Pre-Lab Assignment
Analyzing Genetic Mutations in Breast Cancer

DOCUMENTING FAMILY HISTORY USING A PEDIGREE CHART

Pedigree Charts

A **pedigree chart** is a diagram showing the genetic history of a family over several generations. Pedigrees are often constructed after a family member affected with a genetic disorder has been identified and are used to calculate the probability of future offspring inheriting the disorder.

Pedigrees use a standardized set of symbols: squares represent males and circles represent females. Relationships in a pedigree are shown as a series of lines. Parents are connected by a *horizontal* line and a *vertical* line leads to their offspring. The offspring are connected by a *horizontal* line and listed in birth order from left to right. If an individual is deceased then their symbol will be crossed by a line. Individuals are commonly designated as **affected** (or having the disorder) or **unaffected** (not having the disorder). An example of a family pedigree is shown in *Figure 1*, together with the conventions used to represent different individuals.

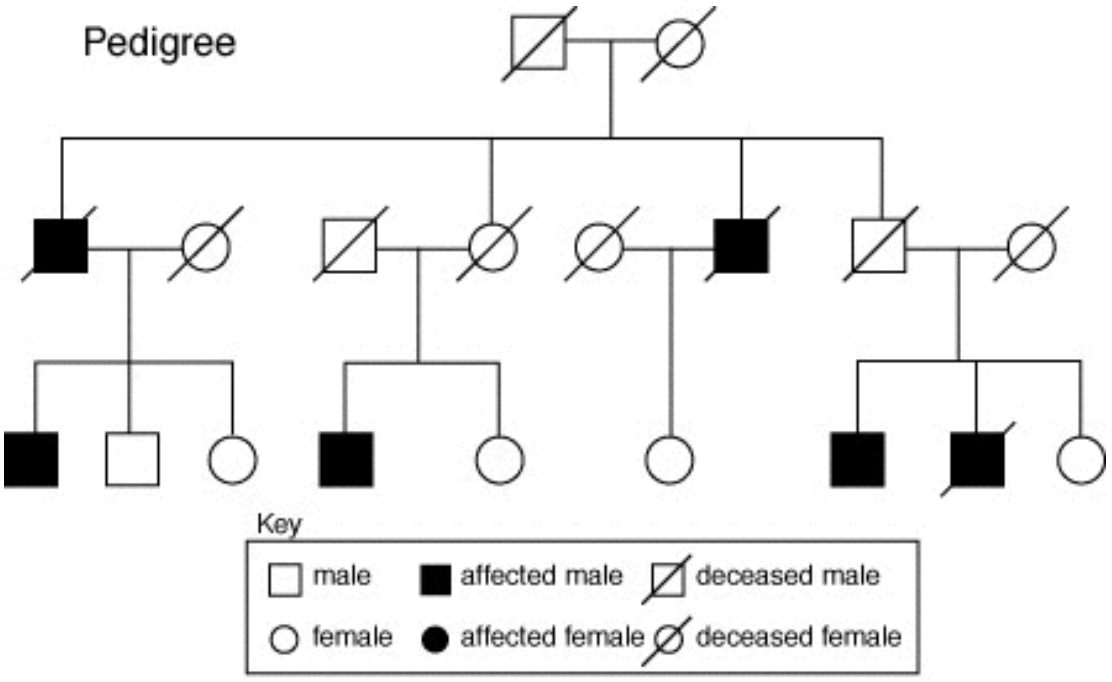

Figure 1: A family pedigree and the conventions used for family members.

You have been provided with a case study "A BRCA Genetic Testing Dilemma". This case study contains the information required to complete a family history for the Lawler family. Knowing extended family history helps to estimate the risks to individual members.

To complete this prelab assignment:

(1) Read the case study and use the "Family History Summary Table" provided to document the Lawler family members names, family relationship, sex, medical conditions and whether they are deceased or not.

(2) Construct a pedigree chart based off the information from the table. When drawing or studying a family pedigree, parents are connected by a horizontal line and a vertical line leads to their offspring. The offspring are connected by a horizontal line and listed in birth order from left to right. As a reminder, *Figure 2* depicts the general guidelines to the symbols used and their representations:

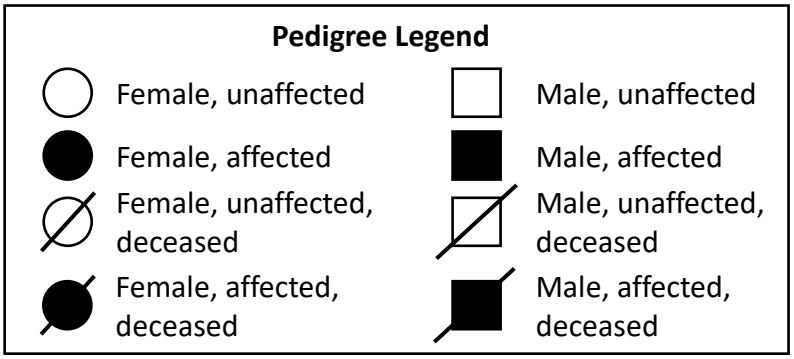

Figure 2: Pedigree legend of symbols.

A Case Study: A BRCA Genetic Testing Dilemma

(Adapted from a true story.)

It was the decision of a lifetime.

Her latest doctor visit showed nothing was wrong. But Deborah Lawler, age 33, was tired of constantly looking for the lump in her breast. Ever since she had learned about the DNA test that could help predict her risk of developing breast cancer, Deb had agonized over whether to have the test, and what to do about the results.

Deb didn't want history to keep repeating itself: Her mother had fought breast cancer when Deb was in high school. Deb's maternal grandfather had told her stories the week prior about his wife who had died from the disease before Deb was born. Deb's uncle Bob, her mother's only brother, had been diagnosed just after his 50th birthday. One of Bob's children and Deb's first cousins, Katherine, had detected breast cancer at the age of 33. The coincidences were too much to ignore.

"It could be growing inside of me right now," she told her mother on the phone in February, pacing in the living room of her Chicago apartment. "We could find it any time." Waiting for an encouraging word, she added, "I could take the test this week." Her mother, not sure what to say, remained silent.

Deb was referring to the breast cancer susceptibility tests—the BRCA tests. Doctors would isolate DNA from Deb's blood and sequence the Breast Cancer Susceptibility 1 and 2 genes to determine whether known cancer-causing mutations are present. BRCA1 and BRCA2 mutations account for about 5% of all breast cancer. The remaining cases are caused by mutations in other genes, environmental exposures, and other unknown factors.

Factors such as excess body weight, lack of exercise, having her first period at a young age, and not having children can increase the risk of breast cancer in all women. If the test finds that Deb carries a cancer-causing mutation in her BRCA genes, her risk of breast cancer would increase dramatically – from 12% (the average lifetime risk for all women) to anywhere between 50-85%. A mutation would also increase her risk of ovarian cancer from the average of 2% to between 16-60%.

Few things in biology are 100%.

If she tested positive for the mutations known to be associated with cancer, she could have both of her ovaries surgically removed before cancer could strike. This would reduce her risk of cancer substantially, but not completely. She could also have her breasts surgically removed through a procedure known as a mastectomy, but even after a mastectomy, there would still be a 10% chance that tiny cancer cells might be hiding in her otherwise healthy tissue.

She could try regular doses of drugs that block estrogen and help prevent the development of breast cancer, but these drugs induce a form of menopause. She and her doctors could practice

increased surveillance to try to catch the cancer early by using twice-yearly mammograms (x-rays of the breast used to detect breast cancer), breast self-exams, blood tests, and at least yearly physical exams with her doctor and other tests to detect potential ovarian cancer.

As they seek to avoid the potentially lethal consequences of a mutant gene, many people turn to relatives who may share the burden of having such a gene. But at a moment when a genetic test can make family ties even more tangible, they are often most strained. Parents who fought cancer might not understand the choices that confront their children, and guilt over giving their children a harmful allele might color their advice. Siblings and cousins who may carry the risky allele might try to persuade others to confront the problem just as they do, while those relatives who inherited functional forms of the genes may seem unqualified to judge those who did not.

Even as she searched for her own answer, Deb, a doctor, found herself navigating her family's strong and conflicting opinions on the imperfect options lying before her. Her father, who once feared he would lose his wife to cancer, encouraged her and her siblings to have the test. Her brother John felt ambivalent about the knowledge the test would bring, even though the risk of breast cancer in men carrying BRCA mutations is also high. Her sister Lori and her husband Jack were also undecided, though they thought that the results may benefit their two young children, Betsy and Kevin, someday. Deb's Aunt Sue who is married to Bob said she hated to see her niece embrace a course of action that was "upsetting the whole family for her own personal gain." Another cousin, Katherine's sister Lynn, declined even to talk about the DNA test—she did not have health insurance and the test was too costly to pay for out-of-pocket, so why even consider it? But for Deb, even with her family's mixed reactions, it was her mother's blessing that she most eagerly sought.

"I have the potential of this amazing gift, of knowing my risk," Deborah told her mother over the phone that winter night. "How can I not do anything about that?"

But biology is rarely a simple thing, and her risk of cancer, even should she test positive for cancer-causing mutations, was far from certain.

Credit: Adapted from Harmon, Amy. "The DNA Age: Cancer Free at 33 but Weighing a Mastectomy." The New York Times. September 16, 2007. Print.

Pre-Lab DATA SHEETS:
Analyzing Genetic Mutations

Name: _____ Date: _____

Laboratory Instructor: _____Section: _____

Family History Summary Table

Family Member Name	Relationship	Sex (Female/Male)	Medical Conditions	Still Living / Deceased

A BRCA Genetic Testing Dilemma
Pedigree Chart Construction

LABORATORY 9:
Analyzing Genetic Mutations in Breast Cancer

Overview

At the end of this lab, students will be able to:

- Analyze genetic information using pedigree charts
- Navigate the National Center for Biotechnology Information (NCBI) website to align DNA sequences using the Basic Local Alignment Search Tool (BLAST)
- Identify changes between different DNA and protein sequences using BLAST

Preparation

You should be familiar with the following concepts and techniques:

- The structure of DNA
- The template strand and coding strand in DNA
- Genomics and bioinformatics
- The association of BRCA1 and BRCA2 genes with cancer risk
- Constructing a pedigree chart

Equipment and Supplies

Equipment
Laptop

INTRODUCTION

Genetics may not be at the forefront of every doctor's mind when a woman presents with cancer. The stage of cancer progress and various treatment options are most likely to be of a higher priority than taking a detailed family history. The **etiology** (cause or set of causes) of a disease can be very complex, involving both genetic and environmental factors. Understanding genes and networks of genes can yield valuable information in understanding inherited diseases or traits such as ovarian, breast and prostate cancers, hemophilia A, or Duchenne's muscular dystrophy. With the advancements in genetics and genomics, obtaining a family history can provide important clues to understand whether the patient has an inherited risk of cancer arising from a genetic mutation.

Genomes and Genomics

DNA, or deoxyribonucleic acid, is the hereditary material in humans and all other organisms. DNA molecules are made of two twisting, paired strands, in the shape of a double helix as shown in *Figure 1*. The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T). The order, or **sequence**, of these bases determines the information available for building and maintaining an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and sentences.

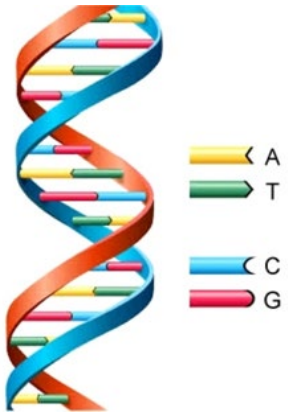

Figure 1: The structure of DNA consists of two strands containing A, T, G, C bases.

The two strands within a DNA double helix are given specific names, which are depicted on the following page in *Figure 2*. The **DNA template strand** refers to the sequence of DNA that is copied during the synthesis of messenger RNA (mRNA). The mRNA contains a sequence of bases divided into triplets called codons that are used to determine the order of amino acids that make up a protein. The sequence of bases in mRNA is *complementary* to the sequence of bases in the DNA template strand. [For example, a cytosine (C) base in DNA is paired with a guanine (G) base in mRNA.] The other DNA strand is called the **DNA coding strand** because it closely matches the coding information contained within the mRNA that is used to generate amino acids. In fact, the

two base sequences are identical *except* for when a thymine (T) base in DNA is replaced by a uracil (U) base in mRNA.

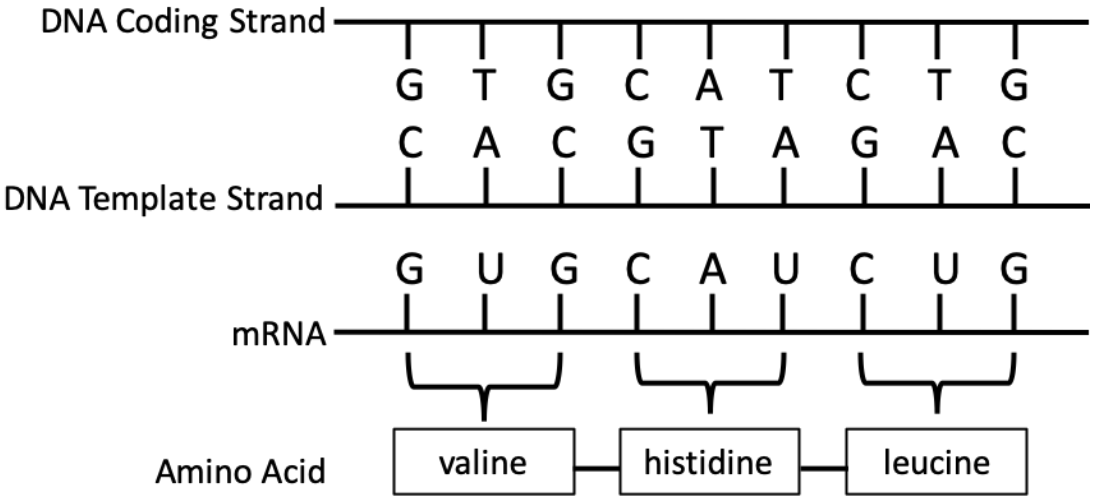

Figure 2: The two DNA strands are called the template strand and coding strand. The sequence of bases in the template strand is use to generate a complementary sequence of bases in the mRNA strand. The base sequence of the coding strand closely matches the sequence of the mRNA strand, except that T in DNA is replaced by U in RNA.

The genetic information in mRNA is organized into triplets of bases called **codons**. Each codon corresponds to an **amino acid.** For example, the codon GUG shown in *Figure 2* corresponds to an amino acid called valine. Similarly, the codon CAU corresponds to a different amino acid called histidine. A protein is composed of a long chain of amino acids that folds into a specific three-dimensional shape.

An organism's complete set of DNA is called its **genome**. Each genome contains all of the information needed to build and maintain that organism. The field of **genomics** is the study of genomes through DNA analysis. Genomics requires the sequencing and analysis of genomes. **Sequencing** simply means determining the exact order of the bases in a strand of DNA. Researchers can use DNA sequencing to search for genetic variations and/or mutations that may play a role in the development or progression of a disease.

The **Human Genome Project**, which was developed at the **National Institutes of Health (NIH)** by the **National Human Genome Research Institute**, produced a very high-quality version of the human genome sequence. The sequence is not that of one person, but is a composite derived from several individuals. Therefore, it is a "representative" or generic sequence. The Human Genome Project was designed to generate a resource that could be used for a broad range of biomedical studies. One such use is to look for the genetic variations that increase risk of specific diseases, such as cancer, or to look for the type of genetic mutations frequently seen in cancerous cells.

The Genetic Code

The **genetic code** provides the set of instructions that tell us how a specific codon of mRNA bases is translated into a particular amino acid. There are 20 different amino acids that are commonly found in proteins. There are also specific codons that provide instructions to **start** or **stop** translation. One representation of the genetic code is shown in *Figure 3*. This version of the genetic code arranges the codons in a circle. Here are the steps for using the genetic code table:

1. Begin with the first base of the codon in the middle of the circle. For example, we will start with **A**.
2. Move outwards to the next circle to select the second base in the codon. We will choose **A G**.
3. Finally, move outwards to the next circle to select the third base of the codon. We will pick **A G C**.
4. The codon **A G C** corresponds to an amino acid called **serine**. This amino acid can also be represented by a three-letter code (Ser) or a one-letter code (S).

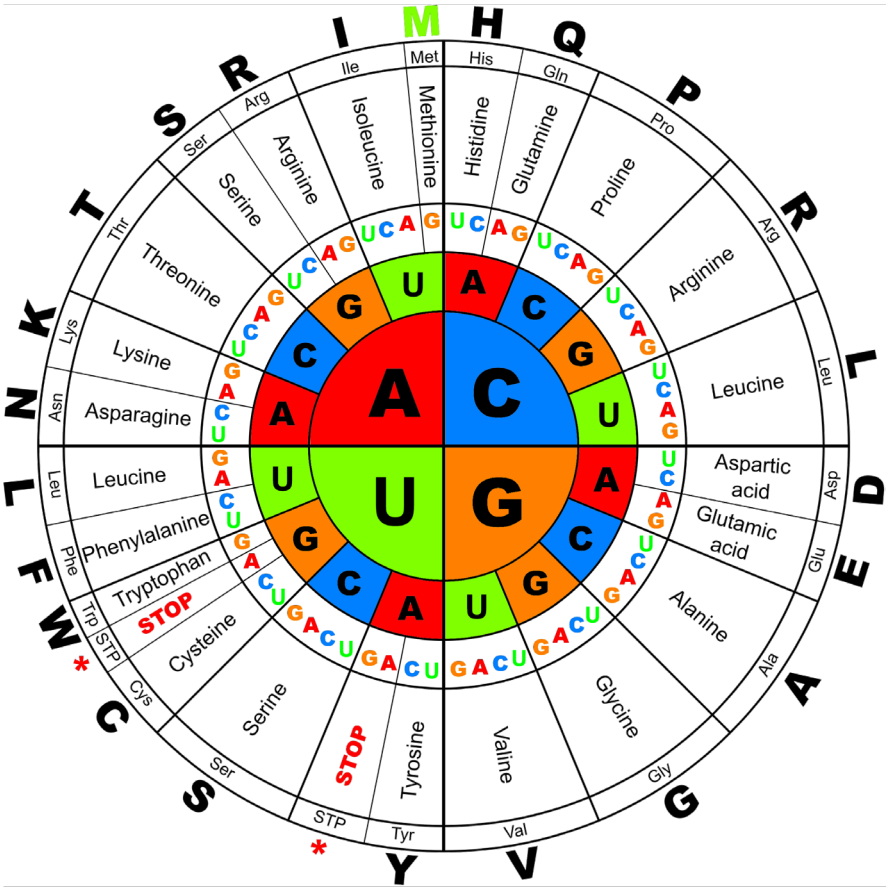

Figure 3: A genetic code table.

Table 1 lists the names of the 20 common amino acids, along with their three-letter codes and one-letter codes. Scientists use these shortened codes in order to write an amino acid sequence more efficiently. In this lab, you will see the amino acid sequence presented in one-letter codes; one example of a sequence is FVCERTLKY. Although the letters may look unfamiliar, you can think of them as representing molecular varieties of amino acids within the protein. This information will be provided in the lab and does not need to be memorized!

Table 1: Alphabetical listing of the 20 common amino acids

Name of Amino Acid	Three letter code	One letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamic Acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Bioinformatics

Bioinformatics is the collection, clarification, storage, and analysis of biological information using computers. This technique is especially applied to molecular genetics and genomics. The **National Center for Biotechnology Information (NCBI)** houses databases of biological information and all the genomes that have been sequences, such as the human genome. The **Basic Local Alignment Search Tool (BLAST)** is an NCBI program that can detect sequence similarities between a query "sample" sequence and reference sequences, that are known to be free of mutations. This comparison is used to confirm the presence or absence of DNA mutations within the query "sample" sequence. BLAST is popular because it can quickly identify regions of similarity or differences between two sequences. The ability to detect differences or changes in an individual's sequence allows us to identify a person's risk of developing disorders or diseases such as breast and ovarian cancer. In today's lab, you will use the DNA coding strand because it shares a similar base sequence to mRNA

BRAC1, BRCA2, and the Risk of Cancer

The names *BRCA1* and *BRCA2* stand for **br**east **ca**ncer susceptibility gene **1** and **br**east **ca**ncer susceptibility gene **2**, respectively. For cells to function properly, they need to be able to repair errors in their DNA. These errors can arise when DNA is being copied, or when DNA becomes damaged following exposure to chemicals or radiation. The breast cancer susceptibility gene (*BRCA1*) encodes a protein that is involved in DNA repair. When a DNA strand is broken, the BRCA1 protein works with other proteins to help repair the break. If these breaks are not repaired, the DNA damage can ultimately lead to cancer. Therefore, *BRCA1* is known as a tumor suppressor, because it helps prevent the formation of tumors (which can arise when DNA errors go unrepaired). Mutations to the *BRCA1* gene can interfere with or terminate the BRCA1 protein's normal function, thus allowing cancer to develop. Similarly, *BRCA2* also acts as a tumor suppressor gene.

The *BRCA1* gene is located on chromosome 17 whereas the *BRCA2* gene is located on chromosome 13, as seen in *Figure 4* on the following page. Together, mutations in these genes account for 5-10% of all breast cancer cases and approximately 45% of all familial (inherited) breast cancer. Some of these changes dramatically increase the risks of breast and ovarian cancer. For the average woman, the lifetime risk of breast cancer is 12%, and the lifetime risk of ovarian cancer is 2%. Certain mutations in *BRCA1* or *BRCA2* can increase these risks to 36-85% for breast cancer and 20-60% for ovarian cancer. It is important to note that *BRCA1* and *BRCA2* mutations also confer increased risk of breast and prostate cancer in men.

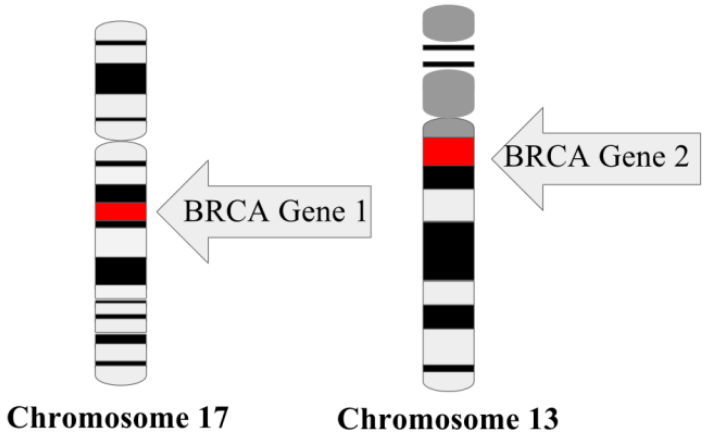

Figure 4: The chromosome locations of the *BRCA1* and B*RCA2* genes.

The BRCA1 gene is very large and contains over 5,700 bases. To analyze a gene of this size, it must be broken down into smaller pieces, sequenced, and then put back together using software. In this experiment, only a small piece of the *BRCA1* gene (600 bases) will be analyzed. *Figure 4* depicts 24 regions of the *BRCA1* gene. These regions are called **exons** because they provide the genetic instructions to make amino acids in the BRCA1 protein. In this experiment, we will be looking at Exons 19-24 within the BRCA1 gene, which are circled in *Figure 5*.

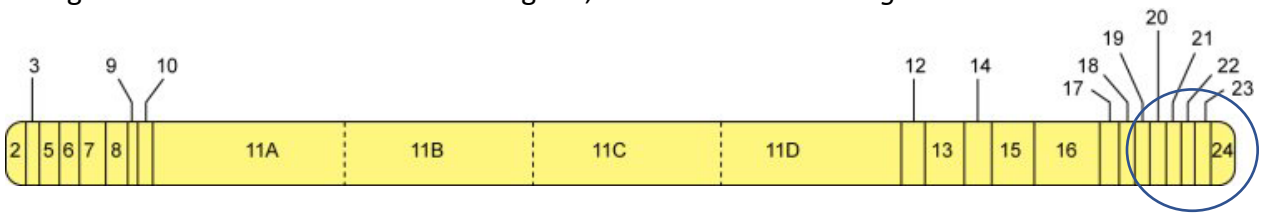

Figure 5: Exons within the *BRCA1* gene. The region of analysis (Exons 19-24) is circled.

BRCA associated cancer is thought to develop in a person with one functional copy of a BRCA gene and one mutant (non-functional) copy. When a new mutation occurs, it deactivates the functional copy of the gene. The need for a second mutation could explain why some people don't get breast or ovarian cancer, even when they have mutant copies of the *BRCA1* and *BRCA2* genes. Inheritance of two mutated copies of *BRCA1* is lethal to an embryo.

Over 1,600 different mutations have been identified in *BRCA1* and over 1,800 have been found in *BRCA2*. Many families have their own type of mutation that stays within the family through inheritance. There are differences in cancer risk associated with different mutations, but we don't know enough about these genes yet to fully understand why this occurs.

LABORATORY EXERCISES

PART A: DNA ALIGNING SEQUENCES WITH BLAST

In this lab, you will learn how genetic tests are performed. To identify mutations in a gene, the individual's DNA sequence is compared to a reference sequence. This comparison is known as a **DNA alignment**. In part A, you will learn how to perform a DNA alignment using a BRCA1 reference sequence and Deb's DNA sequence. To begin:

1. Locate the DNA sequence files from the student laptop desktop. They contain the *BRCA1* reference DNA sequence and six individual Lawler family sequences. Open up the text files for the reference, Deb, and family members DNA sequences and familiarize yourself with the sequences.

2. Go to the NCBI blast website (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Select "nucleotide blast," as shown in **Figure 6**, since we will be comparing a DNA sequence (sequence of nucleotides) to a DNA sequence (sequence of nucleotides).

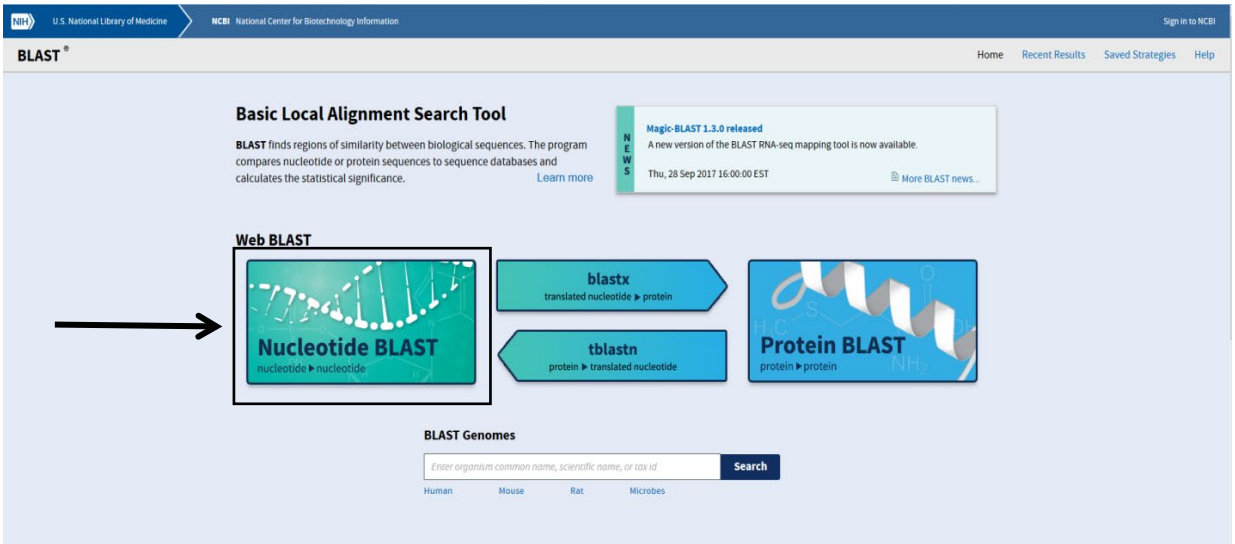

Figure 6: NCBI BLAST home screen.

4. From the nucleotide blast page, click the box "Align two or more sequences" **(Figure 7)**.

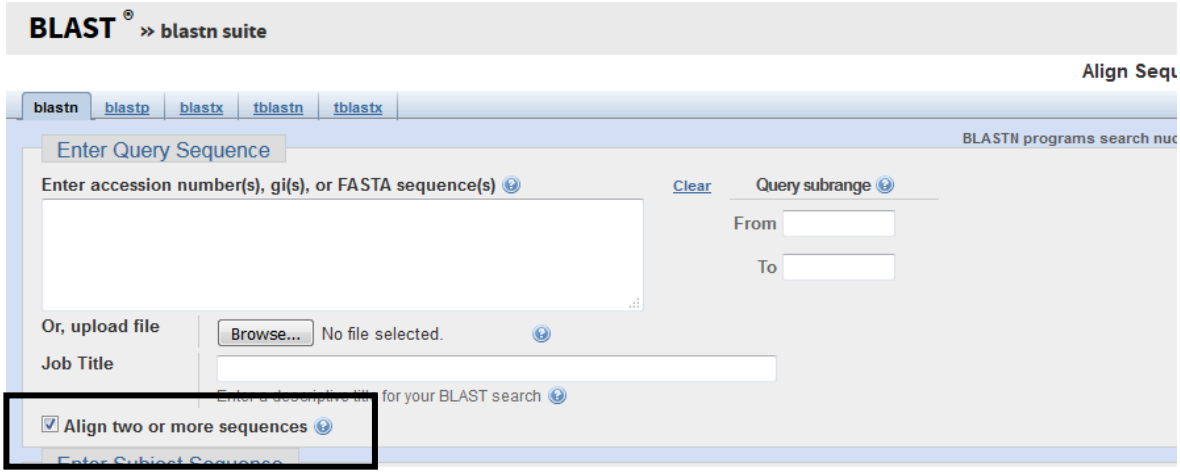

Figure 7: Nucleotide BLAST webpage.

5. A second text box will appear **(Figure 8)**.

Figure 8: Alignment text boxes.

6. Answer **Question 1a and 1b** in the Data Sheets.

7. Now you will add the sequences to each text box. Click the "Choose file" button under Text Box 1. Select the file named "1_Reference _DNA_Sequence_ BRCA1.txt" from the folder in step 1 **(Figure 9)**.

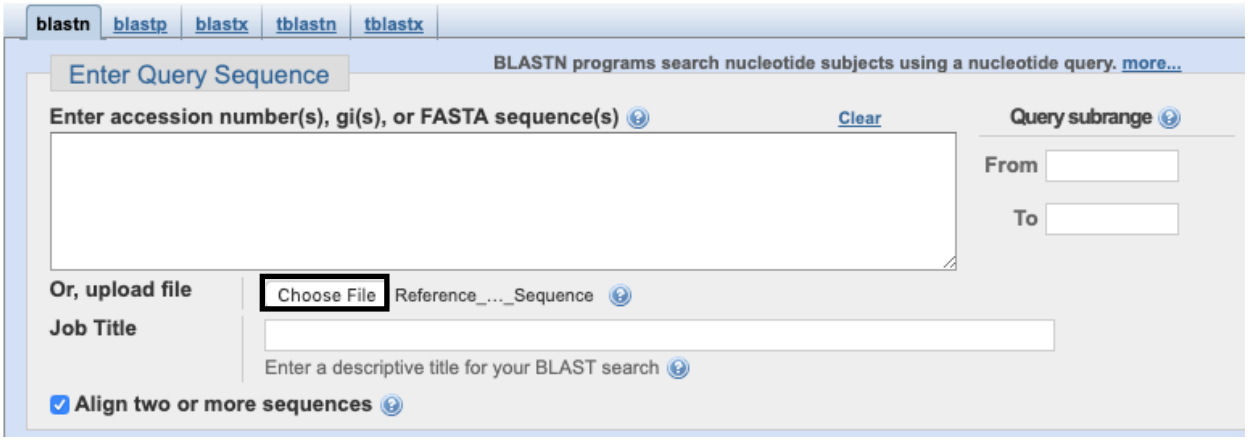

Figure 9: Example of text box including the BRCA1 Reference DNA Sequence.

8. Repeat step 7 for Text Box 2 but choose the file "2_Deb _DNA_Sequence_ BRCA1.txt" instead.

9. Click "BLAST." When your search is complete, a screen containing the BLAST results will be displayed.

10. Click the "Alignments" tab located near the middle of the page (**Figure 10)**.

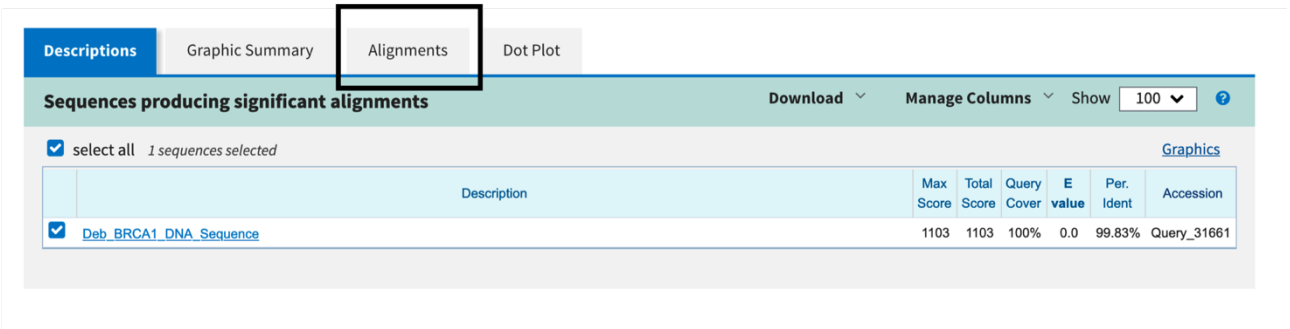

Figure 10: BLAST Results page.

11. Find the Alignment View and use the drop-down menu to choose "Pairwise with dots for identities" as seen in **Figure 11**. The ***query*** is the reference sequence. The pairwise with dots view shows the reference sequence at the top with the subject sequence aligned below (i.e., the family member's sequence or a patient's sequence). **NOTE: *Dots are used to show nucleotides that are identical, and letters are used to highlight nucleotides that differ.***

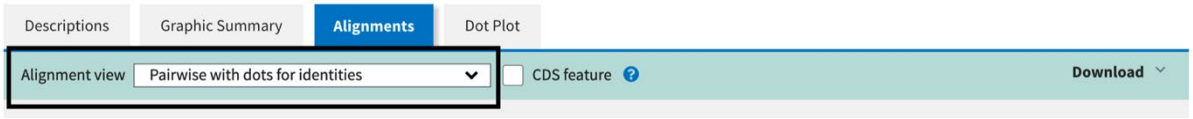

Figure 11: Continuation of BLAST Results page.

12. Scroll down the page to see if there are positions where the ***query*** sequence (which is the ***reference*** sequence) differs from the subject (family member's or patient's) sequence. In other words, look for a place where there is a letter instead of a dot, showing that there has been a change in the nucleotide at that position. Note that the numbers at the beginning and end of the lines refer to the position of the first and last nucleotide as seen in **Figure 12**.

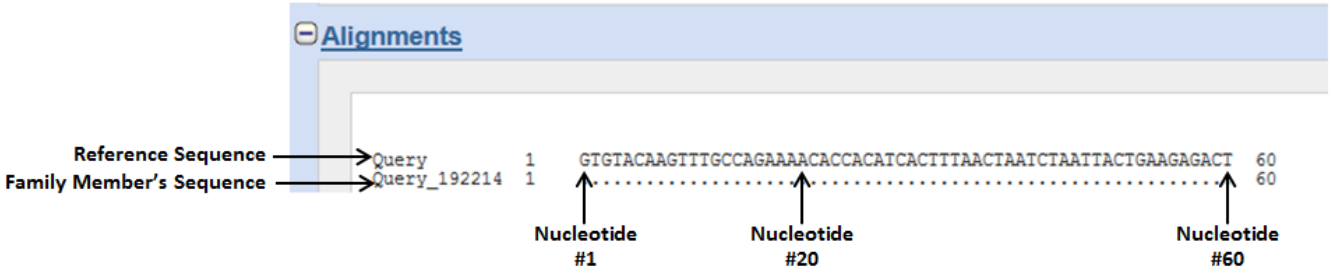

Figure 12: Sequence alignments.

13. Answer **Question 2** in the Data Sheets.

PART B: ANALYZING DIFFERENCES BETWEEN THE DNA SEQUENCES USING BLAST

Now that you have learned how to align sequences, you will perform an alignment of the DNA sequences of all family members being tested to determine whether a mutation in their BRAC1 gene is present.

1. First you will start a new BLAST alignment. Scroll to the top of the page and click the "blastn suite-2sequences" button as seen in **Figure 13**.

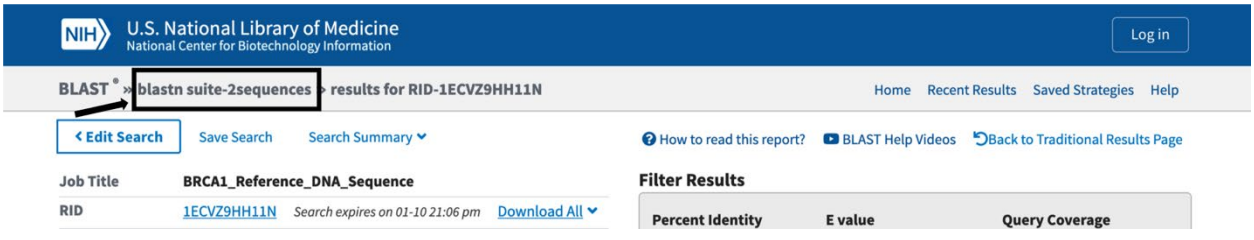

Figure 13: "blastn suite-2sequences" at top of results page.

2. Upload the file "1_Reference _DNA_Sequence_ BRCA1.txt" into Text Box 1 **(Figure 9)**.

3. Upload the file "3_Family _DNA_Sequence_ BRCA1.txt" into Text Box 2. Note, in order to compare the entire family, Deb's sequence will also be included in the new alignment.

4. Click "BLAST."

5. Click the "Alignments" tab located near the middle of the page **(Figure 10)**.

6. Find the Alignment View and use the drop-down menu to choose "Pairwise with dots for identities" **(Figure 11)**.

7. Scroll down the page to see if there are positions where the query (reference) sequence differs from the subject (family member) sequence.

8. Answer **Questions 3 - 5** in the Data Sheets.

PART C: PROTEIN ALIGNMENT

To determine whether any mutations detected in the Part B will have an effect on the structure of the BRAC1 protein, a protein alignment for the family members must be performed. The DNA sequences for each member have already been translated for you resulting in a sequence of amino acids that can now be analyzed. To begin:

1. Locate the Protein sequence files from the student laptop desktop. They contain the *BRCA1* reference protein sequence and six individual Lawler family sequences. Open up the text files for the reference, Deb, and family members Protein sequences and familiarize yourself with the sequences.

2. Click on the "BLAST" button again to return to the beginning page (**Figure 14**).

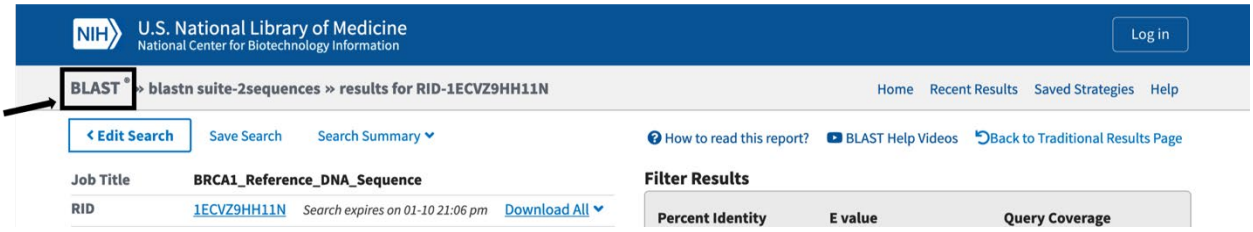

Figure 14: Blast button for new search

3. Select "protein blast" (**Figure 15)** as you will be comparing a protein sequence (sequence of amino acids) to another protein sequence.

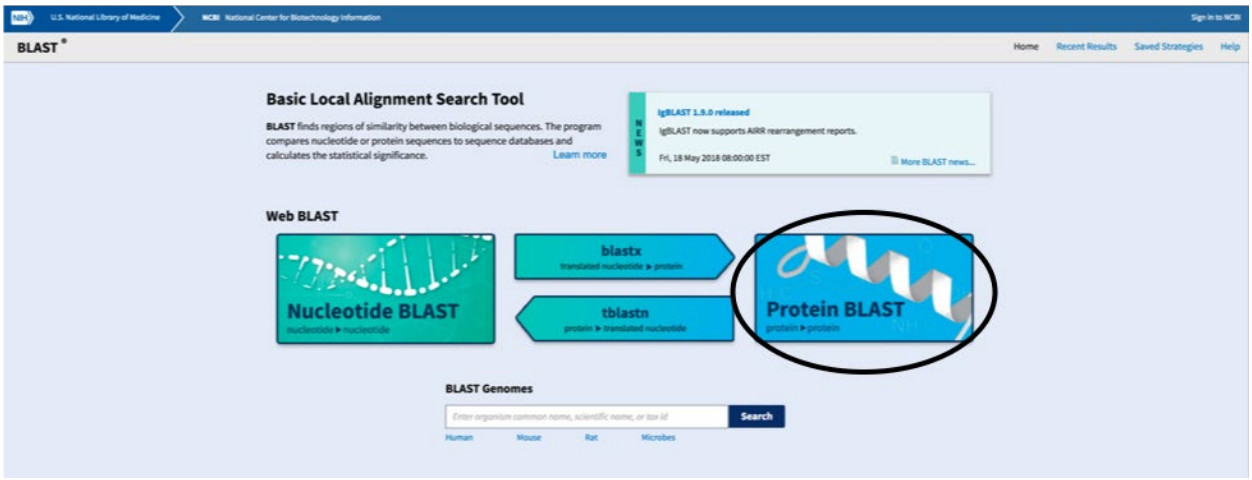

Figure 15: NCBI BLAST home screen.

4. From the protein blast page, click the box to choose the option to "Align two or more sequences" (**Figure 7).**

5. A second text box will appear (**Figure 8**).

6. Upload the file "4_Reference _Protein_Sequence_ BRCA1.txt" into Text Box 1 **(Figure 9)**.

7. Upload the file "6_Family _Protein_Sequence_ BRCA1.txt" into Text Box 2.

8. Click "BLAST."

9. When your search is complete, a screen containing the BLAST results will be displayed.

10. Click the "Alignments" tab located near the middle of the page (**Figure 10)**.

11. Find the Alignment View and use the drop-down menu to choose "Pairwise with dots for identities" (**Figure 11)**. The ***query*** is the reference sequence. The pairwise with dots view shows the reference sequence at the top with the subject sequence aligned below (i.e., the family member's sequence or a patient's sequence). **REMEMBER: *Dots are used to show amino acids that are identical and letters are used to show the amino acids that differ***.

12. Scroll down the page to see if there are positions where the query (reference) sequence differs from the subject (family member's or patient's) sequence. In other words, look for a place where there is a letter instead of a dot, showing that there's been a change in the amino acid at that position. Note that the numbers at the beginning and end of the lines refer to the position of the first and last amino acid as seen in **Figure 16** on the following page.

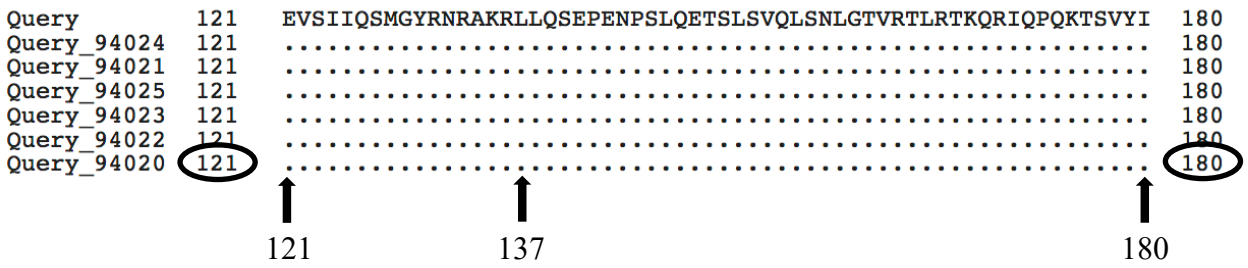

Figure 16: Example of queried alignments.

13. Answer the **Questions 6 – 11** in the Data Sheets.

DATA SHEETS:
Analyzing Genetic Mutations

Name: _____ Date: _____

Laboratory Instructor: _____Section: _____

PART A: DNA ALIGNING SEQUENCES WITH BLAST

Question 1a: What is a reference sequence?

Question 1b: Explain why you think we need to compare DNA sequences of the people in this family to a "reference sequence."

Question 2: Does Deb have a mutation? Are there any differences between the DNA reference sequence (the top sequence marked "query") and the test sequence you entered? What is the location of the base that differs? (You will need to look at the numbers on the side of the alignment.)

Fill out the table below with the answers to the following questions.

Family Member	**Mutations**		**Base Differences** (i.e. A → T)	**Location**
Deb	☐ Yes	☐ No		

PART B: ANALYZING DIFFERENCES BETWEEN THE DNA SEQUENCES USING BLAST

Question 3: Fill out the table below with the answers to the following questions.

Family Member	Mutations		Base Differences (i.e. A → T)	Location
Deb	☐ Yes	☐ No		
Father	☐ Yes	☐ No		
Katherine	☐ Yes	☐ No		
Lori	☐ Yes	☐ No		
Mother	☐ Yes	☐ No		
Uncle Bob	☐ Yes	☐ No		

Question 4: Referencing the table in Question 3, do the family members have any different base mutations in their *BRAC1* gene? If so, what are they?

Question 5: What effect do you think the mutation(s) may have on the protein encoded by the *BRCA1* gene?

PART C: PROTEIN ALIGNMENT

Question 6: Fill out the table below with the answers to the following questions.

Family Member	Mutations		Amino Acid Differences (i.e. K → L)	Location
Deb	☐ Yes	☐ No		
Father	☐ Yes	☐ No		
Katherine	☐ Yes	☐ No		
Lori	☐ Yes	☐ No		
Mother	☐ Yes	☐ No		
Uncle Bob	☐ Yes	☐ No		

WORKED EXAMPLE

Question: You run a protein BLAST and discover there is a mutation that changes the amino acid from Aspartic acid to Glutamic acid in your sequences. Earlier, you ran a nucleotide BLAST and found a nucleotide mutation of Cytosine to Guanine. How can you determine the codon that was mutated in your nucleotide sequences based off of the information received from your protein BLAST?

Answer: We will work through this problem in smaller steps.

STEP 1: List all of the possible mRNA codons for each amino acid using an Amino Acid Codon Table.

Aspartic acid	Glutamic acid
GAU	GAA
GAC	GAG

STEP 2: Convert the nucleotide sequence from the DNA coding strand to mRNA.

The nucleotide sequences you worked with are from the DNA coding strand, however the codons listed in the Amino Acid Codon Table represent mRNA. From the Introduction, we know that the DNA Coding Strand and mRNA are identical except for when a thymine (T) base in DNA is replace by a uracil (U) base in mRNA.

DNA Coding Strand	C	G
mRNA	C	G

Here you can see that our mutation C to G in the DNA Coding Strand will be the same mutation to look for in the mRNA strand.

STEP 3: Determine the mRNA codon that was mutated.

Looking back at our table from Step 1, the only codon containing a cytosine is GAC. If the cytosine of this codon was mutated to guanine, you would get the codon GAG, which is listed under the Glutamic acid side of the table.

STEP 4: Convert the codon from mRNA to the DNA coding strand.

Finally, we can determine the codon in the DNA Coding Strand that was mutated.

mRNA	GAC	GAG
DNA Coding Strand	GAC	GAG

Question 7: What amino acid mutations did you find in Question 6? List all of the mRNA codons for each amino acid in Mutation 1 and Mutation 2.

Mutation 1:

Amino Acid		
Codons		

Mutation 2:

Amino Acid		
Codons		

Question 8: What was the base mutation associated with each amino acid mutation? Convert the nucleotide sequence to mRNA for Mutation 1 and Mutation 2.

Mutation 1:

DNA Coding Strand		
mRNA		

Mutation 2:

DNA Coding Strand		
mRNA		

Question 9: Using the information you determined in Question 7 and Question 8, determine the mRNA codon that was mutated in Mutation 1 and Mutation 2.

Mutation 1:

Mutation 2:

Question 10: Complete the table, including the codons and resulting amino acids (add the full name of the amino acids to the table.) Finally, convert the codon from mRNA to the DNA coding strand for Mutation 1 and Mutation 2.

Data Table 1: Lawler Family Sequence Analysis

	Reference Sequence		**Mutated Sequence 1**	**Mutated Sequence 2**
Amino Acid				
mRNA Codon				
DNA Coding Strand				

Question 11: What are the risks for an individual that has a *BRCA1* mutation compared to an individual with no mutations?