Edvo-Kit #314

In Search of the Cancer Gene

Experiment Objective:

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

See page 3 for storage instructions.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment Components</td>
<td>3</td>
</tr>
<tr>
<td>Experiment Requirements</td>
<td>4</td>
</tr>
<tr>
<td>Background Information</td>
<td>5</td>
</tr>
<tr>
<td><strong>Experiment Procedures</strong></td>
<td></td>
</tr>
<tr>
<td>Experiment Overview and Laboratory Safety</td>
<td>8</td>
</tr>
<tr>
<td>Module I: Construction of a Family Pedigree</td>
<td>10</td>
</tr>
<tr>
<td>Overview of Module II</td>
<td>11</td>
</tr>
<tr>
<td>Module II-A: Agarose Gel Electrophoresis</td>
<td>13</td>
</tr>
<tr>
<td>Module II-B: Staining Agarose Gels with InstaStain® Ethidium Bromide</td>
<td>15</td>
</tr>
<tr>
<td>Module III: Analysis of Autorads to Search of p53 Mutagens</td>
<td>19</td>
</tr>
<tr>
<td>Study Questions</td>
<td></td>
</tr>
<tr>
<td><strong>Instructor’s Guidelines</strong></td>
<td>20</td>
</tr>
<tr>
<td>Pre-Lab Preparations</td>
<td>21</td>
</tr>
<tr>
<td>Experiment Results and Analysis</td>
<td>24</td>
</tr>
<tr>
<td>Study Questions and Answers</td>
<td>25</td>
</tr>
<tr>
<td><strong>Appendices</strong></td>
<td>26</td>
</tr>
<tr>
<td>A EDVOTEK® Troubleshooting Guide</td>
<td>27</td>
</tr>
<tr>
<td>B Bulk Preparation of Agarose Gels</td>
<td>28</td>
</tr>
</tbody>
</table>

Safety Data Sheets can be found on our website: [www.edvotek.com/safety-data-sheets](http://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)

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

REAGENTS & SUPPLIES

- UltraSpec-Agarose™
- Electrophoresis Buffer (50x)
- 10x Gel Loading Solution
- InstaStain® Ethidium Bromide cards
- 1 ml pipet
- Microtipped Transfer Pipets
- X-ray simulated p53 hot spot sequences

Check (✓)

Experiment #314 contains reagents for 6 groups.

STORAGE:

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 *(not included with this kit)*

- Horizontal Gel Electrophoresis Apparatus
- D.C. Power Supply
- Automatic Micropipets (5-50 μl) and tips
- Balance
- Microwave, hot plate or burner
- UV Transilluminator or UV Photodocumentation system
- UV safety goggles
- Disposable vinyl or latex laboratory gloves
- Hot Gloves
- Safety goggles
- Laboratory glassware
- Distilled or deionized water
ABOUT FAMILY PEDIGREES

When drawing 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.

THE ROLE OF GENES IN CANCER

Many contributory factors have been identified to cause the onset of cancer that include exposure to certain carcinogens in our diets and environment. Several forms of cancer have familial predisposition. 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 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.

**Figure 1:** Hereditary and Sporadic Models of Gene Inactivation.
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, Wilms’ 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.

In this experiment, students explore the effects of DNA methylation on restriction enzyme activity. First, DNA will be digested with the restriction enzymes DpnI and DpnII. The restriction fragments are then analyzed using agarose gel electrophoresis. After visualizing the gel, students determine which sample is methylated.

With the advent of molecular biology applications to medicine, gene maps and the chromosomal locations of genes are 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 identification of various cancers and other diseases to DNA sequencing information. This information needs to be handled cautiously to assure confidentiality of patient 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 normal 53,000 molecular nuclear phosphoprotein. Wild type 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 in Figures 1 and 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, 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 mutation and tumor tissue. One 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 from individuals with LFS have only a single wild type p53 allele. Examination of their p53 gene have shown correlations of the cancers to mutations in the protein as described above.

**OVERVIEW OF POLYMERASE CHAIN REACTION**

PCR has two important advantages. The first is sensitivity, which allows for DNA fingerprinting identification using much smaller amounts of DNA since PCR amplifies DNA. The second advantage is the speed of PCR analysis, which allows critical questions to be answered more quickly as compared to Southern Blot analysis.

PCR amplification requires the use of a thermostable DNA polymerase, such as Taq polymerase. Purified from a bacterium known as *Thermus Aquaticus* that inhabits hot springs, Taq polymerase is commonly used in PCR because it remains stable at near-boiling temperatures. Also included in the PCR reaction are the four deoxynucleotides (dATP, dCTP, dGTP, and dTTP) and two synthetic oligonucleotides, typically 15-30 base pairs in length, known as “primers”. These components, together with the DNA to be amplified, are incubated in an appropriate buffer that contains Mg2+. The primers are designed to correspond to the start and end of the DNA to be amplified, known as the “target”.

The PCR reaction mixture (which contains the DNA polymerase, buffer, deoxynucleotides, primers, and template) is subjected to sequential heating/cooling cycles at three different temperatures (Figure 3).

- In the first step, the enzyme reaction is heated to near boiling (92° - 96°C.) to denature or "melt" the DNA. This step, known as "denaturation" disrupts the hydrogen bonds between the two complimentary DNA strands and causes their separation.
- In the second PCR step, the mixture is cooled to a temperature that is typically in the range of 45° - 65°. In this step, known as "annealing", the primers, present in great excess to the template, bind to the separated DNA strands.
- In the third PCR step, known as "extension", the temperature is raised to an intermediate value, usually 72°C. At this temperature the Taq polymerase is maximally active and adds nucleotides to the primers to synthesize the new complimentary strands.
Figure 3: The Polymerase Chain Reaction.
OVERVIEW OF DNA SEQUENCING

During DNA sequence analysis, four separate enzymatic reactions are performed, one for each nucleotide. Each reaction contains DNA polymerase, the single-stranded DNA template to be sequenced and to which the synthetic DNA primer has been hybridized, the four deoxyribonucleotide triphosphates (dATP, dGTP, dCTP, dTTP), 32P labeled or fluorescent nucleotide(s) are added for the detection of growing DNA fragments (Figure 4), and the appropriate buffer for in vitro DNA synthesis.

In sequencing reactions, the “G” reaction contains dideoxyGTP, the “C” reaction dideoxyCTP, the “A” reaction dideoxyATP, and the “T” reaction dideoxyTTP. The dideoxynucleotide concentrations are carefully adjusted so that they are incorporated into a growing DNA strand randomly and infrequently. Once a dideoxynucleotide is incorporated into the growing DNA strand, DNA synthesis is terminated. The site of the dideoxynucleotide incorporation allows one to determine the position of that base. The dideoxynucleotide lacks a 3’-OH group on the ribose ring and as a consequence, DNA synthesis is terminated because the DNA polymerase will not add another nucleotide to the growing strand since a 3’-OH group is absolutely required for DNA chain elongation.

Since a particular reaction will contain millions of growing DNA strands, a “nested set” of fragments is obtained with each fragment being terminated at a different position corresponding to the random incorporation of the dideoxynucleotide.

THE ROLE OF GENES IN CANCER

Figure 4 shows a “nested set” of fragments produced for a hypothetical DNA sequence. The “G” reaction contains the standard reaction mixture (dATP, dCTP, dGTP, dTTP, the DNA polymerase, the appropriate buffer for DNA synthesis, 32P labeled or fluorescent tagged nucleotide(s) and a small amount of dideoxyGTP (ddGTP). The ddGTP (dideoxyGTP) random and infrequent incorporation will produce a “nested set” of fragments which terminate with a ddGTP. The “nested set” is complimentary to the sequence. Similar “nested sets” are produced in the separate “A”, “T”, and “C” reactions. For example, the “A” “nested set” would terminate with a ddATP.

It should be apparent that together the “G, A, T, C” “nested sets” contain fragments ranging in size successively from 19 to 31 nucleotides for the hypothetical sequence in Figure 3. In the figure the “G” reaction contains fragments of 21, 23, 25, 29 and 31 nucleotides in length. The first eighteen nucleotides are contained in the synthetic DNA sequencing primer which are not shown. The rest are added during de novo synthesis by the DNA polymerase.

The reaction products from the G, A, T, and C reactions are separated by electrophoresis using a thin and long vertical polyacrylamide gel. DNA sequencing gels resolve fragments which differ in size by a single nucleotide. After electrophoretic separation is complete, the sequence is determined by either radioactivity or fluorescence. If radioactivity was used, autoradiography is performed. The polyacrylamide gel is placed into direct contact with a sheet of x-ray film. Since the DNA fragments are radioactively labeled with 32P, their position can be detected by a dark exposure band on the sheet of x-ray film. Recent advances have also made it possible to automate DNA sequencing and to avoid the use of isotopic 32P.

**Figure 4: Determining the sequence of a DNA fragment.**
Experiment Overview

EXPERIMENT OBJECTIVE

In this experiment, you will construct a family pedigree that is suspected to have the classic Li-Fraumeni syndrome. DNA samples which have been enzymatically digested will be separated by electrophoresis on an agarose gel as an independent diagnostic test. DNA sequencing x-rays simulating p53 hot spot sequences will also be examined and mutations will be identified.

LABORATORY SAFETY

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!

- Wear gloves and goggles while working in the laboratory.
- Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.
- Wear protective gloves when working with hot reagents like boiling water and melted agarose.
- DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
- Always wash hands thoroughly with soap and water after working 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.
Module I: Construction a Family Pedigree

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.

INFORMATION FOR DEVELOPING THE FAMILY PEDIGREE:

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 (age 40), 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 (Patrick’s sister), Jane, was diagnosed with childhood leukemia and subsequently died at age 2.
• Patrick’s two other brothers, Richard (age 28) and Curtis (age 30), are in good health and free of cancer.
• Valerie’s sister, Nancy (age 38), is free of cancer.
• Nancy’s son, Michael, was diagnosed at age 3 as having sarcoma. Recently, at age 18, he was diagnosed as having osteosarcoma.
• Nancy’s other son, John (age 16), and daughter, Jessica (age 8), are free of cancer.

Valerie has five children:
• Justin (age 16)
• Sheila (age 14)
• Robert (age 10)
• Angela (age 8)
• Anthony (age 6).

All the children show no signs of cancer at this time. Valerie has requested that DNA sequencing be done for each of her children (see Module III).
Overview of Module II

The familial pedigree in Module I 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 biopsy 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 this simulation experiment, 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 also the palindrome CAGCTG for the restriction enzyme. This 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 “control” DNA fragment banding pattern. The DNA obtained from blood lymphocytes 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 DNA samples with the control wild type and DNA markers will be separated by agarose gel electrophoresis, stained, and then analyzed.
Overview of Agarose Gel Electrophoresis and Staining

1. Prepare agarose gel and casting tray.

2. Remove end caps & comb. Place tray into the electrophoresis chamber. Add 1x buffer into the chamber to submerge the gel.

3. Using a variable micropipet, load each sample into consecutive wells.

4. Attach safety cover, connect leads to power source and conduct electrophoresis.

5. After electrophoresis, transfer gel for staining with InstaStain® Ethidium Bromide.

6. Visualize results on a U.V. Trans-illuminator. Gel pattern will vary depending upon the experiment.
Module II-A: Agarose Gel Electrophoresis

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. Carefully **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

**Table A**

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

**IMPORTANT:**
7 x 7 cm gels are recommended. Each gel can be shared by 1 student group. Place well-former template (comb) in the first set of notches.

If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at [www.edvotek.com](http://www.edvotek.com).

**Wear gloves and safety goggles**
Module II-A: Agarose Gel Electrophoresis, continued

8. PLACE gel (on the casting tray) into electrophoresis chamber. POUR 1X Diluted Chamber Buffer into the electrophoresis chamber (See Table B for recommended volumes). Completely SUBMERGE the gel.

9. LOAD 18-20 μl of each sample into the wells in consecutive order as indicated by Table 1.

10. PLACE safety cover. CHECK that the gel is properly oriented. Remember, the 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). For best results, the blue tracking dye should migrate at least 4 cm from the wells.

12. After electrophoresis is complete, REMOVE the gel and casting tray from the electrophoresis chamber and proceed to STAINING the agarose gel with InstaStain® Ethidium Bromide (page 16).

### Table 1: Gel Loading

<table>
<thead>
<tr>
<th>Lane</th>
<th>Tube A</th>
<th>DNA Standard Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tube B</td>
<td>Control DNA</td>
</tr>
<tr>
<td>2</td>
<td>Tube C</td>
<td>Patient Peripheral Blood DNA</td>
</tr>
<tr>
<td>3</td>
<td>Tube D</td>
<td>Patient Breast Tumor DNA</td>
</tr>
<tr>
<td>4</td>
<td>Tube E</td>
<td>Patient Normal Breast Tissue DNA</td>
</tr>
</tbody>
</table>

### Table B: 1x Electrophoresis Buffer (Chamber Buffer)

<table>
<thead>
<tr>
<th>EDVOTEK Model #</th>
<th>Total Volume Required</th>
<th>Dilution</th>
<th>Distilled Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>M6+ &amp; M12 (new)</td>
<td>300 ml</td>
<td>6 ml</td>
<td>294 ml</td>
</tr>
<tr>
<td>M12 (classic)</td>
<td>400 ml</td>
<td>8 ml</td>
<td>392 ml</td>
</tr>
<tr>
<td>M36</td>
<td>1000 ml</td>
<td>20 ml</td>
<td>980 ml</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Electrophoresis Model</th>
<th>M6+</th>
<th>M12 (new)</th>
<th>M12 (classic) &amp; M36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min. / Max. Volt.</td>
<td>Min. / Max.</td>
<td>Min. / Max.</td>
<td>Min. / Max.</td>
</tr>
<tr>
<td>150</td>
<td>15/20 min.</td>
<td>20/30 min.</td>
<td>25 / 35 min.</td>
</tr>
<tr>
<td>125</td>
<td>20/30 min.</td>
<td>30/35 min.</td>
<td>35 / 45 min.</td>
</tr>
<tr>
<td>75</td>
<td>35 / 45 min.</td>
<td>55 / 70 min.</td>
<td>60 / 90 min.</td>
</tr>
</tbody>
</table>
Module II-B: Staining Agarose Gels with InstaStain® Ethidium Bromide

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. DO NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.

2. MOISTEN the gel with a few drops of electrophoresis buffer.

3. Wearing gloves, REMOVE and DISCARD the clear plastic protective sheet from the unprinted side of the InstaStain® card(s). PLACE the unprinted side of the InstaStain® Ethidium Bromide card(s) on the gel. You will need one card to stain a 7 x 7 cm 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® Ethidium Bromide card is in direct contact with the gel surface. STAIN the gel for 3-5 minutes.

6. REMOVE the InstaStain® Ethidium Bromide card(s). VISUALIZE the gel using a mid-range ultraviolet transilluminator (300 nm). DNA should appear as bright orange bands on a dark background.

BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!
Module III: Analysis of Autorads to Search for p53 Mutagens

In this part of the experiment, x-ray results of the wild p53 and samples from Valerie's five children will be read to determine whether or not there are mutations.

<table>
<thead>
<tr>
<th>Valerie's children</th>
<th>Ages</th>
<th>Autorad #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Justin</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Sheila</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Robert</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Angela</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Anthony</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

1. For each of Valerie's children, obtain the appropriate sample autoradiograph and place it on a light box to enhance visualization.

2. The sequencing reactions have all been loaded in order: G-A-T-C.

3. Begin analysis of the DNA sequence at the bottom of the autoradiograph with the circled band, which is an A.

4. Compare the deduced sequence to the wild type sequence shown in the box below.

5. Identify the location of the potential mutant nucleotide. What was the mutation? Is there more than one mutation?

**Wild Type Sequence:**

5’-AGCTTGCTGAGGATCCGAGAACTCTGAT-3’

6. Based on the information obtained from the x-rays, which of Valerie's children have a mutation in their DNA sequence?

**NOTE:** This is a simulation and the DNA sequence is not that of p53. The principles of reading DNA sequences and finding the point mutation is the same.
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:

<table>
<thead>
<tr>
<th>Preparation for:</th>
<th>What to do:</th>
<th>When?</th>
<th>Time Required:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Module II-A:</strong></td>
<td>Prepare QuickStrips™</td>
<td>Up to one day</td>
<td>45 min.</td>
</tr>
<tr>
<td>Agarose Gel</td>
<td>Prepare diluted</td>
<td>before performing</td>
<td></td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>electrophoresis buffer</td>
<td>the experiment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prepare molten agarose</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>and pour gels</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Module II-B:</strong></td>
<td>Prepare staining</td>
<td>The class period</td>
<td>10 min.</td>
</tr>
<tr>
<td>Staining Agarose</td>
<td>components</td>
<td>or overnight after</td>
<td></td>
</tr>
<tr>
<td>Gels</td>
<td></td>
<td>the class period</td>
<td></td>
</tr>
</tbody>
</table>

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Pre-Lab Preparations for Module II-A

AGAROSE GEL ELECTROPHORESIS

Preparation of Agarose Gels

This experiment requires one 0.8% agarose gel per student group. A 7 x 7 cm gel is recommended. 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:
Individual gels can also be prepared prior to conducting the experiment. See Module II 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 quantity. 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 (4° C) for up to two 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.

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.

18-20 μl of the DNA sample will be loaded into each well.
Pre-Lab Preparations for Module II-B

STAINING AGAROSE GELS WITH INSTASTAIN® ETHIDIUM BROMIDE

InstaStain® Ethidium Bromide provides the sensitivity of ethidium bromide while minimizing the volume of liquid waste generated by staining and destaining a gel. An agarose gel stained with InstaStain® Ethidium Bromide is ready for visualization in as little as 3 minutes! Each InstaStain® card will stain 49 cm² of gel (7 x 7 cm).

Use a mid-range transilluminator (Cat. #558) to visualize gels stained with InstaStain® Ethidium Bromide. **BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!**

- The EdvoQuick™ DNA Ladder should be visible after staining even if other DNA samples are faint or absent. If bands appear faint, repeat staining with a fresh InstaStain® card for an additional 3-5 min. If the ladder is not visible, troubleshoot for problems with electrophoretic separation.
- Ethidium bromide is a listed mutagen. Wear gloves and protective eyewear when using this product. UV protective eyewear is required for visualization with a UV transilluminator.
- InstaStain® Ethidium Bromide cards and stained gels should be discarded using institutional guidelines for solid chemical waste.

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.
Experiment Results and Analysis

MODULE I - PEDIGREE

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).

MODULE II - AGAROSE GEL ELECTROPHORESIS

<table>
<thead>
<tr>
<th>Lane</th>
<th>Tube</th>
<th>Sample</th>
<th>Molecular Weights (in bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>DNA Standard Maker</td>
<td>-------------------------</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>Control DNA</td>
<td>4282</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>Patient Peripheral Blood DNA</td>
<td>4282, 3000, 1282</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>Patient Tumor DNA</td>
<td>3000, 1282</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>Patient Breast Normal DNA</td>
<td>4282, 3000, 1282</td>
</tr>
</tbody>
</table>

MODULE III - ANALYSIS OF AUTORADS

Sheila (#2), Angela (#4), and Anthony (#5) have a normal DNA sequence for p53. Justin (#1) and Robert (#3) have a mutation in their DNA sequence, thus increasing their chance for developing a cancer at some point during their lifetimes.
Please refer to the kit insert for the Answers to Study Questions
Appendices

A. EDVOTEK® Troubleshooting Guide
B. Bulk Preparation of Agarose Gels

Safety Data Sheets:
Now available for your convenient download on www.edvotek.com/safety-data-sheets
### Appendix A

**EDVOTEK® Troubleshooting Guides**

<table>
<thead>
<tr>
<th>PROBLEM:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bands are not visible on the gel.</td>
</tr>
<tr>
<td>After staining the gel, the DNA bands are faint.</td>
</tr>
<tr>
<td>DNA bands were not resolved.</td>
</tr>
<tr>
<td>There is no separation between DNA bands, even though the tracking dye ran the appropriate distance.</td>
</tr>
<tr>
<td>There’s not enough sample in my QuickStrip™.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CAUSE:</th>
</tr>
</thead>
<tbody>
<tr>
<td>The gel was not prepared properly.</td>
</tr>
<tr>
<td>The gel was not stained properly.</td>
</tr>
<tr>
<td>Malfunctioning electrophoresis unit or power source.</td>
</tr>
<tr>
<td>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.</td>
</tr>
<tr>
<td>The QuickStrip™ has dried out.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ANSWER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ensure that the electrophoresis buffer was correctly diluted.</td>
</tr>
<tr>
<td>Repeat staining.</td>
</tr>
<tr>
<td>Contact the manufacturer of the electrophoresis unit or power source.</td>
</tr>
<tr>
<td>Repeat staining protocol.</td>
</tr>
<tr>
<td>Destain the gel for 5-10 minutes in distilled water.</td>
</tr>
<tr>
<td>Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 V).</td>
</tr>
<tr>
<td>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.</td>
</tr>
<tr>
<td>Add 40 µL water, gently pipet up and down to mix before loading.</td>
</tr>
</tbody>
</table>
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%)  

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 Module II-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.