BRCA Chronicles: Breast Cancer Genetics MiniLab

Student Guide

Cat# M3054

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A special thank you to Crystal McDowell for her contribution that made this MiniLab possible.

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Laboratory Safety

1. Wear lab coats, gloves, and eye protection as required by district protocol.
2. Use caution with all electrical equipment such as PCR machines and electrophoresis units.
3. Heating and pouring molten agarose is a splash hazard. Use caution when handling hot liquids. Wear eye protection and gloves to prevent burns.
4. Wash your hands thoroughly after handling biological materials and chemicals.

Introduction - Part I: Something to Think About

"Whew!" Hannah sighed in relief as she slid into her seat just as the bell rang for her seventh period genetics class. One more tardy and she would get detention for sure.

Mrs. Davis immediately welcomed the class and asked everyone to take a moment to consider the question she had displayed on the board.

Hannah read the question to herself, "Should someone with a family history of a genetic disorder seek genetic testing for the condition?" Hannah immediately felt a little uneasy.

"Okay, everyone. We have been discussing bioethics. Mark, please describe what we mean by that term," Mrs. Davis asked.

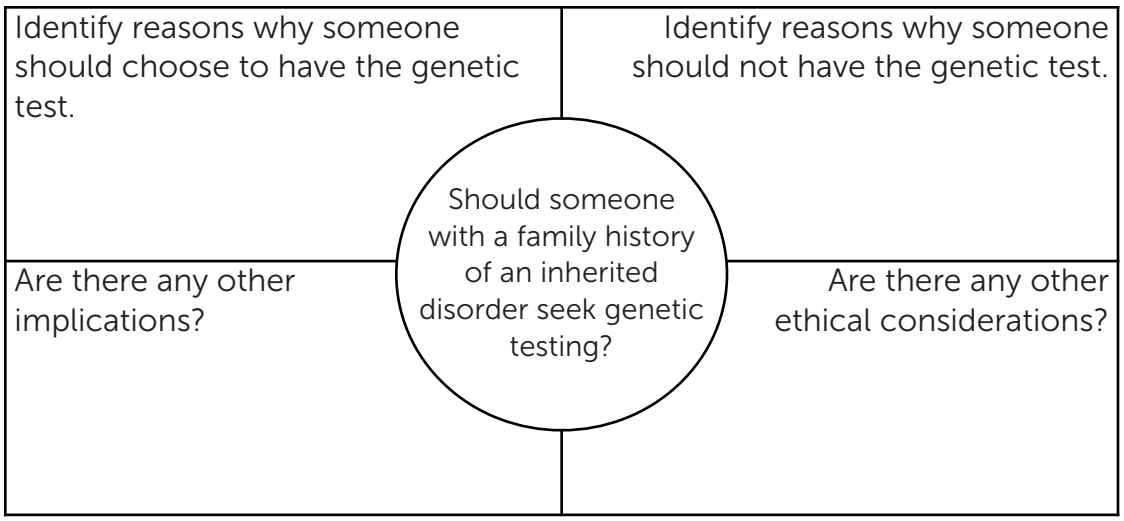
"It is kind of like what we should or should not do or what might be right or wrong but related to biology," Mark said. We had completed an exercise the day before identifying the difference between bioethical questions and scenarios versus other types of questions and situations, so Mark pretty much summed up what the rest of us took away from that activity.

"That is a good way to think about it and if you remember from yesterday, many of our ethical questions were asking, 'Should we do something.' We have to make decisions regarding biological issues based on an analysis of the effects or impacts. So, before we start that thinking process, let's see where everyone stands right now. I have a piece of tape on the floor. This end of the tape is a 'Yes' for choosing to have the genetic test and that end of the tape is a 'No.' There are 10 separate marks on the tape. Go stand at the mark that represents what you would choose to do if the first mark is a definite yes and the last mark is a definite no," Mrs. David instructed the class.

Hannah did not know what to do. This question was definitely affecting her on a personal level. Her older sister, Rachel, had just been diagnosed with breast cancer at the age of 30. Her mom, Deborah, was diagnosed with breast cancer when she was 42, only five years after Hannah had been born. Her other sister, Sarah, is 25 and does not have breast cancer thus far. They had all watched what their mom had gone through during her treatments. Knowing that Rachel is now facing the same battle was definitely taking its toll and what about Sarah and herself? Hannah went to stand at number 5. While she kind of wanted to know if she could develop cancer, she was also only 18 and she did not know if she wanted to constantly be wondering about when it might develop.

"Okay, class. Now that we see where we initially stand, let's think about the ethical considerations of genetic testing. Take a few moments and complete the graphic organizer and then we will share some of the advantages and disadvantages of genetic testing. Feel free to use your device to obtain information about both perspectives, the impact of choosing to get tested and the impact of electing not to get tested," Mrs. Davis instructed.

Critical Analysis: Imagine you are in Hannah's class. **Obtain and evaluate information** or think of your own reasons for choosing whether or not to undergo genetic testing.



Mrs. Davis gave her students some time to think about the bioethical question individually, in small groups and then as a class. Hannah listened intently as students shared lots of reasons for each perspective. As the class was ending Mrs. Davis asked if anyone had any questions.

Hannah raised her hand. "Yes, Hannah," Mrs. Davis acknowledged.

"Mrs. Davis, isn't there a genetic test for breast cancer?" Hannah asked.

"Yes. There are a number of genetic tests and options for identifying different alleles that could increase a person's chances of developing breast cancer," Mrs. Davis replied just as the bell rang.

Hannah knew the answer before she asked it, but the class definitely had given her a lot to think about.

Part II: Finding Answers

"Good afternoon, Hannah, Sarah, Rachel and Mrs. Alterman. My name is Dr. Chaney. I am a genetic counselor. It looks like you are interested in learning more about genetic testing for breast cancer. Is that correct?"

"Yes," Mrs. Alterman replied. "I am a breast cancer survivor and, Rachel, has recently been diagnosed with breast cancer. Hannah has expressed interest in finding out if she is at risk, along with her sister, Sarah."

"Well, you are taking the right first step. When a family history has been determined, it is good to see a genetic counselor to learn more about what might be causing the cancer and to determine if family members are good candidates for genetic testing. The next step is for us to gather some more information about your family history and to conduct a physical examination. Let's continue to collect family history. I am going to make some notes and construct at least a three-generation pedigree to help us see any patterns of breast cancer in your family. We can then conduct physical exams on Sarah and Hannah just as a precautionary screening. Can I ask you a few questions?"

Everyone nodded in agreement.

Critical Analysis: Imagine you are Dr. Chaney and **evaluate** the responses to the following questions. **Use the information** to **construct a model pedigree** of the occurrence of breast cancer in the space provided on the next page.

Alterman Family History Notes

Question 1: Do you have any other family members who have been diagnosed with breast cancer? And if so, can you give me their age of onset or when they were diagnosed?

Answer 1: Mrs. Alterman replied, "Yes. My father, Nathanael Goldberg, was also diagnosed in his forties. No one in my mother's family was diagnosed. My husband's family has no history of breast cancer." Rachel noted that there is no history of breast cancer in her husband's family either. Rachel has a daughter who is five and a son who is three. Sarah and Hannah are not married.

Note: Deborah was diagnosed when she was 42. Rachel was recently diagnosed at the age of 30. Sarah is 25 and has not been diagnosed. Hannah is 18 and has not been diagnosed.

Question 2: Has anyone in your family ever been diagnosed with ovarian cancer?

Answer 2: No.

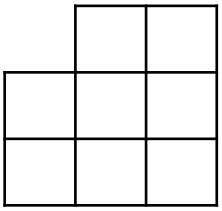
Question 3: Sometimes there are population-specific mutations known as **"founder"** mutations that tend to affect certain populations in higher frequency than others. Is there anything unique you can tell me about your ancestry?

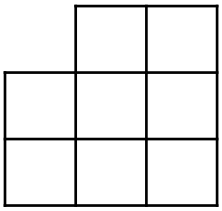
Answer 3: Our family is Jewish, specifically Ashkenazi or eastern European.

Based on this information, **develop a model of a pedigree** illustrating all four generations below.

Based on the pattern in the pedigree, do you think it is likely that breast cancer is inherited in this family? If yes, **predict** the mode of inheritance.

Given the family history, use a Punnett Square to **predict** the likelihood of Hannah or Sarah inheriting breast cancer. What is the likelihood that Rachel's children could have inherited breast cancer?





"The good news is that the physical examinations show no signs of breast cancer in Hannah or Sarah right now," Dr. Chaney explained. "However, your family history reveals a lot of information that we should discuss."

"When an individual cannot be identified clinically as having breast cancer but there is a family history of the condition, another option is genetic testing using genomic biomarkers. You may have heard of BRCA1 and BRCA2 which are tumor suppressor genes that normally help to repair damaged DNA and help to prevent cancer. The problem is when mutations occur in these genes and other genes that have been identified to be linked to breast cancer. BRCA1 and BRCA2 account for 13% of breast and ovarian cancer biomarkers. They are known as highly penetrant because they predict a high likelihood of disease when identified through genetic testing. Fifty percent of biomarkers are unexplained and there are many others that have been identified as well. It is important to also note that only 10% of breast cancers may be hereditary. There are lots of other factors that play a role as well. As a genetic counselor, I have to weigh all this information before determining whether family members are good candidates for biomarker testing.

In your family's case, it appears that all of you are candidates for BRCA2 biomarker testing.

Narrowing down to this particular biomarker is cheaper and quicker than DNA sequencing. Mutations in BRCA2 are more likely when both males and females are affected whereas identifying BRCA1 mutations tends to be used when families have incidents of both breast and ovarian cancer. We would be looking at a very specific region of the DNA on chromosome 13 rather than the entire genome.

That is a lot of information to process. Do you have any questions?" Dr. Chaney asked.

"Why did you ask about our ancestry?" Hannah inquired.

"Excellent question. Sometimes, throughout history groups of individuals from an initially larger population found communities that are smaller. Incidences of certain genes tend to increase in those smaller populations and then individuals pass those genes or mutations of those genes to others in the population. Two founder mutations in BRCA1 and one in BRCA 2 have been identified in the Ashkenazi Jew population. The penetrance or likelihood of disease tends to be higher in the BRCA1 founder mutations than those of the BRCA2. These mutations have been well-documented and it is believed that 3% of individuals in this population carry a founder mutation. It increases your family's likelihood of testing positive for a BRCA2 mutated allele." Dr. Chaney explained.

"Since Rachel and I have been diagnosed and may carry the allele, what does that mean for Sarah, Hannah and even Rachel's children?" Deborah asked.

"Mutations in BRCA1 and BRCA2 have been identified as Autosomal Dominant. They are autosomal because each gene is not found on a sex chromosome. BRCA1 is on chromosome 17 and BRCA2 is on chromosome 13. It is considered dominant, meaning you only need to have one of the mutated alleles to increase your chances of developing breast cancer. Individuals with only one of the mutated alleles for BRCA2 have an estimated 49% chance of developing breast cancer. Those at greatest risk have an 83% chance of breast cancer by age 80. There are many factors involved that determine the degree of risk. Unfortunately, your family has a number of those risk factors," Dr. Chaney continued to explain.

"Should we get tested?" Hannah asked.

"Ultimately, that decision is up to each of you. Your family history shows that you are candidates for BRCA2 testing. You meet a number of the criteria:

- You have a history of breast cancer in your family.
- Men in your family have had breast cancer; in this case, one.
- Your family is of Ashkenazi (Eastern European) Jewish descent.
- Usually, we say three or more women have had breast cancer, particularly those diagnosed prior to age 50 but you have two.

Since both your mom and one of your sisters have already been diagnosed, we can test them and Sarah and you. It is always better to have someone who is diagnosed tested because if they

test positive for the biomarker, the test is more valid because they have been confirmed to have the disease. Then we can compare the pattern in their DNA to yours and to Sarah's to see if you two have that marker as well. We do not advise testing children under the age of 18 because there are really no effective preventive measures in children so young and they are not old enough to provide informed consent. Each individual should decide how much information they want to know about their lifetime cancer risk. By the time they could develop cancer, new treatments may be available and there is no need to add that level of anxiety.

People who test positive have the benefit of exploring better preventative strategies, implementing strategies to decrease the risk, and exploring other targeting therapies. At the same time, a positive test can increase anxiety. A positive test means you are at higher risk for developing breast cancer. A negative test does not mean you will not develop breast cancer; it means the risk may be more similar to the general population.

I am going to provide you with some materials about the benefits and risks associated with biomarker testing. I want you all to read over the materials and make a list of questions that we can discuss at our next meeting. After we discuss all your questions, I will go over the informed consent document. We do not want you to agree to testing until you feel you understand all the benefits and risks."

Critical Analysis: After reading Dr. Chaney's explanation, answer the following questions.

1. Provide evidence for why the BRCA2 biomarker test is the best choice for the Alterman family. Why would Hannah's niece and nephew not be included in the test?

2. What is a founder mutation? Why does this type of mutation support that the Altermans are good candidates for biomarker testing?

3. The BRCA2 gene is 70,000 bases and includes 27 exons or coding regions. Most mutations in this gene result from premature truncations due to a nonsense or frameshift mutation. Explain what is meant when a gene is truncated. How could either a nonsense or frameshift mutation cause a gene to be truncated?

4. The BRCA2 founder mutation in Ashkenazi (eastern European) Jewish ancestry is noted as 6174delT. Describe how you think that mutation affected the original DNA sequence.

5. Based on the information provided, who do you think should be tested and why?

Part III: Deciding Yes to the Marker Test

As a Molecular Genetics Technologist, you will be performing the genetic marker test today for the Alterman family. All four women decided to be tested and signed an informed consent document. Blood samples were taken from each woman, DNA was extracted from the white blood cells, and the DNA was amplified using Polymerase Chain Reaction. The samples were then prepared using a restriction digest using the biomarker and a restriction enzyme to cut the DNA into different fragment sizes.

Below are the protocols used by this lab for running the DNA samples using gel electrophoresis. Since you are new to your position, follow the directions below exactly and listen to further instructions provided by your supervisor.

Part I: Electrophoresis

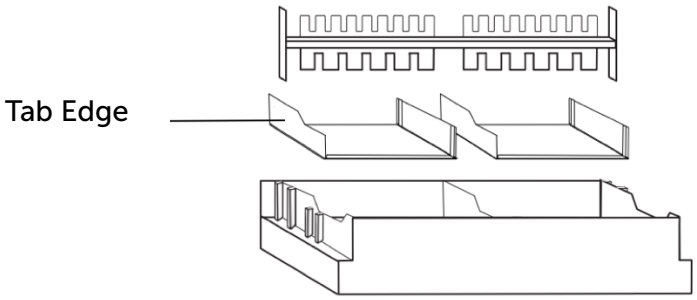
Materials

1 MiniOne® Casting System
1 MiniOne® Electrophoresis System
1 agarose GreenGel™ cup (1.5 %)
6 DNA sample aliquots
135 mL of running buffer
1 micropipette (2-20µL)
10 pipette tips

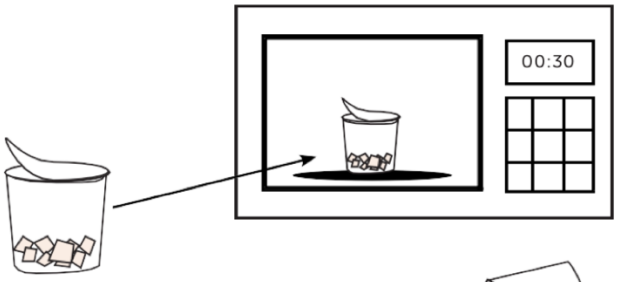
Lane #	Sample Name	Volume
1	MiniOne® marker (100, 300, 500, 1K, 2K bp) (M1M)	10 µL
2	Sarah Alterman (S)	10 µL
3	Rachel Epstein Alterman (R)	10 µL
4	Deborah Goldberg Alterman (D)	10 µL
5	Hannah Alterman (H)	10 µL
6	Negative Control (N)	10 µL

How to Cast a Gel

1. Place the MiniOne® Casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 6-well side facing down.

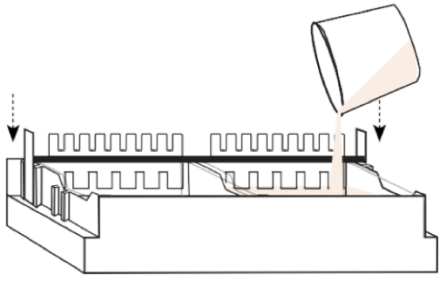


2. **Partially** peel the film off a GreenGel™ cup and microwave for 25-30 seconds. Allow to cool for 15 seconds. DO NOT microwave more than 5 gel cups at a time.



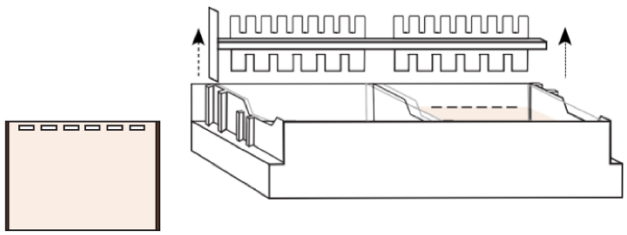
Safety requirement: Adult supervision required if students are handling gel cups!

3. One gel cup is for making one agarose gel! Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 minutes or until opaque.



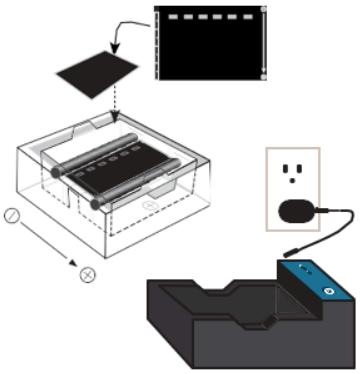
DO NOT disturb the gel until time is up.

4. Carefully remove comb when gel is ready. Remove gel tray with solidified gel from Casting Stand and wipe off any excess agarose from the bottom of the tray.



How to Load a Gel

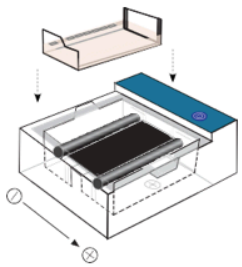
1. Ensure the black viewing platform is in the gel tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**



2. Plug the power supply into the wall and carefully insert the other end into the back of the MiniOne® Carriage.

3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.

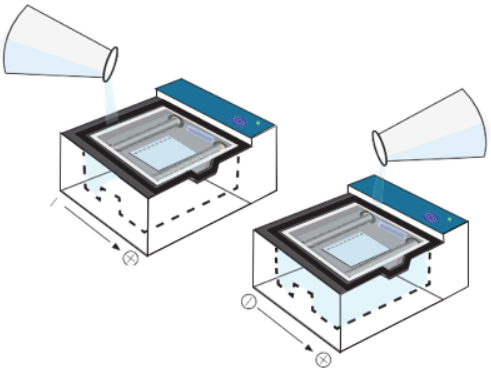
4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.



Note: Place gel tank with the gel on the gel tray into carriage **before** pouring buffer in.

5. Turn the low intensity blue LED on by pressing the button on the carriage.

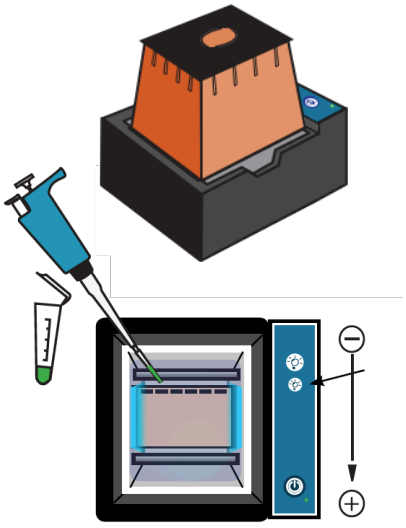
6. Measure 135 mL of TBE running buffer and pour into **one side** of the gel tank. Watch the air push out between the gel tray and viewing platform. Once air has been removed from under the gel tray, pour remaining buffer into the **other side of the gel tank.**



7. Place photo hood on the carriage.

8. Press the power button which should now be a solid green light. If **green light is solid,** turn off the unit and proceed to loading gels.

9. Turn the low intensity blue light on by pressing the button on the carriage to help visualize the wells when loading.



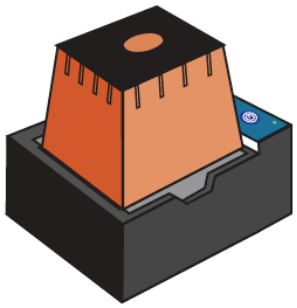
10. Load 10 µL per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**

Run, Visualize and Capture Image

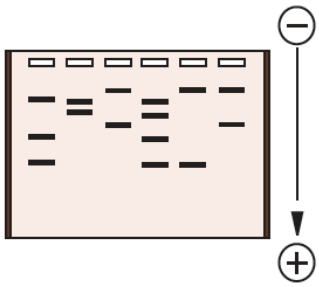
1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the button. The green LED next to the button will turn on.

   The green power LED will not turn on if:

   - **The tank is not properly placed inside the carriage.**
   - **There is no buffer in the tank.**
   - **The buffer is too diluted.**
   - **The photo hood is not on the carriage.**
   - **There is too little running buffer.**
   - **The power supply is not plugged in. Check by turning on the blue LEDs.**
   - **If the green power LED is blinking, please refer to the troubleshooting steps in the MiniOne® Electrophoresis Instruction Manual**



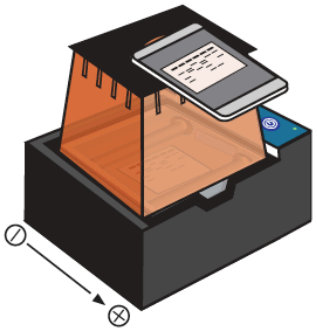
2. Have students periodically check the migration of the bands (~every five minutes). (Use the low intensity for viewing during the run. Light will weaken the fluorescent DNA signal.)



3. Allow the gel to run **25 - 30 minutes** or until DNA separation is sufficient. Keep in mind small DNA samples run faster so it's important to periodically check where your bands are. After your run is complete, turn off the power by pressing the button.

4. Document your results.

   Wipe off the condensation from the inside of the hood with a soft cloth if necessary, then place the hood back on the carriage. **Turn on** the high intensity light. Place your cell phone or camera directly on the photo hood to take a picture of the DNA. **DO NOT** zoom in as this will result in blurry pictures. (The photo hood is already at the optimal focal length for a smart device.)



5. Clean up. Follow teacher's instructions on disposal and clean up.

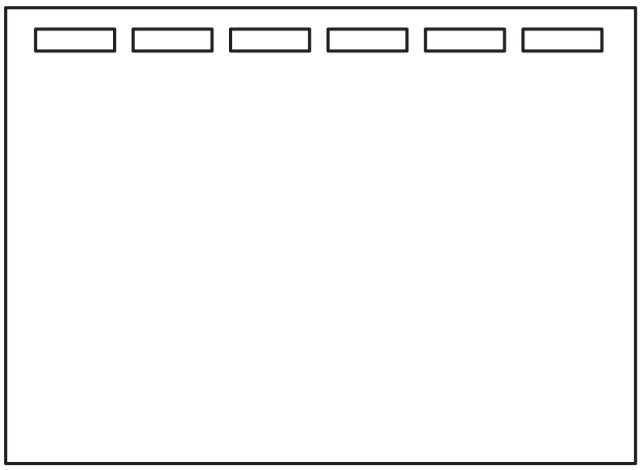
Clean Up

Note: All reagents in this lab can be disposed of as non-hazardous waste.

1. After collecting data and documenting results, remove the photo hood and unplug the power supply from the wall and from the back of the MiniOne® Carriage. Remove the clear running tank from the carriage and remove the gel and tray from the running tank.
2. Pour the used running buffer down the drain or into a waste beaker. Throw the gel away BUT SAVE THE GEL TRAYS. Rinse the clear plastic running tank, gel tray, comb, and casting system with DI or distilled water. Allow the tanks to fully air dry before storing.
3. Use a paper towel or Kimwipe™ to gently wipe the gold rivets in the carriage (where the electrodes connect) to ensure all moisture is removed. Wipe up any buffer that may have spilled into the black carriage. Follow any additional directions the instructor gives for clean up and storage.

Part II: Results

What does your gel look like? Record images of the gel in the gel below



Lane 1: _____
Lane 2: _____
Lane 3: _____
Lane 4: _____
Lane 5: _____
Lane 6: _____

Part III: Data Analysis

1. Using a ruler, measure the distance traveled from the bottom of the well to the bottom edge of each fragment band (Fragments 1 - 5, this is Distance A in Table 1. Then measure the distance from the bottom of the well to the bottom edge of the gel, this distance B (this is the same for all 5 fragments). Calculate the Rf value for each fragment by dividing the A and B values. Record your data in Table 1.

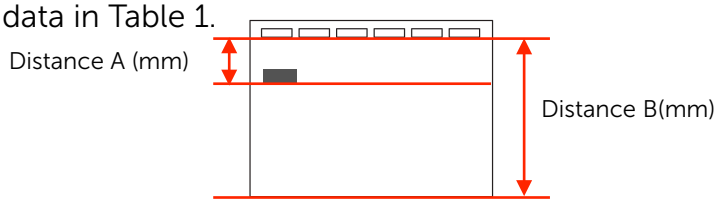


Table 1				
DNA Size Markers	Fragment length in base pairs (bp)	A = Distance Migrated (mm)	B = Distance to Reference Point (mm)	R_f (A/B)
Fragment 1	2000			
Fragment 2	1000			
Fragment 3	500			
Fragment 4	300			
Fragment 5	100			

2. Plot a standard curve (fragment size vs Rf) on Log-Y (semi-log) paper. Plot Rf values on the X-axis, and fragment size on the Y-axis.
3. Next, calculate the Rf for the patient samples by measuring the A and B values for each fragment, and dividing the two values, as you previously did in Step 1. Record your values in Table 2 below.

Table 2				
DNA Sample	Fragment	A = Distance Migrated (mm)	B = Distance to Reference Point (mm)	R_f (A/B)
Tube 2 - Sarah	Fragment 1			
	Fragment 2			
Tube 3 - Rachel	Fragment 1			
	Fragment 2			
Tube 4 - Deborah	Fragment 1			
	Fragment 2			
Tube 5 - Hannah	Fragment 1			
	Fragment 2			

4. Use your standard curve that you drew in Step 2 to determine the fragment lengths of each of your DNA samples. Record in Table 3.

Table 3			
DNA Sample	Fragment	Fragment length calculated from the graph (bp)	Allele Present
Tube 2 - Sarah Alterman	Fragment 1		
	Fragment 2		
Tube 3 - Rachel Alterman Epstein	Fragment 1		
	Fragment 2		
Tube 4 - Deborah Goldberg Alterman	Fragment 1		
	Fragment 2		
Tube 5 - Hannah Alterman	Fragment 1		
	Fragment 2		

5. Look up each fragment length calculated from the graph and find the number of corresponding alleles present from Table 4. For example, if you calculated that the fragment length is 800 bp, the number of alleles present would be 7. Record in Table 3.

Table 4	
Fragment Length in Base Pairs:	Allele:
200	Allele 1
300	Allele 2
400	Allele 3
500	Allele 4
600	Allele 5
700	Allele 6
800	Allele 7
1050	Allele 8

1. Based on the banding pattern results from the gel you ran and the information provided above, pretend you are Dr. Chaney. How would you explain the results to Mrs. Alterman and her daughters?

2. Based on your knowledge of genetics, which two alleles does Hannah's father possess?

Part V: Leaving a Legacy

After learning about her family's test results, Hannah became very interested in learning more about breast cancer research and particularly new methods for testing. Although she still did not know her own fate, she used the information to become informed about risk-reducing strategies and measures that were options for her, Sarah and her niece and nephew. She had always been interested in biology but her family's experience led her to declare her major in molecular biology as she headed off to college. She was determined to become a part of the solution as she aimed for a career in cancer research.

Analysis and Extension:

1. Why do you think Hannah's experience has motivated her to pursue a molecular biology degree and a possible career in cancer research?

2. Watch the short video clip below about Nancy Wexler and her experience both personally and professionally with Huntington's disease. **Obtain and evaluate additional information** about Dr. Wexler and her lifelong work and battle with Huntington's disease. Why do you think she considers this work and this disease her family's legacy?

(Huntington's Disease and Nancy Wexler - https://www.youtube.com/watch?v=Yf8gh4oDAXk)

3. Science should be objective, but that does not mean it is not sometimes personal. How do both Hannah's and Dr. Wexler's experiences show how one's personal experiences can be impacted by their understanding of science?

4. **Extension:** Research other forms of testing for breast cancer like Next Generation Sequencing and Liquid Biopsies. What innovative measures are being used to help with testing and treatments?

Appendix A - References

Ashkenazi Jewish Surnames

https://en.wikipedia.org/wiki/Category:Ashkenazi_surnames

Jewish Feminine Names

https://en.wikipedia.org/wiki/Category:Jewish_feminine_given_names

Jewish Given Names

https://en.wikipedia.org/wiki/Category:Jewish_given_names

CDC. Genetic testing for hereditary breast and ovarian cancer. https://www.cdc.gov/genomics/disease/breast_ovarian_cancer/testing.htm

Genetic testing. https://www.breastcancer.org/genetic-testing

Petrucelli N, Daly MB, Pal T. BRCA1- and BRCA2-Associated Hereditary Breast and Ovarian Cancer. 1998 Sep 4 [Updated 2022 May 26]. In: Adam MP, Mirzaa GM, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1247/

Walsh MF, Nathanson KL, Couch FJ, Offit K. Genomic Biomarkers for Breast Cancer Risk. Adv Exp Med Biol. 2016;882:1-32. doi: 10.1007/978-3-319-22909-6_1. PMID: 26987529; PMCID: PMC5016023.

Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5016023/

Appendix B - What is Gel Electrophoresis?

Gel electrophoresis is a technique used in many areas of science to analyze the components of complex chemical mixtures. Mixtures of DNA, RNA, proteins, or dyes can be separated into their individual components based on molecular size and electrical charge using a separation matrix within an electric field.

The gel used in gel electrophoresis is a tangle of polymers forming a three-dimensional matrix with water-filled pores through which molecules migrate. A higher density of polymers creates smaller pores. Like the holes in a sieve or colander, the size of the pores has to be the appropriate size for the molecules being separated. Gels can be made from different substances depending on the application. One of the most commonly used and effective materials is agarose, a polymer extracted from seaweed. Agarose gels are formed (or cast) by pouring molten (melted) agarose into a tray where it solidifies into the desired shape as it cools. A comb is placed while the agarose is molten and then removed after it solidifies to create wells where the samples are loaded.

After the gel solidifies it is placed in an electrically conductive buffer between parallel positive ((+) anode) and negative ((-) cathode) electrodes

A voltage is applied between the electrodes, creating a uniform electric field within the gel. Molecules in the wells begin to move under the influence of the electric field: positively charged molecules migrate toward the (-) cathode and negatively charged molecules migrate toward the (+) anode.

The speed of a molecule's movement in an electric field is determined by the strength of its electric charge relative to its molecular weight. This is quantified as the charge to mass ratio. Speed of movement within a gel is also influenced by the size of the molecule relative to the pores in the gel. The polymers in the gel are like an obstacle course: smaller molecules maneuver easily through the pores, traveling faster and farther than large, bulky molecules. However, a large molecule can move faster through a gel than a smaller molecule when the strength of its charge relative to its mass is significantly higher. Shape can also affect how a molecule moves through the gel. Long spaghetti-like molecules will move slower than compact molecules, which slip easily through the pores. Molecules of the same size, shape, and charge will move together and form a distinct band. If multiple types or sizes of molecules are present in the sample, they will separate from each other and each will form a distinct band.

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