Lab: DNA Fingerprinting to

Locate Inherited cancer genes

**Background:**

Li-Fraumeni syndrome is a rare cancer disease that affects young family members and results in high mortality rates. 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.

Upon a monthly breast self-examination, Valerie Brown, age 36, found a small irregular mass in her breast. She was concerned because she knew that hermother 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, Valeries 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. 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.

You will be loading 5 samples. The table below shows what is in each tube.

|  |  |  |  |
| --- | --- | --- | --- |
| **Lane** | **Tube Color** | **Tube Contents** | **Meaning** |
| 1 | Orange | Standard DNA Fragments | Shows length of DNA bands |
| 2 | Purple | Control DNA | DNA from tissue culture that is known to be normal. Not from actual patient. |
| 3 | Blue | Patient Peripheral Blood DNA | To show if cancer has spread or mutation has occurred/spread. |
| 4 | Yellow | Patient Tumor DNA | DNA taken from the small mass in Valerie’s breast. |
| 5 | Green | Patient Breast Normal DNA | Normal tissue from actual patient. Used to show comparison with tumor DNA.  |

**Materials**:

* Five tubes of DNA samples (see table above)
* Microcentrifuge
* Agarose gel
* Gel electrophoresis chamber
* 1X TAE buffer solution
* Power source
* 2-20µL micropipette
* Small micropipette tips
* Beaker for used tips
* Quik View stain
* Zip-lock bag
* square of acetate sheet

**Procedures**:

1. Obtain five tubes of DNA. Do not open the vials containing the DNA samples as they can become contaminated.
2. Record observations of DNA on student data sheet.
3. 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.
4. Obtain an agarose gel from your teacher. (handle all gels with gloves on)
5. Place the gel tray on the platform in the gel electrophoresis chamber. Remember, the wells should be closest to the (-) cathode end of the box, where the black lead is connected.
6. Pour 1X TAE buffer in the chamber until it just covers the wells.
7. Move the electrophoresis chamber next to the power supply box that you will be using.
8. With the 2-20µL pipette, load 15µL of each sample in separate wells. To prevent contamination, change pipette tips for each DNA sample that is added to the well.
9. **On the student data sheet, fill in the diagram of the gel. Label the wells with the corresponding source of DNA (one well will remain empty).**
10. Secure the lid on the gel box. Connect the electrical leads to the power supply.
11. When all groups are ready, turn on the power supply, set the voltage to 200V and push run to begin the electrophoresis.
12. Allow the DNA to migrate through the agarose gel for approximately 20-30 minutes. Watch the gel to ensure that the DNA is moving and does not move off the end of the gel.
13. While the DNA samples are migrating through the gel, **answer the analysis questions on the student data sheet.**
14. When it is time to stop, turn off the power and disconnect the leads from the power supply.
15. Life the casting tray out of the gel box and CAREFULLY slide your gel into a plastic staining tray.

**\*\*\* CAUTION\*\*\* Ward’s QuikView is a strong stain. DO NOT TOUCH with your bare hands or get on clothing.**

1. CAREFULLY dispense about 500µL of concentrated QuikView to the surface of the gel between your well and the dark blue DNA “spots”.
2. Let the gel sit in the stain until there is about 10 minutes left of class.
3. Using the large micropipette put the stain back in the bottle- it can be reused for the next class
4. Rinse your gel with distilled water and then 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.
5. Clean up your lab station to be dismissed.

**DAY 2**

1. Collect your gel. Do not attempt to take the gel out of the baggie as it will rip
2. Put your gel on a light table so that you can “read” the bands of DNA
3. On an acetate sheet, make a record of your agarose gel. To do this, 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.
4. 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.
5. Complete the rest of your analysis questions.

Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Period: \_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**DNA Fingerprint lab: Student Data Sheet**

**Pre-lab questions:**

1. A) What is the overall charge of DNA?

B) To which pole (end of DNA chamber) will DNA travel to?

C) Towards which side of the chamber (+ or -) should the wells be closest to?

**Data:**

1. Describe the samples of DNA (physical properties).
2. Are there any observable differences in the samples of DNA?

**Diagrams**:

Cathode (-)

Cathode (-)

Start time:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

End Time:

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Anode (+)

Anode (+)

|  |  |  |
| --- | --- | --- |
| **DNA Source** | **Number of DNA bands** | **Distance Migrated by each fragment (mm)** |
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**Analysis Questions: (Day 1)**

1. DNA samples were loaded in wells and “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.
2. Which fragments are expected to travel the shortest distance? Explain.

**Analysis Questions: (Day 2)**

1. Based on the above analysis, does the patient carry the gene for Li-Fraumeni disease? How do you know this?

(Answer on separate sheet of paper and staple to this handout)