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**Includes EDVOTEK's All-NEW
DNA Standard Marker**

- Better separation
- Easier band measurements
- No unused bands

NEW DNA Standard ladder sizes:
6751, 3652, 2827, 1568, 1118, 825, 630



Edvo-Kit #

115

Edvo-Kit #115

Cancer Gene Detection

Experiment Objective:

In this experiment, students will gain an understanding of the p53 tumor suppressor gene and its role in familial cancers.

See page 3 for storage instructions.

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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)

- A Standard DNA Marker
- B Control DNA
- C Patient Peripheral Blood DNA
- D Patient Breast Tumor DNA
- E Patient Normal Breast Tissue DNA

Check (✓)

- ☐
- ☐
- ☐
- ☐
- ☐

REAGENTS & SUPPLIES

- UltraSpec-Agarose™
- Electrophoresis Buffer (50x)
- Practice Gel Loading Solution
- FlashBlue™ DNA Stain
- InstaStain® Blue cards
- 1 ml pipet
- Microtipped Transfer Pipets

- ☐
- ☐
- ☐
- ☐
- ☐
- ☐
- ☐

Includes EDVOTEK's All-NEW DNA Standard Marker

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- Easier band measurements
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NEW DNA Standard ladder sizes:
6751, 3652, 2827, 1568, 1118, 825, 630



Experiment #115 is designed for 8 gels if stained with FlashBlue™ or InstaStain® Blue (both included) or 16 gels if stained with SYBR® Safe or InstaStain® Ethidium Bromide (not included).

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

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.

Requirements

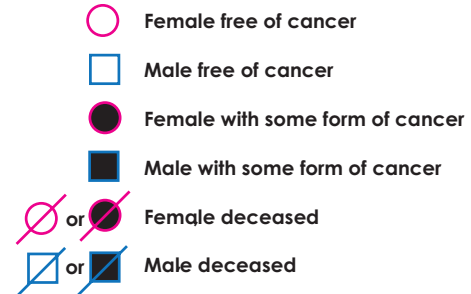
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Small plastic trays or large weigh boats (for gel destaining)
- DNA visualization system (white light)
- Distilled or deionized water

Background Information

ABOUT FAMILY PEDIGREES

When drawing or studying a family pedigree, the following are general guidelines to the symbols used and their representations:

- A Circle represents a female.
- A square represents a male.
- A shaded circle or square refers to a person having some form of cancer.
- An open (non-shaded) square or circle represents a person who is free of cancer.
- A circle or square (either shaded or open) with a diagonal slash through it represents a person who is deceased.



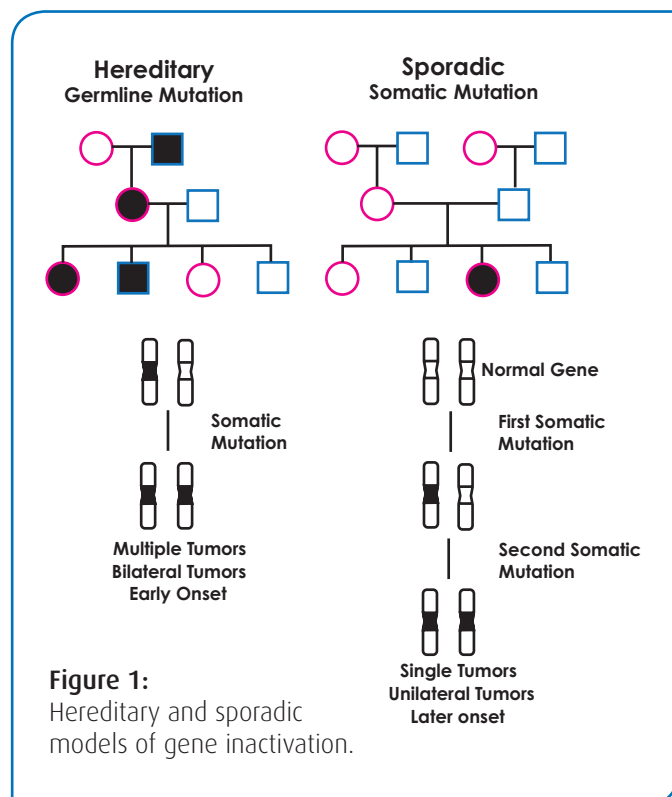
In Li-Fraumeni syndrome, the pattern of cancers in family pedigrees suggest dominant inheritance. It is a genetic predisposition leading to specific types of cancers. Typically, the onset of cancer is at an early age, with multiple primary tumors.

CANCER GENE DETECTION

Many contributory factors have been identified to cause the onset of cancers, that include exposure to certain carcinogens in our diets and environment. Several forms of cancer have familial predispositions. These cancers appear to be linked to inherited mutation of suppressor genes, such as p53.

Familial cancers constitute a very small fraction of the total reported cancers and they occur in dominant inherited patterns. Mutations that are directly inherited are referred to as germline mutations. Such mutations can be detected in familial pedigrees. A second type of mutation, known as somatic mutations, do not have direct genetic links and are acquired during the life of the individual. Patterns of typical hereditary and sporadically acquired nonhereditary pedigrees appear in Figure 1.

In a germline with an inherited mutation, a single somatic mutation within a suppressor gene will result in the inactivation of both alleles. By contrast, normal inherited suppressor genes, that are free of mutations, will require two sequential mutations to initiate tumors. This model is referred to as the "Two-hit" hypothesis.



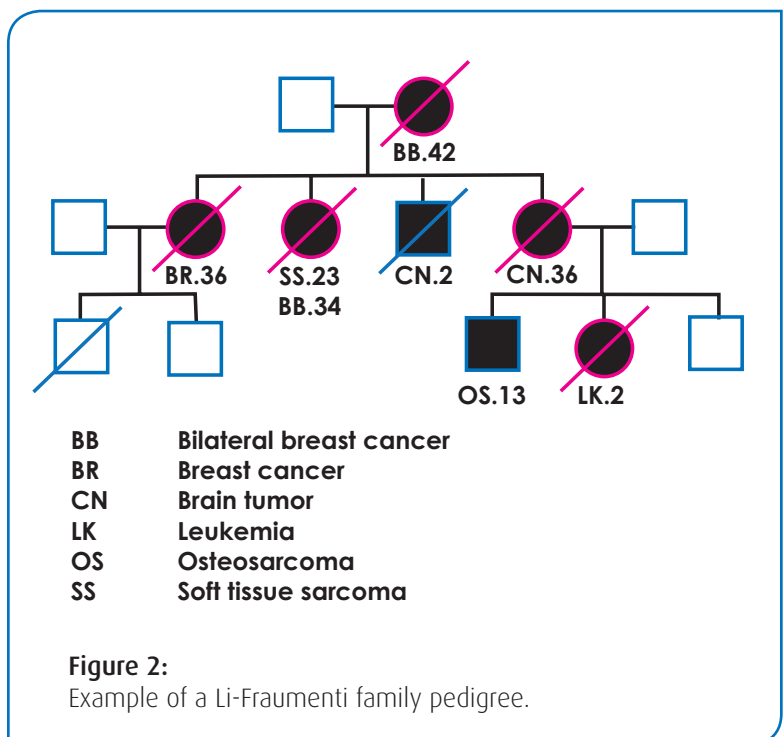
Historically, some of the first genes identified include the retinoblastoma (RB) gene, Wilm's' tumor (WTI), neurofibromatosis type II gene and Li-Fraumeni syndrome. In Li-Fraumeni syndrome, a notable feature in family pedigrees, include a sarcoma patient and at least two immediate relatives with other cancers before the age of 45, as well as multiple cancers in other family members. This is illustrated in Figure 2.

With the advent of molecular biology applications to medicine, gene maps and the chromosomal locations of genes are becoming available as tools for the identification of predisposition for various diseases. The procedures used to obtain such information include DNA isolation and the analysis of point mutations in hot spot areas in cancer-related genes, such as p53. Several methods of analysis for the detection of point mutations in genes include DNA sequencing.

The Human genome project has provided information to link to the identification of many various cancers and other diseases to DNA sequence information. This information needs to be handled cautiously to assure confidentiality of patients' genetic profiles.

The study of inherited cancers has given cancer molecular biologists the opportunity to search for genes that are critical in normal cell development and carcinogenesis. At the molecular level, cancer formation is characterized by alterations in both dominant oncogenes and tumor suppressor genes, such as p53. Suppressors are normal cellular proteins that are involved in limiting cell growth. By contrast, oncogenes are involved in promoting the growth of cells.

In recent years, the p53 tumor suppressor protein has become the center of many cancer biology studies. Because it appears to be of major significance, there is great impetus to study how this gene functions in normal cells compared to cancer cells. The gene for the p53 protein is located on the short arm of chromosome 17. It encodes a 53,000 molecular nuclear phosphoprotein. Wild type (normal) p53 functions as a cell regulator. There is now well-documented evidence that normal p53 is a sequence-specific DNA-binding protein that is a transcriptional regulator. Upon introduction of mutations, p53 loses its ability to bind to DNA. By contrast, p53 that have mutations in specific hot spots promote uncontrolled cell growth and therefore function as oncogenes. For a tumor suppressor gene such as p53 to play a role in transformation in cancer, both alleles need to be altered, as shown Figure 2.



The p53 protein can be divided into three domains. The first is the amino terminus region which contains the transcriptional activation region. The second is the central region within the protein where the majority of critical "hot spot" mutations are located. These "hot spots" are sites where mutations are detected in high frequencies. They are between exons 5 through 8 where 95% of the mutations occur. Within this region there are five subregions where point mutations are detected in human cancers. The third region of the p53 protein is the carboxyl section that is the most complex section that contains the oligomerization and nuclear localization sequences.

Examples of hot spots include codons 165 and 175 in exon 5; 196 and 213 in exon 6; 245 and 248 in exon 7; 273 and 282 in exon 8; all are within the p53 protein. Several of these mutations result in an altered p53 protein conformation. In turn, these changes can result in increased stability of the mutant protein and the ability to bind to the normal p53 protein and inactivate it. It is of interest to note that there are correlations between the mutation and tumor tissue. One such example is the mutation at amino acid 175 which is common in colon carcinoma but is rarely observed in lung carcinoma.

The inherited Li-Fraumeni syndrome as it has become to be known is rare. When it does occur it affects young family members and results in high mortality rates. Two physicians, Li and Fraumeni first described the syndrome after examining death certificates of 648 childhood sarcomas. It was discovered in four families where siblings and cousins had childhood sarcomas. Further analysis showed more than 50% of the affected families had extended phenotypes that included brain, breast cancers and leukemias. Cells in the individuals with LFS have a single wild type p53 allele. Examination of the p53 has shown a correlation to mutations in the protein as described above.

CONSTRUCTING A FAMILY PEDIGREE

A first step in the search and assignment of Li-Fraumeni syndrome is to establish the family pedigree of the patient.

The first part of the experiment is based on the information made available as part of a diagnosis by the family physician and the oncologist. The pedigree information that you will develop is for a young woman who is suspected to have the Li-Fraumeni syndrome.

Upon monthly breast self-examination, Valerie Brown, age 36, found a small irregular mass. She was concerned because she knew that her mother had a mastectomy when she was in her late thirties. Valerie made an appointment with her physician, who referred her to a specialist at a local cancer center, where she was diagnosed as having breast cancer. As part of the medical work-up, the oncologist had inquired about her family history of cancer. Upon consultation with her mother, Valerie learned that her father and his family appeared to be free of cancer. However, in Valerie's mother's family, several cases of cancer have occurred.

With the information given below, chart the family pedigree.

- Her mother, Diane, was diagnosed and treated for breast cancer at the age of 39.
- Valerie did not know that Diane had a sister, Mabel, who died at age 2 of a brain tumor.
- Diane's brother, James underwent surgery, followed by chemotherapy for colon cancer.
- Her maternal grandmother, Elsie, died at age 42 from bilateral breast cancer.
- Her maternal grandfather, Elmer, was free of cancer and is 88 years old.
- Her maternal cousin, Patrick (son of James), died of brain cancer at 14.
- Her cousin, Jane, aged 2 who is Patrick's sister was diagnosed with childhood leukemia and subsequently died.
- Patrick's two other brothers, Robert, 28 and Curtis, 30, are in good health and free of cancer.
- Valerie's sister, Nancy is free of cancer.
- Nancy's son, Michael was diagnosed at the age of 3 as having sarcoma. Recently, at the age of 18, he was diagnosed as having osteosarcoma.
- Nancy's other son, John, and daughter, Jessica, are free of cancer.

Valerie has five children: Justin (16), Sheila (14), Robert (10), Angela (8), and Anthony (6), none of whom show any signs of cancer at this time. She was interested in the p53 diagnostic test to determine if she inherited mutations.

The familial pedigree strongly suggests Li-Fraumeni syndrome. In such a case, a secondary diagnostic test is normally conducted. In this scenario, Valerie provides a sample of blood and tumor tissue to conduct DNA analysis for the p53 gene. Normally the procedure is to amplify the gene using polymerase chain reaction. This is followed by one of several methods to detect the presence of a point mutation at the hot spots.

In the simulation experiment which follows, Valerie's DNA has already been digested with a restriction enzyme that recognizes the mutant sequence at the simulated hot spot site at nucleotide 165 which is the palindrome CAGCTG. A restriction enzyme was used as a probe to cut the simulated amplified gene for Valerie's DNA sample, together with a normal control and a set of standard DNA marker fragments. Digestion of the normal amplified DNA will give a characteristic DNA fragment banding pattern. The DNA obtained from blood lymphocyte will give an altered band pattern representing one normal allele and the second which is the mutant. The DNA analysis from the tumor tissue will show only the pattern for the tumor allele. The predigested samples with the control wild type and DNA markers will be separated by agarose gel electrophoresis and stained.

Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will gain an understanding of the p53 tumor suppressor gene and its role in familial cancers.

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.

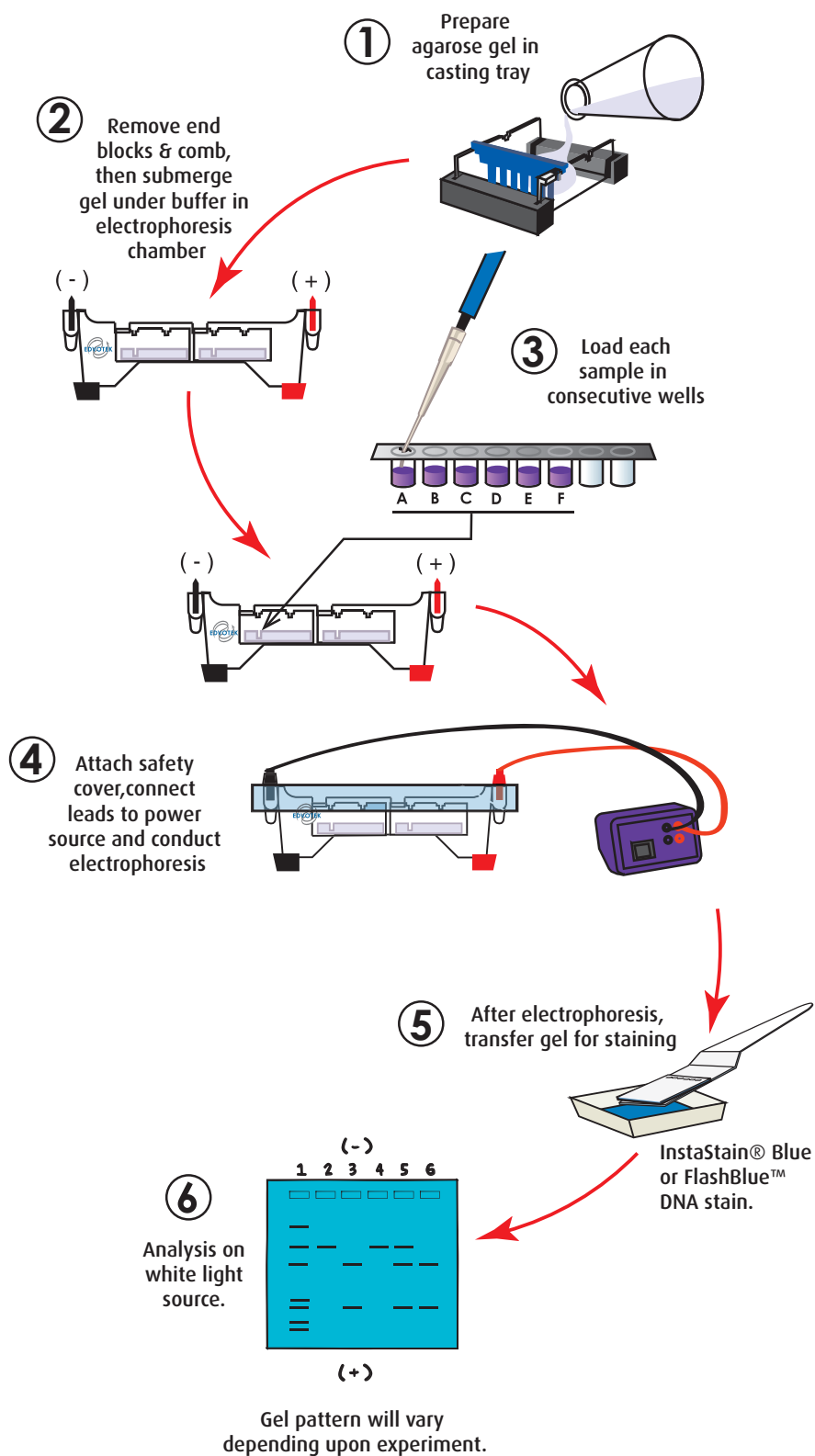
During the Experiment:

- Record your observations.

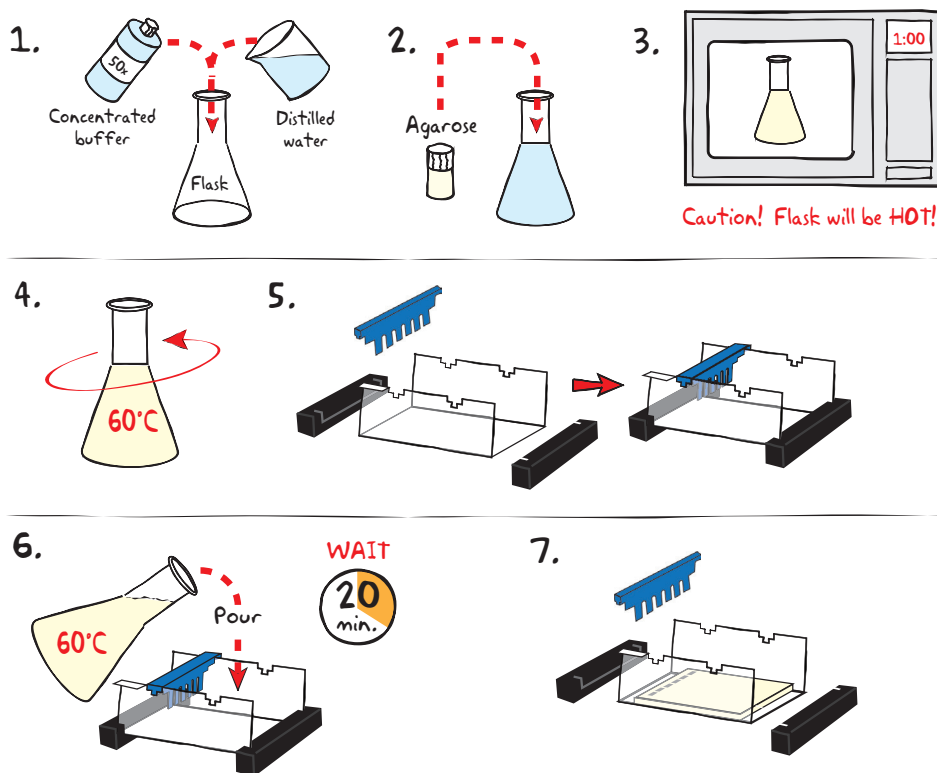
After the Experiment:

- 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



Module I: 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

CASTING THE AGAROSE GEL

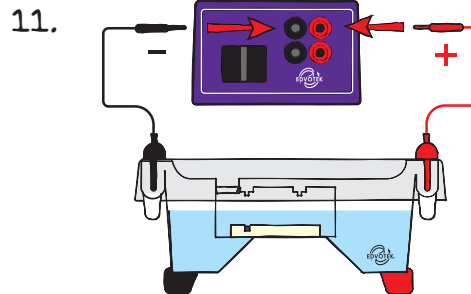
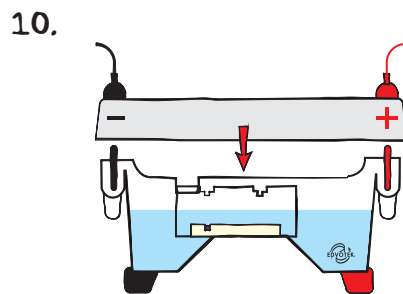
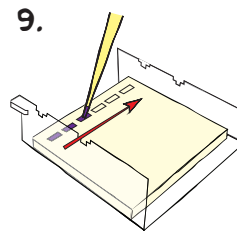
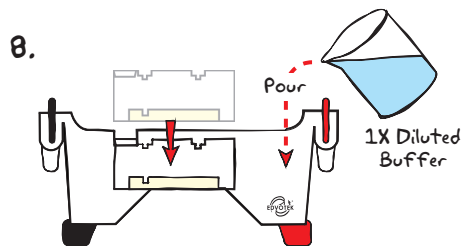
- DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
- MIX** agarose powder with buffer solution in a 250 ml flask (refer to Table A).
- DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** 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).
- COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
- 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.
- 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.
- 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 |

Module I: Agarose Gel Electrophoresis



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- Better separation
- Easier band measurements
- No unused bands

NEW DNA Standard ladder sizes:
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Wear gloves
and safety goggles

REMINDER:

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

RUNNING THE GEL

- 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.
- PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35 µl) into the well in the order indicated by Table 1, at right.
- PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
- 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.
- After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to instructions for **STAINING** the agarose gel.

Table 1: Gel Loading

| Lane | Tube | Sample |
|------|--------|----------------------------------|
| 1 | Tube A | Standard DNA Marker |
| 2 | Tube B | Control DNA |
| 3 | Tube C | Patient Peripheral Blood DNA |
| 4 | Tube D | Patient Breast Tumor DNA |
| 5 | Tube E | Patient Normal Breast Tissue DNA |

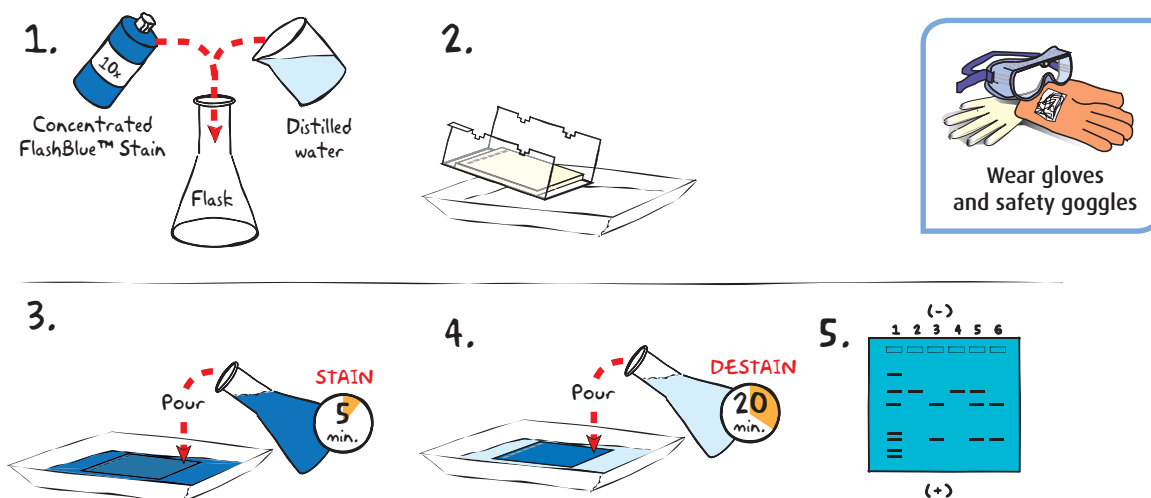
Table B
1x Electrophoresis Buffer (Chamber Buffer)

| 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)

| Volts | Electrophoresis Model | | |
|-------|-----------------------|--------------------------|------------------------------------|
| | 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. |

Module II-A: Staining Agarose Gels Using FlashBlue™

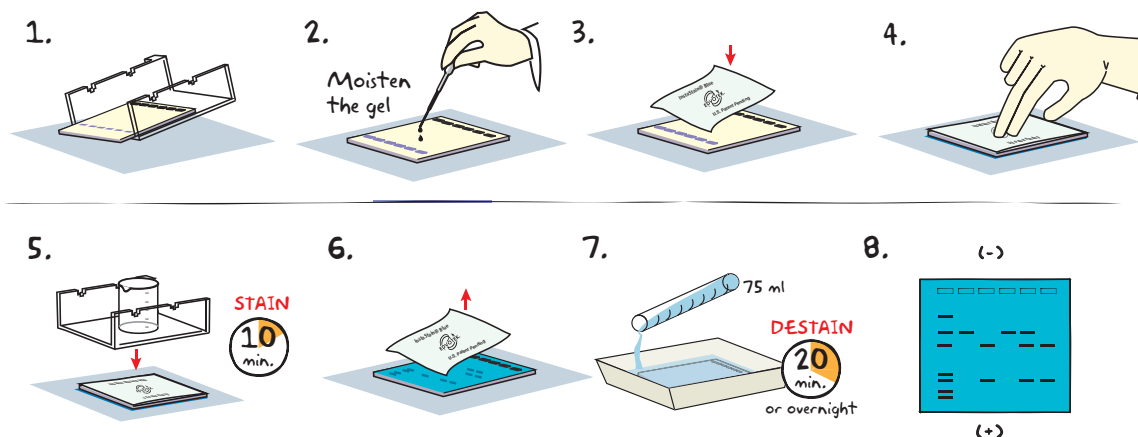


1. **DILUTE** 10 ml of 10x concentrated FlashBlue™ with 90 ml of water in a flask and **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1x FlashBlue™ stain solution. **STAIN** the gel for 5 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 5 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
4. **TRANSFER** the gel to a second small tray. **COVER** the gel with water. **DESTAIN** for at least 20 minutes with gentle shaking (longer periods will yield better results). Frequent changes of the water will accelerate destaining.
5. Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

ALTERNATIVE PROTOCOL:

1. **DILUTE** one ml of concentrated FlashBlue™ stain with 149 ml dH₂O.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.

Module II-B: Staining Agarose Gels Using InstaStain® Blue



1. Carefully **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray on to a piece of plastic wrap on a flat surface.
2. **MOISTEN** the gel with a few drops of electrophoresis buffer.
3. Wearing gloves, **PLACE** the blue side of the InstaStain® Blue card on the gel.
4. With a gloved hand, **REMOVE** air bubbles between the card and the gel by firmly running your fingers over the entire surface. Otherwise, those regions will not stain.
5. **PLACE** the casting tray on top of the gel/card stack. **PLACE** a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Blue card is in direct contact with the gel surface. **STAIN** the gel for 10 minutes.
6. **REMOVE** the InstaStain® Blue card. If the color of the gel appears very light, reapply the InstaStain® Blue card to the gel for an additional five minutes.
7. **TRANSFER** the gel to a small, clean gel-staining tray. **COVER** the gel with about 75 mL of distilled water and **DESTAIN** for at least 20 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. To accelerate destaining, warm the distilled water to 37°C and change it frequently.
8. Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.



Wear gloves
and safety goggles

NOTE:
DO NOT STAIN
GELS IN THE
ELECTROPHORESIS
APPARATUS.

ALTERNATIVE PROTOCOL:

1. Carefully **SLIDE** the agarose gel from its casting tray into a small, clean tray containing about 75 mL of distilled/deionized water or used electrophoresis buffer. The gel should be completely submerged.
2. Gently **FLOAT** the InstaStain® Blue card(s) on top of the liquid with the stain (blue side) facing toward the gel. Each InstaStain® Blue card will stain 49 cm² of gel (7 x 7 cm).
3. **COVER** the tray with plastic wrap to prevent evaporation. **SOAK** the gel in the staining liquid for at least 3 hours. The gel can remain in the liquid overnight if necessary.
4. Carefully **REMOVE** the gel from the staining tray. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

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Study Questions

1. What is the difference between tumor suppressors and oncogenes?
2. What are the effects of hot spots in p53 protein structure?
3. Why does Valerie's tumor DNA sample have fewer bands than the peripheral blood?
4. What is the purpose of the control lane?
5. Can a physician proceed with diagnosis based on molecular biology data?

Instructor's Guide

ADVANCE PREPARATION:

| Preparation for: | What to do: | When? | Time Required: |
|---|---|--|----------------|
| Module I: Agarose Gel Electrophoresis | Prepare QuickStrips™ | Up to one day before performing the experiment | 45 min. |
| | Prepare diluted electrophoresis buffer | | |
| | Prepare molten agarose and pour gels | | |
| Module II: Staining Agarose Gels | Prepare staining components | The class period or overnight after the class period | 10 min. |

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Pre-Lab Preparations: Module I

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 Module I in the Student's Experimental Procedure. Students will need 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. EDVOTEK Series 100 experiments are designed for students who have had previous experience with micropipetting techniques and agarose gel electrophoresis.

If students are unfamiliar with using micropipets, we recommend performing Cat. #S-44, Micropipetting Basics or Cat. #S-43, DNA DuraGel™ prior to conducting this advanced level experiment.

FOR MODULE I Each Student Group should receive:

- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™
- QuickStrip™ Samples

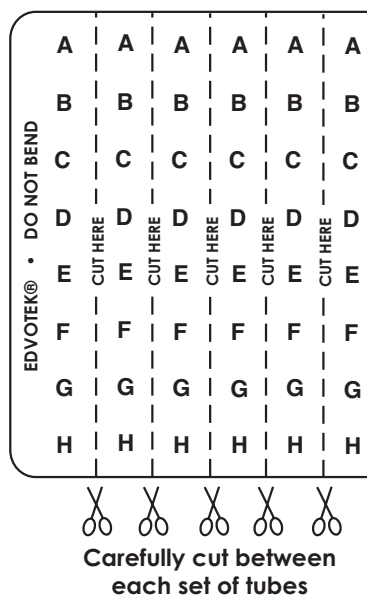
SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

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

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.

If using SYBR® Safe or InstaStain® Ethidium Bromide for DNA visualization, each QuickStrip™ is shared by two groups. 18 µl of the DNA sample will be loaded into each well. Proceed to visualize the results as specified by the DNA stain literature.



Pre-Lab Preparations: Module II

MODULE II-A: STAINING WITH INSTASTAIN® BLUE

The easiest and most convenient DNA stain available is InstaStain® Blue. InstaStain® Blue does not require the formulation, storage and disposal of large volumes of liquid stain. Each InstaStain® Blue card contains a small amount of blue DNA stain. When the card is placed in water, the DNA stain is released. This solution simultaneously stains and destains the gel, providing uniform gel staining with minimal liquid waste and mess.

You can use a White Light Visualization System (Cat. #552) to visualize gels stained with InstaStain® Blue.

MODULE II-B: STAINING WITH FLASHBLUE™

FlashBlue™ stain is optimized to shorten the time required for both staining and destaining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to “equilibrate” in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background. A white light box (Cat. #552) is recommended for visualizing gels stained with FlashBlue™.

- Stained gels may be stored in destaining liquid for several weeks with refrigeration, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed of down the drain.

MODULE II: PHOTODOCUMENTATION OF DNA (OPTIONAL)

Once gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.

**FOR MODULE II-A
Each Student Group
should receive:**

- 1 InstaStain® card per 7 x 7 cm gel

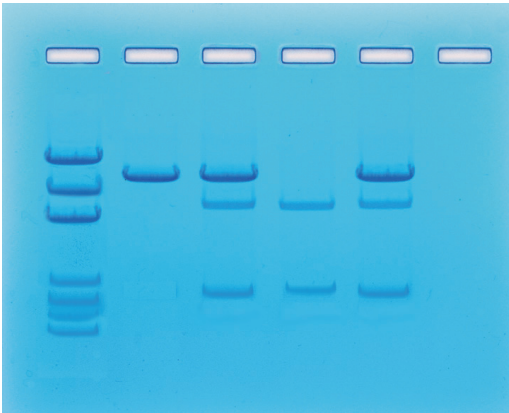


Wear gloves
and safety goggles

**FOR MODULE II-B
Each Student Group
should receive:**

- 10 ml 10X concentrated FlashBlue OR 100 mL 1x diluted FlashBlue
- Small plastic tray or weight boat
- Distilled or deionized water

Experiment Results and Analysis



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

Includes EDVOTEK's All-NEW DNA Standard Marker

- Better separation
- Easier band measurements
- No unused bands

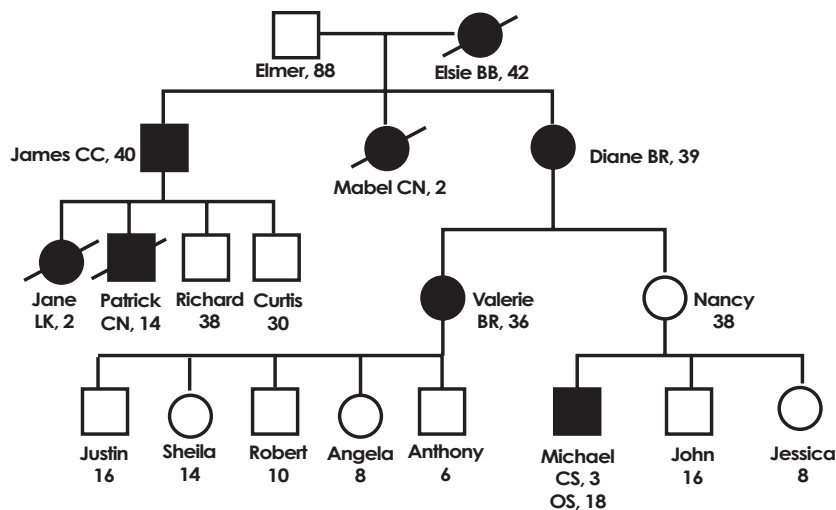
NEW DNA Standard ladder sizes:
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| Lane | Tube | Sample | Molecular Weights (in bp) |
|------|------|------------------------------|---------------------------|
| 1 | A | DNA Standard Markers | ----- |
| 2 | B | Control DNA | 4282 |
| 3 | C | Patient Peripheral Blood DNA | 4282 3000 1282 |
| 4 | D | Patient Tumor DNA | 3000 1282 |
| 5 | E | Patient Breast Normal DNA | 4282 3000 1282 |

Explanation of Gel Result:

DNA obtained from tissue culture and normal patient cells (without an inherited p53 mutation in the PCR amplified DNA product) will not have the restriction enzyme site and therefore the amplified DNA will not be cut and will appear as one band (lane 2). The patient DNA from the tumor sample has a mutation in both p53 genes at this particular site and therefore the tumor PCR amplified DNA will be cut to yield two DNA fragments (Lane 4). The peripheral blood and non-tumor patient DNA will have the same pattern (lanes 3 and 5) where there are three bands due to the combined DNA patterns from the normal gene (like lane 2) and the mutant gene (like lane 4).



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

Appendices

- A EDVOTEK® Troubleshooting Guide
- B Bulk Preparation of Electrophoresis Buffer and Agarose Gels
- C Data Analysis Using a Standard Curve

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

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Appendix A

EDVOTEK® Troubleshooting Guides

| PROBLEM: | CAUSE: | ANSWER: |
|---|---|--|
| Bands are not visible on the gel. | The gel was not prepared properly. | Ensure that the electrophoresis buffer was correctly diluted. |
| | The gel was not stained properly. | Repeat staining. |
| | Malfunctioning electrophoresis unit or power source. | Contact the manufacturer of the electrophoresis unit or power source. |
| After staining the gel, the DNA bands are faint. | The gel was not stained for a sufficient period of time. | Repeat staining protocol. |
| | The background of gel is too dark. | Destain the gel for 5-10 minutes in distilled water. |
| DNA bands were not resolved. | Tracking dye should migrate at least 3.5 cm (if using a 7x7 cm tray), and at least 6 cm (if using a 7x14 cm tray) from the wells to ensure adequate separation. | Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 V). |
| DNA bands fade when gels are kept at 4°C. | DNA stained with FlashBlue™ may fade with time | Re-stain the gel with FlashBlue™ |
| There is no separation between DNA bands, even though the tracking dye ran the appropriate distance. | The wrong percent gel was used for electrophoretic separation. | Be sure to prepare the correct percent agarose gel. For reference, the Ready-to-Load™ DNA samples should be analyzed using a 0.8% agarose gel. |
| There's not enough sample in my QuickStrip™. | The QuickStrip™ has dried out. | Add 40 µL water, gently pipet up and down to mix before loading. |

Appendix B

Bulk Preparation of Electrophoresis Buffer and 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) |

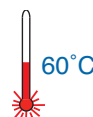
Batch Agarose Gels (0.8%)

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

Data Analysis Using a Standard Curve

Agarose gel electrophoresis separates biomolecules into discrete bands, each comprising molecules of the same size. How can these results be used to determine the lengths of different fragments? Remember, as the length of a biomolecule increases, the distance to which the molecule can migrate decreases because large molecules cannot pass through the channels in the gel with ease. Therefore, the migration rate is inversely proportional to the length of the molecules—more specifically, to the \log_{10} of molecule's length. To illustrate this, we ran a sample that contains bands of known lengths called a "standard". We will measure the distance that each of these bands traveled to create a graph, known as a "standard curve", which can then be used to extrapolate the size of unknown molecule(s).



Figure 3:
Measure distance migrated from the lower edge of the well to the lower edge of each band.

1. Measure and Record Migration Distances

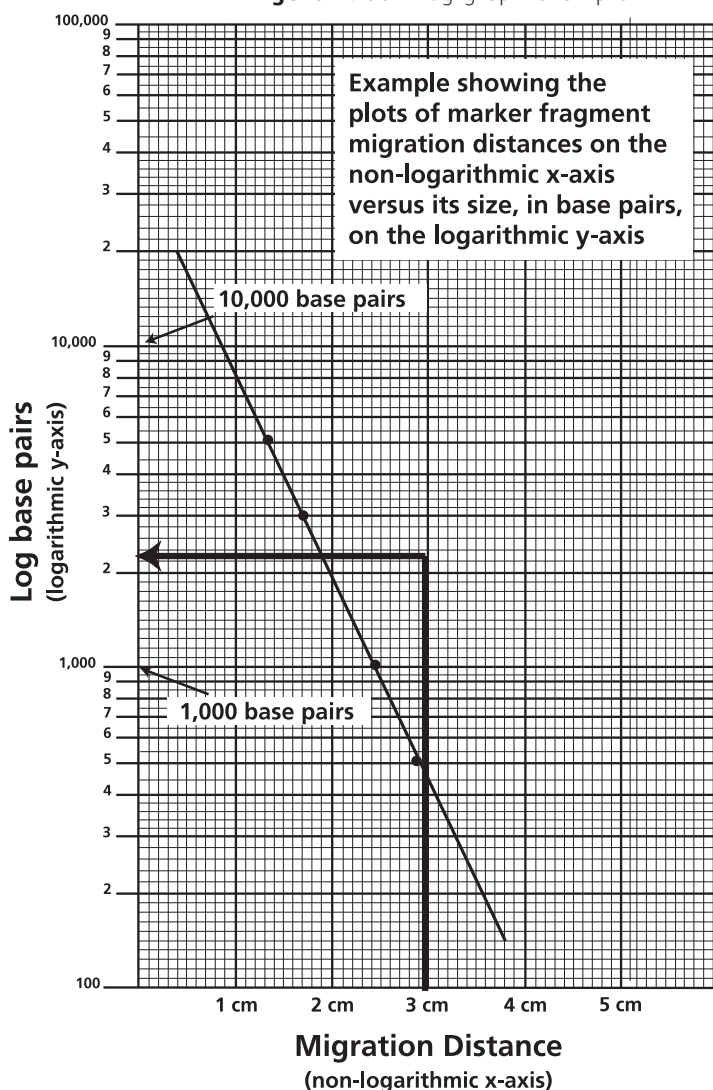
Measure the distance traveled by each Standard DNA Fragment from the lower edge of the sample well to the lower end of each band. Record the distance in centimeters (to the nearest millimeter) in your notebook. Repeat this for each DNA fragment in the standard.

Measure and record the migration distances of each of the fragments in the unknown samples in the same way you measured the standard bands.

2. Generate a Standard Curve.

Because migration rate is inversely proportional to the \log_{10} of band length, plotting the data as a semi-log plot will produce a straight line and allow us to analyze an exponential range of fragment sizes. You will notice that the vertical axis of the semi-log plot appears atypical at first; the distance between numbers shrinks as the axis progresses from 1 to 9. This is because the axis represents a logarithmic scale. The first cycle on the y-axis corresponds to lengths from 100-1,000 base pairs, the second cycle measures 1,000-10,000 base pairs, and so on. To create a standard curve on the semi-log paper, plot the distance each Standard DNA fragment migrated on the x-axis (in mm) versus its size on the y-axis (in base pairs). Be sure to label the axes!

Figure 4: Semilog graph example



Appendix C

Data Analysis Using a Standard Curve

After all the points have been plotted, use a ruler or a straight edge to draw the best straight line possible through the points. The line should have approximately equal numbers of points scattered on each side of the line. It is okay if the line runs through some points (see Figure 4 for an example).

3. Determine the length of each unknown fragment.

- Locate the migration distance of the unknown fragment on the x-axis of your semi-log graph. Draw a vertical line extending from that point until it intersects the line of your standard curve.
- From the point of intersection, draw a second line, this time horizontally, toward the y-axis. The value at which this line intersects the y-axis represents the approximate size of the fragment in base pairs (refer to Figure 4 for an example). Make note of this in your lab notebook.
- Repeat for each fragment in your unknown sample.

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Appendix C

