution?
   
   No ions are contained in distilled water. Ions are required for conductivity of the fluid and therefore, the ability of the molecules to migrate through the gel.
Appendices

A  0.8 % Agarose Gel Electrophoresis Reference Tables
B  Quantity Preparations for Agarose Gel Electrophoresis
C  Agarose Gel Preparation Step by Step Guidelines
0.8% Agarose Gel Electrophoresis Reference Tables

Table A.1 Individual 0.8%* UltraSpec-Agarose™ Gel

<table>
<thead>
<tr>
<th>Size of Gel (cm)</th>
<th>Amt of Agarose (g)</th>
<th>Concentrated Buffer (50x) (ml)</th>
<th>Distilled Water (ml)</th>
<th>Total Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 x 7</td>
<td>0.23</td>
<td>0.6</td>
<td>29.4</td>
<td>30</td>
</tr>
<tr>
<td>7 x 10</td>
<td>0.39</td>
<td>1.0</td>
<td>49.0</td>
<td>50</td>
</tr>
<tr>
<td>7 x 14</td>
<td>0.46</td>
<td>1.2</td>
<td>58.8</td>
<td>60</td>
</tr>
</tbody>
</table>

* 0.77 UltraSpec-Agarose™ gel percentage rounded up to 0.8%

Table A.2 Individual 0.8%* UltraSpec-Agarose™ Gel

<table>
<thead>
<tr>
<th>Size of Gel (cm)</th>
<th>Amt of Agarose (g)</th>
<th>Diluted Buffer (1x) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 x 7</td>
<td>0.23</td>
<td>30</td>
</tr>
<tr>
<td>7 x 10</td>
<td>0.39</td>
<td>50</td>
</tr>
<tr>
<td>7 x 14</td>
<td>0.46</td>
<td>60</td>
</tr>
</tbody>
</table>

The recommended electrophoresis buffer is Tris-acetate-EDTA, pH 7.8. The formula for diluting EDVOTEK (50x) concentrated buffer is one volume of buffer concentrate to every 49 volumes of distilled or deionized water. Prepare buffer as required for your electrophoresis unit.

Table B Electrophoresis (Chamber) Buffer

<table>
<thead>
<tr>
<th>EDVOTEK Model #</th>
<th>Total Volume Required (ml)</th>
<th>Dilution 50x Conc. Buffer (ml) + Distilled Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M6+</td>
<td>300</td>
<td>6 + 294</td>
</tr>
<tr>
<td>M12</td>
<td>400</td>
<td>8 + 392</td>
</tr>
<tr>
<td>M36</td>
<td>1000</td>
<td>20 + 980</td>
</tr>
</tbody>
</table>

Time and Voltage recommendations for EDVOTEK equipment are outlined in Table C.1 for 0.8% agarose gels. The time for electrophoresis will vary from approximately 15 minutes to 2 hours depending upon various factors. Conduct the electrophoresis for the length of time determined by your instructor.
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.

### Batch Agarose Gels (0.8%) (0.8%)

For quantity (batch) preparation of 0.8% agarose gels, see Table E.1.

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. Refer to Appendix A 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.

---

**Table D**

<table>
<thead>
<tr>
<th>Concentrated Buffer (50x) (ml)</th>
<th>Distilled Water (ml)</th>
<th>Total Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>2,940</td>
<td>3000 (3 L)</td>
</tr>
</tbody>
</table>

**Note:** The UltraSpec-Agarose™ kit component is often labeled with the amount it contains. In many cases, the entire contents of the bottle is 3.0 grams. 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.1**

<table>
<thead>
<tr>
<th>Amt of Agarose (g)</th>
<th>Concentrated Buffer (50X) (ml)</th>
<th>Distilled Water (ml)</th>
<th>Total Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>7.5</td>
<td>382.5</td>
<td>390</td>
</tr>
</tbody>
</table>

---

**60°C**
Principles and Practice of Agarose Gel Electrophoresis

Appendix C

Agarose Gel Preparation - Step by Step Guidelines

Preparing the Gel bed

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.
   A. Using Rubber dams:
      • Place a rubber dam on each end of the bed. Make sure the rubber dam fits firmly in contact with the sides and bottom of the bed.
   B. Taping with labeling or masking tape:
      • Extend 3/4 inch wide tape over the sides and bottom edge of the bed.
      • Fold the extended tape edges back onto the sides and bottom. Press contact points firmly to form a good seal.

2. Place a well-former template (comb) in the set of notches at the middle of the bed. Make sure the comb sits firmly and evenly across the bed.

Casting Agarose Gels

3. Use a flask or beaker to prepare the gel solution.

4. Refer to the appropriate Reference Table (i.e. 0.8%, 1.0% or 2.0%) for agarose gel preparation. Add the specified amount of agarose powder and buffer. Swirl the mixture to disperse clumps of agarose powder.

5. With a lab marking pen, indicate the level of the solution volume on the outside of the flask.

6. Heat the mixture to dissolve the agarose powder.
   A. Microwave method:
      • Cover the flask with plastic wrap to minimize evaporation.
      • Heat the mixture on High for 1 minute.
      • Swirl the mixture and heat on High in bursts of 25 seconds until all the agarose is completely dissolved.
   B. Hot plate method:
      • Cover the flask with aluminum foil to minimize evaporation.
      • Heat the mixture to boiling over a burner with occasional swirling. Boil until all the agarose is completely dissolved.

Continue heating until the final solution appears clear (like water) without any undissolved particles. Check the solution carefully. If you see “crystal” particles, the agarose is not completely dissolved.

If gel trays and rubber end caps are new, they may be initially somewhat difficult to assemble. Here is a helpful hint:

Place one of the black end caps with the wide “u” shaped slot facing up on the lab bench.

Push one of the corners of the gel tray into one of the ends of the black cap. Press down on the tray at an angle, working from one end to the other until the end of the tray completely fits into the black cap. Repeat the process with the other end of the gel tray and the other black end cap.

At high altitudes, use a microwave oven to reach boiling temperatures.
7. Cool the agarose solution to 60°C with careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume marked in step 5.

After the gel is cooled to 60°C:

- If you are using rubber dams, go to step 9.
- If you are using tape, continue with step 8.

8. Seal the interface of the gel bed and tape to prevent agarose solution from leaking.
   - Use a transfer pipet to deposit a small amount of the cooled agarose to both inside ends of the bed.
   - Wait approximately 1 minute for the agarose to solidify.

9. Place the bed on a level surface and pour the cooled 60° C agarose solution into the bed.

10. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.

Preparing the gel for electrophoresis

11. After the gel is completely solidified, carefully and slowly remove the rubber dams or tape from the gel bed. Be especially careful not to damage or tear the gel wells when removing the rubber dams. A thin plastic knife, spatula or pipet tip can be inserted between the gel and the dams to break possible surface tension.

12. Remove the comb by slowly pulling straight up. Do this carefully and evenly to prevent tearing the sample wells.

13. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.

14. Fill the electrophoresis apparatus chamber with the appropriate amount of diluted (1x) electrophoresis buffer (refer to Table B on the Appendix page provided by your instructor).

15. Make sure that the gel is completely submerged under buffer before proceeding to loading the samples and conducting electrophoresis.
Each lane represents an individual’s make up for a particular gene. Each protein is coded by two genes that are inherited from both parents. If one of the genes is mutated, the person can still generate the correct protein (from the other non-mutant gene) and will not show a full blown clinical condition. The mutant gene can be inherited in a Mendelian pattern; if both genes have the same critical mutation, the individual will be a carrier of disease. In this experiment analysis is based on the size of the gene.

**PLEASE NOTE: THIS IS FROM A DIFFERENT LAB THAT USES THE SAME STANDARD MARKER DYES AS EDVOTEK KIT 101**

Note: This technique has a ± 10 - 15% margin of error.
need to measure distance of band that went towards neg. pole and extrapolate size by going over measured distance on X-axis, going up to line and putting cursor on line - read size from coordinates on bottom left of graph
Basic Microchemical Techniques: Gel Electrophoresis Tips

To make a 1X buffer: add 10 ml concentrated buffer (50X) + 490 ml distilled water. (Prepare approximately 400 ml per electrophoresis chamber and 100 ml per gel - this will give you some extra)

To prepare a 1% agarose gel (caution: gel bed volume < 50 ml): add .5 g agarose powder to 49.5 ml of 1X electrophoresis buffer in a 250 ml beaker.

Practice loading solution: Pipette 180 ul distilled H₂O into a 1.5 ml microtube and add 20 ul loading dye. Touch the pipette tip containing the dye to the meniscus of the water before expelling the dye. Close the tube top and flick the tube vigorously to mix.

Methylene Blue based stains are about half as sensitive as ethidium bromide but has the great advantage of not being mutagenic so you can use it in your classrooms. SYBR Safe stain is a great alternative, having almost the sensitivity of ethidium bromide combined with the safety of methylene blue based stains.

Add 1 ul SYBR Safe Stain per 10 ml of agarose after the agarose has cooled and before you pour it.

http://www.edvotek.com