

Lab: DNA Fingerprinting-Cancer Gene Detection

Objectives:

1. To demonstrate the skills necessary to do molecule separation using gel electrophoresis.
2. To apply what they have learned to a cancer scenario.
3. To distinguish between healthy and mutated DNA when separated by gel electrophoresis.
4. To use evidence to conclude if a sample of DNA contains a mutated, cancer causing gene.

Background:

Li-Fraumeni syndrome is a rare cancer-causing disease that affects young family members and results in high mortality rates. It is suspected when at least two immediate relatives develop various cancers before the age of 45. A first step in the search and assignment of Li-Fraumeni syndrome is to establish the family pedigree of the patient. We will be looking at a young woman who is suspected to have the Li-Fraumeni syndrome.

The Human Genome Project has provided information to link the identification of many types of cancers and other diseases to DNA sequence information. (Edvotek) Cancer has been found to be linked to mutations in a tumor suppressor genes such as one called p53. These genes usually keep cells from dividing too rapidly and forming cancer. When they are mutated, they can no longer do this job and cell division goes out of control. This gene is located on chromosome 17. Each person has two of these genes, one from each of their parents. For this mutation to cause cancer, the gene on both of these chromosomes needs to be mutated.

The name of this syndrome comes from the two physicians who first recognized this as a syndrome (Li and Fraumeni). They recognized it after examining death certificates for 648 people who died of childhood cancers. The various cancers include sarcoma, brain and breast cancers and leukemia.

Scenario:

Upon monthly breast self-examination, Valerie Brown, age 36, found a small irregular mass in her breast. 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.

Here is some maternal family history:

- 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 cancer gene.

In the simulation experiment which follows, Valerie's DNA has already been digested with a restriction enzyme that recognizes the mutant sequence. 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.

5 Test Tubes:

1. **Standard DNA Marker Fragments ("DNA Ladder") = Orange Tube**
2. **Control DNA = Purple Tube**
3. **Patient Breast Tumor DNA = Yellow Tube**
 - a. The DNA analysis from the tumor tissue will show only the pattern for the tumor allele.
4. **Patient Peripheral Blood DNA = Blue Tube**
 - a. The DNA obtained from blood lymphocytes (peripheral blood) will give an altered band pattern representing one normal allele and the second which is the mutant.
5. **Patient Normal Breast Tissue DNA = Green Tube**
 - a. Digestion of the normal amplified DNA will give a characteristic DNA fragment banding pattern.

Prelab Questions:

Use the information in the background section to answer the following questions.

1. **When is Li-Fraumeni Syndrome suspected?**
2. **What is the job of a tumor suppressor gene?**
3. **What is the name of this gene?**
4. **Where did the name for the syndrome come from?**
5. **Create a pedigree for this family. You may have to research the rules for creating a pedigree. See 7.4 in the textbook for background information.**

Your task: You are about to perform a procedure known as DNA fingerprinting. The data obtained may allow you to determine if samples of DNA carry the gene for Li-Fraumeni syndrome which causes cancer.

Read the procedure very carefully and answer the embedded questions (answer them mentally and then once the gels are running you can write them down in your lab notebook using complete sentences).

- Choose roles:
 - 1 Director,
 - 2 Materials Managers (1 for the gel box, 1 for the samples),
 - 1 Data Collector

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PROCEDURE – Day 1

SAMPLE PREPARATION:

- Obtain five tubes of DNA. Do not open the vials containing your DNA samples, you could contaminate them!
 1. Write down the names of the 5 samples.
 2. Describe the samples of DNA (physical properties).
 3. Are there any observable differences in the samples of DNA?
- Verify that the caps are closed on all tubes. Then, pulse (2 seconds) in the micro- centrifuge to get all DNA solution to the bottom of the tube. Remember to balance the tubes!

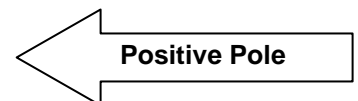
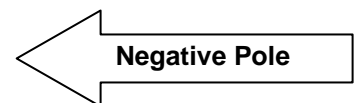
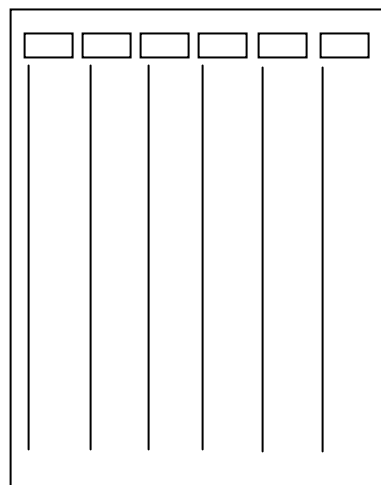
GEL BOX PREPARATION:

- Obtain the gel box with the agarose gel from your teacher.
 4. Describe the texture and color of the solid gel.
 5. The electrophoresis apparatus creates an electrical field (positive and negative ends of the gel). DNA molecules are negatively charged. **To which pole (+ or -) of the electrophoresis field would you expect your DNA to migrate?**
 6. **What color represents the negative pole? The positive pole?**
- Check the gel tray on the platform in the gel electrophoresis box. The wells should be at the (-) cathode end of the box, where the black lead is connected.
- Pour buffer in the gel electrophoresis box until it just covers the wells (125 ml).
- Gently remove the dams and the comb from the solidified gel. Take care that you do not tear the wells.
- Move the gel box next to the power supply box you will be using.

LOADING SAMPLES:

- With the P-20 pipette, load 15 μ L of each sample in separate wells. To prevent contamination, change pipette tips for each DNA sample you add to the well! Also, avoid touching the tip of the pipette with your hand.

7. Draw and fill in this diagram of the gel, labeling the wells with the corresponding source of DNA (In this order: Standard DNA fragments, Control DNA, Patient's Peripheral Blood DNA, Patient Breast Tumor DNA, Patient's Normal Breast Tissue DNA). You should have three wells that remain empty.



8. Why must care be taken to avoid touching or reusing the pipette tips?

RUN ELECTROPHORESIS:

- Secure the lid on the gel box. Connect the electrical leads to the power supply.
- When both you and your partner groups are ready, turn on the power supply, set the voltage to 200V and push run to begin the electrophoresis.
- Allow the DNA to migrate through the agarose gel for approximately 20-30 minutes. Watch your gel to ensure that the DNA is moving and does not move off the end of the gel!
- While your DNA samples are moving through the gel, consider the following questions:

Analysis Questions:

- 9. DNA samples were loaded in wells and were “forced” to move through the gel matrix. Which size fragment, small or large, would you expect to move toward the opposite end of the gel most quickly? Explain.
- 10. Which fragments are expected to travel the shortest distance? Explain.

- When it is time to stop, turn off the power and disconnect the leads from the power supply.

STAIN/DESTAIN

- Lift the casting tray out of the gel box and CAREFULLY slide your gel into a plastic staining tray.
- *****CAUTION***** FastBlast is a strong stain, Do NOT touch with your hands or get on clothing.
- CAREFULLY dispense 30mL (using the large micropipette) of concentrated FastBlast to the surface of the gel between your wells and the dark blue DNA “spots”.
- Let the gel sit in the stain for 3 minutes.
- Put the stain back in the bottle—it can be reused for the next class.
- GENTLY move gel to larger container
- Rinse your gel with 500 mL of warm tap water for 10 seconds. Pour the water down the drain, being careful not to disturb your gel.
- Repeat the previous step and agitate the large container for 5 minutes.
- Repeat the previous step and wait for 5 minutes.
- Put your gel into a zip-lock bag and seal. Mark with your name and period and put the baggie on the cart in the front of the classroom.
- Clean up for a lab stamp.

DATA ANALYSIS - Day 2

- Collect your gel. Do not attempt to take the gel out of the baggie as it will rip.
- Put your gel on a light table so that you can “read” the bands of DNA
- On an acetate sheet, make a record of your agarose gel. Place the acetate on top of the gel and with a permanent marker record the locations of the DNA bands and the wells. Be as precise and detailed as possible. **Attach your acetate to this lab sheet.**
- Completely clean up your lab station. Ensure that all materials are back in their designated locations. Your gel can be disposed of in the trash.

11. Draw the following chart in your lab notebook and fill it in.

DNA Source	Number of DNA bands	Distance Migrated by <u>each</u> fragment (mm)

12. Which DNA sample(s) has the smallest DNA fragment? How do you know this?

13. Based on the above, analysis, does the patient carry the gene for Li-Fraumeni disease? Use evidence to explain how you know this.