 

**HIGH SCHOOL**

**Biotechnology**

**Lesson 5: Electrophoresis Discovery Cancer Gene Detection**

***The testing of Gena’s child Elizabeth***

**Pre-requisite knowledge:** Cell structure/function, DNA and RNA structure, protein synthesis, basic understanding of genetics and inheritance, principles of electrophoresis for detecting DNA fragments.

**Objective:** Students will…

* Perform a biotechnology lab using electrophoresis to sort out fragments of DNA to determine the presence of a mutated gene.
* Learn how the p53 gene can influence whether a person gets cancer.
* Compare the results of the experiment as a scenario of testing Gena’s child Elizabeth Jozwik.

**Materials – one kit will be sufficient for 6 groups of students)**

* Edvotek Cancer Gene Detection Kit #115 (www.edvotek.com)
* Horizontal gel electrophoresis apparatus
* D.C. power supply
* Automatic micropipettes with tips
* Balance
* Microwave or hot plate, hot gloves
* 250 ml flasks or beakers
* safety goggles and disposable laboratory gloves
* small plastic trays or large weigh boats (for gel de-staining)
* DNA visualization system (light box or white light)
* Distilled or de-ionized water

**Time needed:**

* 30 minutes prep time to prepare the gels – Day 1
* 60 minute class period to load the DNA samples and run the electrophoresis. – Day 2
* 3 hour or overnight staining and de-staining
* 15 -20 minutes to observe and discuss the results – Day 3

**National Standards met:** S1, S5, S6, S7

**Lesson Overview:**

Day 1

* Tell the students that today they will be using a current biotechnology procedure that can help to identify the possible presence of a mutated p53 gene. Since this gene is a tumor suppressing gene it can influence whether a person will acquire cancer.
* Gena’s daughter Elizabeth Jozwik, has decided to be tested since she is pregnant and is concerned over her future health as a mother.
* Students will pour the electrophoresis gels to be used during the next class period. Students must read the direction sheet that describes this activity.
* Have students read the procedure for the next day while the gels are hardening so they will be prepared for the next lab session.
* Gels, on their plastic supports, should be placed into zip-lock bags with a small amount of buffer and placed in the refrigerator until the next class. The zip-lock bags can be labeled for each group of students.

Day 2

* Place the gels into the electrophoresis chambers and cover them with buffer.
* Load the gels with the DNA samples, run the electrophoresis
* During the electrophoresis, hand out the student sheets on the Over view of Cancer. The students can read this sheet.
* Discuss the information about cancer. A homework assignment may be given to research the many types of cancer therapies or to answer the questions.
* Stain the gels with methylene blue and decolorize overnight.

Day 3

* Observe the gels and determine the outcome of the experiment.
* Discuss the options for Elizabeth based on her diagnostic results.

**Procedure:**

*Teacher should read the Edvotek kit #15 manual and also can refer to the* [*www.edvotek.com*](http://www.edvotek.com) *website for additional information.*

Make copies of page 12 in the Edvotek manual to give out to students so they may view a flow chart of the experiment.

**Day 1 – Agarose gel preparation**

Recommended gel size is 7 x 7 cm or 7 x 14 cm

5 sample wells will be needed, put the well forming template in first set of notches, near the negative side of the gel (black line) as sample will migrate toward the positive (red) side.

Dilute the concentrated buffer to 1X with distilled water. This is used to make the agarose gels and to put in the chambers for electrophoresis.

1. Using masking tape, close off the open ends of a clean and dry casting tray by carefully wrapping the tape around the whole tray so that no liquid can leak out. (p 16 in manual)

2. Place a well-forming comb in the first set of notches at the end of the bed. Make sure the comb sits firmly and evenly across the bed.

3. Prepare the 0.8% agarose gel with directions given on pp 17-19 of manual.

The amount to be made will be determined by your tray size and electrophoresis equipment.

4. After the gels harden, each student group should label a zip-lock bag with their names and insert the gel with the tray and comb. Pour a small amount of 1X buffer into the bag and zip tightly. Place in the refrigerator until the next class period.

**Day 2 – Run the electrophoresis**

Before the next class period, remove the gels from the refrigerator so they can warm up to room temperature.

1. Remove the gels from the zip-lock bags and place the tray into the electrophoresis chamber (be sure to line up the black and red electrode sides correctly. Cover the gel with 1X buffer until the gel is completely covered.

2. Load the 5 samples into lanes 1-5 with tubes A-E. Use 35 micro liters of each sample. Tell students that the patient is Elizabeth Jozwik.

Sample A contains standard DNA fragments

Sample B is a control DNA from tissue that is known to be normal Sample C is Elizabeth’s peripheral blood DNA

Sample D is a patient’s tumor DNA

Sample E is DNA from Elizabeth’s breast tissue

3. Following the directions on pages 20-21, run the electrophoresis.

4. Gel staining and de-staining is described on pages 21-25.

**Day 3 – read and discuss the results (Instructor’s Guide, pp 27-34)**

Students should drain off the de-ionized water that was used to decolorize their gels. Then observe the results on a white light box.

Discuss the results with the class. You may use the following study questions to aid in your discussion, or students may do them for homework.

**Study Questions**

1. Differentiate between the following terms: cancer, benign tumor, metastasis.

2. What is the difference between tumor suppressor genes and oncogenes?

3. What is the difference between a germ line mutation and a somatic mutation? With which type should Elizabeth be concerned?

4. What are the effects of hot spots in p53 protein structure (for advanced students who might like to research this questions)

5. Why do lanes 3 and 5 show both bands that are seen in lanes 2 and 4?

6. What is the purpose of the control lane?

7. Can a physician proceed with diagnosis based on this molecular biology data?

**Teacher Answer Sheet**

*(questions 2,4,6 and 7 are answered on page 34 of Edvotek manual)*

1. Cancer is uncontrolled cell growth where the cells divide at an abnormally accelerated rate. A benign tumor is one that stays within the mass and is operable by surgery. Metastasis is a process where the cancer cells invade and destroy other tissues in the body.

2. Tumor suppressors, such as p53, are normal cellular proteins that are involved in limiting growth of cells. If a person has a mutation in one of the alleles, he or she is susceptible to unrestricted cell growth (tumors) if the second allele is subsequently damaged by mutation. Oncogenes are involved in promoting cell growth.

3. A germ line mutation is one which is directly inherited and can be followed by studying a family pedigree. A somatic mutation does not have genetic links and is acquired during the lifetime of a person.

Elizabeth must be concerned with both types of mutations. If she inherits one mutated allele from her family then she must be concerned if her other normal allele becomes mutated from exposure to a mutagen during her lifetime. According to the “Two-hit” hypothesis, a normal suppressor gene will need two mutations to promote.

4. Hot spots are sites of amino acid residues in a protein structure that is often the target of mutations. In, p53 some of these mutations result in conformational changes which in turn can result in stability of the mutant protein. In addition, some of the p53 mutants aggregate with the normal p53 protein, resulting in inactivation.

5. Lanes 3 and 5 show both bands seen in lanes 2 and 4 because Elizabeth has one normal allele (lane 2) and one mutated allele (lane 4)

This should initiate class discussion on germ line mutation and if the mutated allele could be from that inheritance. Also, Elizabeth may need to be careful and protect herself from carcinogens since she only has one non-mutated allele for the p53 gene.

6. In all experiments, especially biomedical analysis, controls are essential to ensure that the results obtained are not due to artifacts or aberrations of the reaction and to be sure the method is giving accurate results.

7. A physician makes medical diagnoses based on a variety of independent sources of information. In this case, the family pedigree, data obtained from DNA analysis and DNA sequencing can be used to help in the diagnosis. At this time, most molecular biology, DNA-based diagnostic tests are not approved by the FDA and are therefore used as supplemental data by physicians.

**Overview of Cancer**

Cancer is an uncontrolled division of cells where the cells invade and destroy healthy tissue and may also spread to other tissues in the body, a process called metastasis. It is considered to be the result of a problem with gene expression where both an oncogene (controls the rate of the cell cycle, therefore promoting cell growth) and a tumor suppressing gene such as p53, (produces normal cellular proteins that limit cell growth) are mutated. These mutations are determined by family history (germ line mutations are inherited through families), and numbers of exposures and lengths of exposure to certain carcinogens (causing somatic mutations that do not have genetic links and are acquired during the life of the person.) Carcinogens are cancer causing agents such as chemicals, x-rays, UV light, tobacco, radiation, and certain viruses.

Diagnosis may be done by radiographic imaging and by a pathologist examining tissue samples from tumors. During this histological examination the abnormal cells are graded, 1-3, (describing the degree of resemblance of the tumor to the surrounding benign tissue) and through a process called staging, 1-4, (describes the degree of invasion of the body by the tumor). There are blood tests for some forms of cancer and DNA testing may be done to look for mutations in particular genes.

Cancer treatments will involve some or a combination of the following procedures: surgery, chemotherapy, radiotherapy, angiogenesis inhibitors, biological therapy, the use of specific antibodies, bone marrow transplants and gene therapy.

Healthy people should undergo screening techniques on a regular basis to detect any tumors before they become apparent. A mammogram, colonoscopy, complete blood count, and PSA (prostate specific antigen) are examples of some screening tests. Some cancers can now be prevented with the use of vaccines.

The p53 gene, in recent years, has become involved in many cancer biology studies. This gene is located on the short arm of chromosome 17. It produces a protein that normally functions as a cell regulator by binding to DNA and regulating transcription. When mutated, the p53 protein cannot bind to DNA and therefore promotes uncontrolled cell growth, functioning as an oncogene. Both alleles of this gene must be altered to cause cancer.

**Experimental Results and Analysis**

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Lane Tube

1 A Standard DNA Fragments

2 B Control DNA

3 C Patient Peripheral Blood DNA

4 D Control Tumor DNA

5 E Patient Breast Normal DNA

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**Explanation of Gel Result:**

Sample B is DNA from tissue culture that is known to be normal (i.e. has no mutation). After PCR and amplification of the segment containing the p53 gene, the DNA is cut with a restriction enzyme. Since the control DNA has no mutation, it does not contain the site for the enzyme and therefore does not cut. Elizabeth, on the other hand has one normal p53 allele and one mutated p53 allele and you see a representation of both patterns in lane #’s 3 and 5.

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