4 Understanding Genetic Tests to Detect *BRCA1* Mutations

Introduction

Students begin this lesson by working through a pedigree chart and Punnett squares for the Lawler family, attempting to track the *BRCA1* mutation across generations. Based on the decisions about who should be tested for the *BRCA1* mutation, students then use the bioinformatics tool known as **BLAST** (**Basic Local Alignment Search Tool**) to compare individual DNA and protein sequences to **reference sequences** that are known to be free of *BRCA1* mutations associated with cancer. At the end of the lesson, students compile class information from the Lawler family in order to revise their pedigree charts and Punnett squares. In *Lesson Four*, students learn how ***laboratory technicians*** might use bioinformatics tools in their career.

Learning Objectives

At the end of this lesson, students will know that:

- Reference sequences, which are known to be free of cancer-causing mutations, are used to determine whether patient DNA sequences contain mutations.
- The bioinformatics tool BLAST (Basic Local Alignment Search Tool) can be used to determine whether patient DNA sequences contain mutations.
- Bioinformatics tools are used by people in many careers, including laboratory technicians.

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

- Analyze genetic information using pedigree charts and Punnett squares.
- Navigate the NCBI in order to align sequences using the Basic Local Alignment Search Tool (BLAST).
- Identify changes between DNA and protein sequences using BLAST.

Key Concepts

- Comparisons of the similarities and differences among nucleotide or protein sequences can be done using BLAST.
- When performing a genetic test, the DNA (or protein) sequence from a patient is compared to a known reference sequence to determine whether there are any disease-causing **mutations** present in the patient sequence.
- Once sequence data has been analyzed, it falls to genetic counselors and physicians to explain the results and help families determine how to use the information.
- Bioinformatics tools are used by people in many careers, including lab technicians.

Class Time

One to two class periods of 50 minutes each (up to 100 minutes total). If students are familiar with pedigrees and Punnett squares, the lesson can be completed in one class period. If students have not completed the BLAST exercise during class, the remainder of the assignment may be assigned as homework.

Prior Knowledge Needed

- Basic Mendelian inheritance, including dominant and recessive traits.
- Understanding of the relationship between DNA and proteins.
- Where to find single letter abbreviations for each amino acid. A helpful reference showing **codons** and the one-letter abbreviations for each amino acid can be found in the *Appendix*.
- The difference between **genotype** and **phenotype**.
- How to use a Punnett Square.

Common Misconceptions

- All genes are either dominant or recessive.
- All **mutations** are deleterious.
- People who inherit any mutation in *BRCA1* will develop cancer.

Materials

Materials	Quantity
Copies of Student Handout—*Careers in the Spotlight* (handed out in *Lesson One*)	1 per student
Copies of Student Handout—*Lawler Family Phenotype Pedigree: Who Has Breast Cancer?*	1 per student
Class set of Student Handout—*Instructions for Aligning Sequences with BLAST*	1 per student (class set)
Copies of Student Handout—*Aligning Sequences with BLAST Worksheet* [**Note:** This worksheet is for students' answers to lesson questions]	1 per student
Teacher Answer Key—*Lawler Family Phenotype Pedigree*	1
Teacher Answer Key—*Aligning Sequences with BLAST*	1

Computer Equipment, Files, Software, and Media
Computer with internet access and projector to display PowerPoint slides. ***Alternative:*** Print PowerPoint slides onto transparencies and display with overhead projector.
Lesson Four PowerPoint Slides—*Understanding Genetic Tests to Detect* BRCA1 *Mutations*. Available for download at: http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing.
A student version of lesson materials (minus teacher answer keys) is available from NWABR's Student Resource Center at: http://www.nwabr.org/students/student-resource-center/instructional-materials/introductory-bioinformatics-genetic-testing.
Teachers will need to provide the DNA and protein sequences for: 1) Deb, 2) Lori, 3) Katherine, 4) Deb's mother, 5) Deb's father, and 6) Deb's Uncle Bob (labeled "Uncle"). These sequences should be in an electronic format in a central location where students will have access to them during class. These sequences can be found at: http://nwabr.org/sites/default/files/IntroLesson4_BRCA1sequences_0.doc.
Computer lab with internet access and a word processing program such as Microsoft Word® or Google Docs.

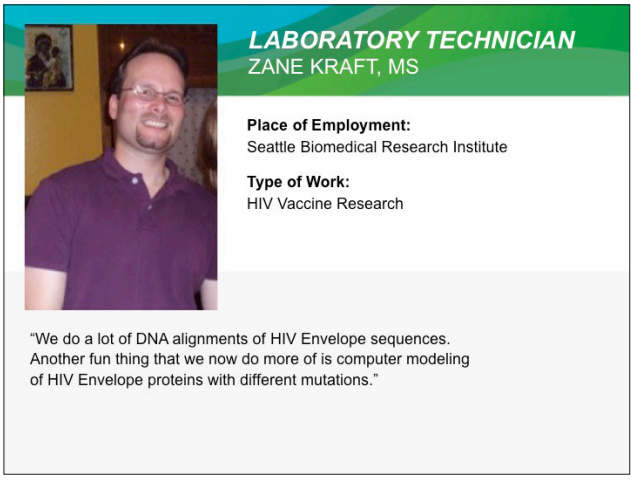
Teacher Preparation

- Load the classroom computer with the *Lesson Four* PowerPoint slides.
- Teachers will need to provide the DNA and protein sequences for: 1) Deb, 2) Lori, 3) Katherine, 4) Deb's mother, 5) Deb's father, and 6) Deb's Uncle Bob (labeled "Uncle"). These sequences should be in an electronic format in a central location where students will have access to them during class. These sequences can be found at: http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing.
- Make copies of Student Handout—*Instructions for Aligning Sequences with BLAST*, one per student. This handout is designed to be re-used as a class set.
- Make copies of Student Handout—*Lawler Family Pedigree: Who Has Breast Cancer?* and Student Handout—*Aligning Sequences with BLAST Worksheet*, one per student. These worksheets are designed for students to complete with their answers to the lesson questions.
- Student Handout—*Lawler Family Pedigree: Who Has Breast Cancer?* should be completed by students as a homework assignment before launching this lesson.

Procedure

WARM UP

1. As students enter the classroom, display PowerPoint ***Slide #1***. This slide highlights laboratory technician Zane Kraft.



Understanding Genetic Tests: ***Slide #1***

2. Have students retrieve Student Handout—*Careers in the Spotlight* from *Lesson One*.
3. Students should think about, and write down, the kind of work a laboratory technician might do (Laboratory Technician Question #1). This will be revisited at the end of the lesson, including how a laboratory technician might use bioinformatics in his or her job.
4. Tell students to keep their *Careers in the Spotlight* handout available for future lessons.

PART I: Lawler Family Pedigree

5. Explain to students the ***aim of this lesson***.

   Lesson Aim:

   - To understand how genetic tests are performed by comparing the DNA and protein sequences of a family to determine who is carrying the *BRCA1* mutation.

   Some teachers may find it useful to write the aim on the board. Teachers may also wish to discuss the *Learning Objectives* of the lesson, which are listed at the beginning of this lesson.

6. Go over the homework assignment from the previous night, Student Handout—*Lawler Family Pedigree*. Suggested answers can be found on Teacher Answer Key—*Lawler Family Pedigree*.
7. Make sure to reinforce that most information is available through the case study, but further information can be gained through genetic testing. Our next step will be to use the National Center for Biotechnology Information (NCBI) website to "run" a genetic test for individuals in the Lawler family. Students will use resources available at the NCBI to understand the kind of information obtained by genetic testing.

More information about how *BRCA1* functions as a tumor suppressor gene, and why some people refer to *BRCA1* inheritance as dominant rather than recessive, can be found at the end of the lesson in the *Teacher Background* section and in the *Appendix* section "*BRCA1:* Is it Dominant or Recessive?" Additional information is also available in the *BRCA1* animation featured in *Lesson 5*, available on the Bio-ITEST website.

8. Ask students who they think should be tested. Take a class vote and record their answers.

9. After considering various individuals, tell students that the following six people have consented to have the genetic test to find out if they have a mutation in their *BRCA1* gene:
   1. Deb
   2. Deb's sister Lori
   3. Deb's cousin Katherine
   4. Deb's mother
   5. Deb's father
   6. Deb's Uncle Bob

10. [**Optional:** Teachers may want to have a class set of plastic bags, each with a cotton swab inside. Each bag should be labeled with the name of one of the six individuals willing to be tested. When students decide on the individual they will be testing, they receive a plastic bag and swab for that person.]

11. Tell students that we are not able to test the following people:

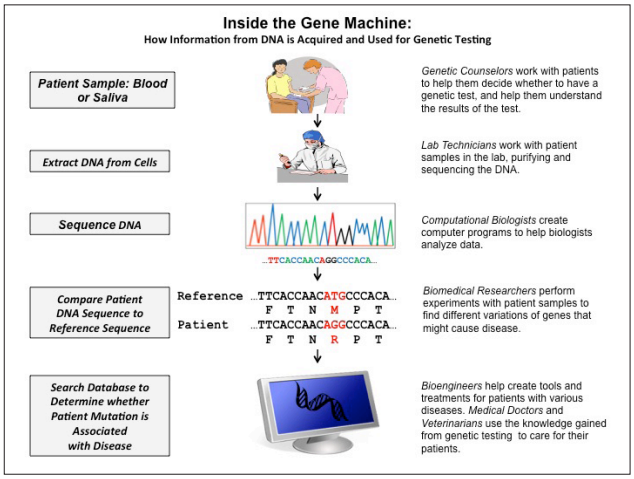
Person	Reason
Deb's cousin Lynn	Doesn't want to know. Did not give consent.
Lori's children	Lori did not give consent for them.
Deb's brother John	Doesn't want to know. Did not give consent.
Deb's Aunt Jackie	Doesn't want to know. Did not give consent.
Deb's grandparents	Deceased.

12. Have each student choose one of the six people who has consented to the test. Make sure that all six individuals will be tested by at least two or three students.

PART II: Inside the Gene Machine

11. Show ***Slide #2***, Inside the Gene Machine.

Understanding Genetic Tests: ***Slide #2***

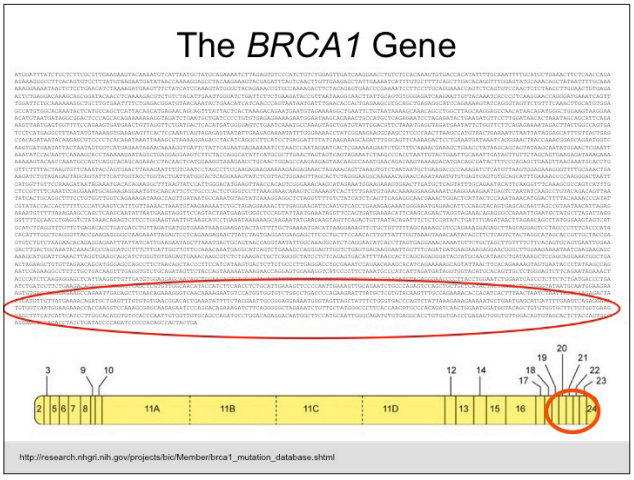


12. Walk students through the steps shown on the slide. Deb, Lori, and the other family members who chose to be tested would begin this process with either a blood or saliva sample.

13. Point out that a lab technician is highlighted in Step #2.

14. Focus students on Steps #3 and #4. Remind students that the DNA sequence shown is made up of the four nucleotides represented by A, T, C, and G. Step #4 shows a one-letter amino acid abbreviation for every three nucleotides. Reinforce this element of the relationship between DNA and proteins for students, if needed.

15. Show ***Slide #3***. The *BRCA1* gene is very large—over 5,700 nucleotides—as seen in this PowerPoint slide, *The BRCA1 Gene*. Tell students that laboratory technicians analyze large genes by breaking them into small pieces, sequencing all of the pieces, and using software to put the sequences together. Tell students that the sequences they will receive from members of the Lawler family will be only a portion (600 nucleotides long) of the entire *BRCA1* gene.



Understanding Genetic Tests: ***Slide #3***

16. Advance the animation by clicking the forward arrow on the computer projecting the PowerPoint slides; a red circle will appear so you can focus student attention on the region of the *BRCA1* gene they will be examining.

17. Click the forward arrow again on ***Slide #3***, and an image representing the 24 **exons** of the *BRCA1* gene will appear. The red circle around Exons 19-24 highlights the region of the gene studied in this lesson. Remind students that their DNA sequences will not start with the start **codon** ATG, as the DNA sequence is found at the end of the gene.

Exon: A nucleic acid sequence that is found in the mature form of an RNA molecule after portions ("introns") of a precursor RNA have been removed.

18. Finally, remind students that each person has two copies of most genes including the *BRCA1* gene. Tell students that they will only be studying the gene sequence from one copy of each family member's *BRCA1* gene. Laboratory technicians have already studied the DNA sequence of the second copy and found no mutations.

BLAST: Basic Local Alignment Search Tool. A bioinformatics tool used to compare DNA or protein sequences to one or more other sequences, or to compare a DNA or protein sequence to a collection of sequences found in databases, such as the Nucleotide or Protein databases at the NCBI.

Nucleotide BLAST: A BLAST performed with nucleotide sequences.

Reference sequence: A sequence that has been chosen for the purpose of comparison. At the National Center for Biotechnology Information (NCBI), reference sequences are chosen because they are of high quality and are thought to accurately represent the sequence from the original organism. In genetic testing, a reference sequence is a known and well-studied DNA or protein sequence that does not contain any mutations or changes that are associated with disease. These sequences are used for comparison with patient sequences during genetic testing.

Protein BLAST: A BLAST performed with protein sequences.

BLAST alignment: The results of a BLAST comparison of DNA or protein sequences.

The Breast Cancer Information Core (or BIC) was started by an international consortium of breast cancer researchers. Similar to the NCBI, it is a central repository for all kinds of information about BRCA-related cancer. Among all the *BRCA1* sequences available, they have agreed upon one as a reference sequence. All of the sequences in the BIC are also deposited at the NCBI, where the reference sequence was obtained.

PART III: Aligning Sequences with BLAST

19. Tell students that one of the tools in the bioinformatics toolkit they will be using is called **BLAST** – **B**asic **L**ocal **A**lignment **S**earch **T**ool. BLAST can be used to compare the sequences of two or more proteins or nucleic acid molecules, or to compare a single sequence to a collection of sequences in a database. Additional information about BLAST can be found in the *Appendix*.

20. Specifically, students will be performing a **Nucleotide BLAST**, comparing Lawler family *BRCA1* DNA sequences to a DNA **reference sequence** from the NCBI, and a **Protein BLAST**, comparing Lawler family BRCA1 protein sequences to a protein **reference sequence**.

21. The results of a BLAST are in the form of an alignment to identify regions of similarity and regions of difference, to determine if there are any mutations in the Lawler family sequences.

22. Provide students with this analogy, if needed:

    Picture a factory that manufactures necklaces. Each necklace is made up of four different beads, and is about 6,000 beads long. Market research has found that one sequence of beads sells much better than any other sequence, so the factory only wants to produce the best-selling necklace. Occasionally, a few beads on a necklace are strung incorrectly. How can the factory determine if the necklace is strung correctly?

    One way, analogous to a **BLAST Alignment**, would be to line up one necklace with the correct sequence of beads (the reference sequence) and compare other necklaces to it by lining them up at ***exactly the same starting point***. The workers don't need to pay attention to the places where the beads align correctly—the colors are the same—just the places where the beads do not match the reference necklace.

    A BLAST alignment works in a similar way, although the actual method to align the sequences is not exactly the same. Our reference sequence is made up of four different nucleotides (A, T, C, and G), and is about 6,000 nucleotides long. The sequence from each person in the family who consented to have the genetic test will be compared to a reference sequence that is known to be free of any *BRCA1* mutations.

    Similarly, with proteins, one could think of a necklace with 20 different beads (i.e., 20 different amino acids) and the necklace is about 2,000 beads long. To determine if the necklace is strung correctly, they are compared to a reference sequence. In this case, the reference sequence is a protein sequence.

23. Ask students, "***Where does the reference sequence come from? If we search the NCBI for* BRCA1 *(as we did in an earlier lesson) over 10,000 sequences are returned. Where do we begin?***"

    Tell students that we know the reference sequence should be: a) from humans and b) free of any cancer-causing mutations. Some people may refer to this as a "wild type" sequence, but this could be misleading. There are so many minor variations in many of our genes, comparing individuals in a population and picking just one as a "wild type" may not make sense. Instead, biomedical researchers [***see sidebar***] agree to standardize their experiments and to describe their findings by comparing all results to an agreed-upon single reference sequence.

24. Pass out Student Handout—*Instructions for Aligning Sequences with BLAST* and Student Handout—*Aligning Sequences with BLAST Worksheet* to students. Explain that the *Instructions* are a re-useable class set, while the *Worksheet* is the place for students to record their individual answers. Students will need computers with internet access to complete the handouts.

25. Tell students where to find the electronic versions of the DNA and protein reference sequences, and the DNA and protein sequences for Deb, Lori, Katherine, Mother, Father, and Uncle.

26. Students will analyze the DNA sequences first, and then look at the protein sequences.

27. Ask students, "***Which sequence would you expect to be longer, the DNA nucleotide sequence or the protein amino acid sequence?***"

28. Since it takes three nucleotides to code for one amino acid, the nucleotide sequence should be three times the length of the amino acid sequence. A DNA sequence this long, however, is unwieldy to work with, so students will align only a portion of the total DNA sequence.

29. Remind students that the gene is very large, and they will be aligning only a portion of the DNA nucleotide sequence.

30. Allow students to work independently through Student Handout—*Instructions for Aligning Sequences with BLAST*.

[**Note:** Scientists use the one letter amino acid abbreviations for protein sequences. See the *Appendix*, "Codons and Amino Acid Chemistry" and "Amino Acid Abbreviations and Chemistry Resource" for a codon table and list containing the one letter codes, if needed.]

PART IV: Putting it all Together

31. Draw a table on the board similar to the one students filled out in the Student Handout—*Aligning Sequences with BLAST Worksheet*, and review it with students:

	Reference Sequence	Mutated Sequence
DNA Coding Strand	ATG	AGG
DNA Template Strand	TAC	TCC
mRNA Codon	AUG	ACC
Amino Acid	Methionine (M)	Arginine (R)

[**Note:** Some biology textbooks refer to the DNA coding strand as the "sense strand" and the DNA template strand as the "anti-sense strand," "non-coding strand," or "complimentary strand."]

32. Tell students that this sort of methionine-to-arginine mutation at position 1775 is abbreviated M1775R. This convention for naming mutations uses the single-letter abbreviations for amino acids and the amino acid position number for the protein reference sequence. The first letter (in this case "M" for methionine) is the amino acid found in the reference sequence, at position number 1775 (i.e. amino acid number 1775 of 1863). The second letter (in this case, "R" for arginine) is the amino acid found in the mutated protein sequence.

33. This is called a **substitution** mutation, when one amino acid is substituted for another. This is one of the more common types of mutations that can occur in the *BRCA1* gene and is associated with cancer.

Substitution [mutation]: A change in a DNA or protein sequence, when one nucleotide or amino acid is changed or substituted for another.

34. Have students return to Student Handout—*Lawler Family Pedigree*. With the additional information gained through the genetic testing, students should update their pedigrees and Punnett squares.

35. Some questions to ask include:

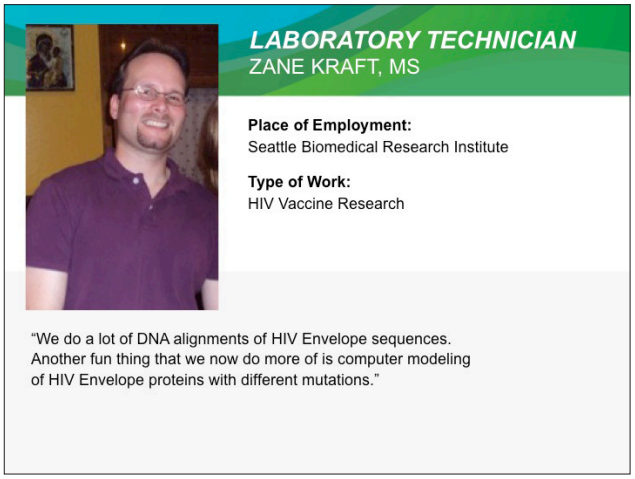
- Should Lori consider having her children tested?
  No. She does not carry the mutation so her children will not have inherited it.
- What is the chance that Deb's brother has the mutation?
  Since his mother has tested positive for the mutation, he has a 50/50 chance of having inherited the mutation.
- Which Punnett square (a, b, or c) would be most accurate?
  Punnett square b. The mother is ***heterozygous*** *for the mutation, and the father is* ***homozygous*** *recessive.*
- Even without consenting to being tested, Katherine's sister could now know that she has a 50/50 chance of having inherited the mutated gene. What bioethical principles may be involved here?
  Respect for Persons: *Knowledge of her possible predisposition for breast cancer was imposed on her without her consent. She was not given the choice to receive that information. The principle of* ***Maximize Benefits/ Minimize Harms*** *is also at play, since maximizing benefits for Deb (who does want to know the outcome of the genetic test) also increases the harm to family members who do not want to know but share genetic ties.*

Closure: *Careers in the Spotlight*

36. Tell students that in today's lesson, they have "looked into the Gene Machine" to understand how bioinformatics tools can be used to perform genetic tests. So far, the BRCA1 protein has been represented as a sequential, linear model. Tomorrow, students will explore the three-dimensional shape of the molecule to see how a simple substitution of one amino acid can change the shape, and consequently, the function of the protein.

37. Return to the picture of the lab technician from the *Careers in the Spotlight*, ***Slide #4***.

Understanding Genetic Tests: ***Slide #4***



Understanding Genetic Tests: ***Slide #5***

38. Show ***Slide #5***, which provides job information for a laboratory technician. Review this information with students.

39. Ask students, "***What more do we know about lab technicians after today's lesson?***" Point out that lab technicians do much of the physical work "inside the Gene Machine" before the information becomes electronic. This includes:

    - Collecting, handling, and storing patient tissue samples that are given over for genetic testing.
    - Purifying and sequencing the DNA.
    - Caring for and servicing the laboratory machinery.

40. Ask students to answer Laboratory Technician Question #2 on their *Careers in the Spotlight* handout, which has students explain how this lesson has changed their understanding of the kind of work a laboratory technician does.

41. Ask students to also answer Laboratory Technician Question #3 on their *Careers in the Spotlight* handout, which has students explain how a laboratory technician might use bioinformatics in his or her work. Tell students to keep their *Careers in the Spotlight* handout available for future lessons.

Homework

The following are suggested homework activities to follow this lesson. It is highly recommended that the reading for the Socratic Seminar in *Lesson Six* be provided as homework to allow more time in class for the Socratic Seminar activity.

A. Pass out the reading for the Socratic Seminar in *Lesson Six*: Student Handout—*Categorizing Genetic Tests* and/or Student Handout—*Weighing the Risks and Benefits of Direct-to-Consumer Genetic Testing*. The handouts can also be passed out as homework to accompany the reading and then be used as entry tickets for that class session. These are the reading and support materials for the Socratic Seminar that students will participate in during *Lesson Six*. Students may need two days to prepare fully.

B. Students can also update Student Handout—*Lawler Family Pedigree as homework*.

C. As a reflective exercise, ask students to write about the activities they learned in *Lesson Four* in their lab notebooks, on another sheet of paper, or in a word processing program like Microsoft Word® or Google Docs which they then provide to the teacher as a printout or via email. This can serve as an entry ticket for the following class. Have them complete these prompts:

a. Today I learned that…

b. An important idea to think about is…

c. Something that I don't completely understand yet is…

d. Something that I'm really confident that I understand is…

[**Note:** Suggested scoring for reflection: +5 points if all four prompts are complete.]

Extension: Amino Acid Chemistry

- Assign the extension questions in Part III of Student Handout—*Instructions for Aligning Sequences with BLAST* concerning amino acid chemistry. Show ***Slide #6*** and point out the following differences between arginine and methionine:

a. In its ionized form, arginine has a positive charge, while methionine is uncharged.

b. The "size" of the R-groups differs. Arginine is larger.

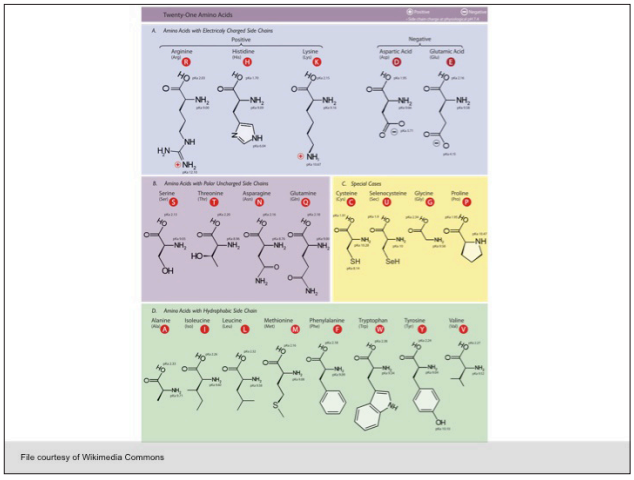
c. Methionine contains a sulfur.

d. Arginine contains two amino groups.

e. Amino acid substitutions can affect the structure of a protein by changing the interactions between the side-chains of nearby residues as well as those whose side-chains come into contact through protein folding.

f. Non-polar amino acids like methionine tend to be buried inside the protein (i.e., away from water), while polar amino acids like arginine are more likely to be exposed on the protein surface, in contact with water or other aqueous solvents.

Understanding Genetic Tests: ***Slide #6***



Teacher Background: Is *BRCA1* Dominant or Recessive?

Unfortunately, traditional Mendelian inheritance terms like "dominant" and "recessive" do not apply well in the case of *BRCA1*. When looking at a family pedigree, inheritance of *BRCA1* alleles appears to be ***autosomal dominant***: only one parent is affected and inheriting a single mutated copy of the gene brings with it inheritance of an ***increased risk of cancer***. However, at the molecular level, the *BRCA1* protein is a ***tumor suppressor***, requiring both copies of the *BRCA1* gene to be mutated for cancer to develop. An at-risk individual typically inherits a single mutated copy of the *BRCA1* gene, and at some point during that person's lifetime, the second copy may become mutated, leading to cancer. If two mutated copies of *BRCA1* are inherited, the embryo will not develop. *BRCA2* is also a tumor suppressor. For more information, see the *Appendix*, "*BRCA1*: Is it Dominant or Recessive?" and the *BRCA1* animation highlighted in Lesson 5 and available on the Bio-ITEST Genetic Testing website.

Autosomal dominant: A pattern of inheritance in which a single copy of a gene (allele) is sufficient to result in a particular phenotype (in contrast to autosomal recessive).

Tumor suppressor: A gene that encodes a protein involved in the suppressing tumors, thus protecting the cell from one step in the pathway to cancer. When both copies of this gene are mutated, either by inheritance or during the life of an organism, the cell can progress to cancer, usually in combination with other genetic changes.

Teacher Background: Reference Sequences

When discussing reference sequences with students, it is important to help students understand that it is not always as simple as the "wild type" and "mutant" forms of the genes they learn about in Mendelian genetics. Wild populations can show a large amount of genetic diversity. Some nucleotides can be substituted for others and still encode the same amino acid. Some amino acids are specified by multiple codons. In other cases, nucleotide substitutions produce codons that specify different amino acids, but the protein function remains the same either because of where the change occurs in the protein or because the amino acids have similar chemical properties.

The *BRCA1* gene is large and the exact sequence can vary between individuals. As described above, nucleotide changes can occur that are unrelated to breast cancer. In order to distinguish between genetic changes that are linked to cancer and genetic changes with little or no effect, researchers typically compare new *BRCA1* sequences with existing reference sequences and with databases of *BRCA1* variants.

Glossary

Allele: An alternative form of a gene, located at a specific position on a specific chromosome. A single allele is inherited from each parent.

Autosomal dominant: A pattern of inheritance in which a single copy of a gene (allele) is sufficient to result in a particular phenotype (in contrast to autosomal recessive).

Autosomal recessive: A pattern of inheritance in which both copies of a gene (allele) are required to result in a particular phenotype (in contrast to autosomal dominant).

BLAST: Basic Local Alignment Search Tool. A bioinformatics tool used to compare DNA or protein sequences to one or more other sequences, or to compare a DNA or protein sequence to a collection of sequences found in databases, such as the Nucleotide or Protein databases at the NCBI.

BLAST alignment: The results of a BLAST comparison of DNA or protein sequences.

Codon: Series of three nucleotides in a row that specifies the genetic code information for a particular amino acid when translating a gene into protein. For example, the codon CCG codes for the amino acid Proline (P). Also called a nucleotide triplet.

Database: A collection of related data that is stored, managed, and retrieved in an organized way.

Exon: A nucleic acid sequence that is found in the mature form of an RNA molecule after portions ("introns") of a precursor RNA have been removed.

Genotype: The genetic make-up of a cell, organism, or individual, usually with reference to a particular trait or phenotype.

Heterozygous: In a diploid organism (i.e., an organism with two copies of each chromosome), heterozygous refers to the presence of two different alleles at a particular gene locus (in contrast to homozygous).

Homozygous: In a diploid organism (i.e., an organism with two copies of each chromosome), homozygous refers to the presence of identical alleles at a particular gene locus (in contrast to heterozygous).

Intron: Any nucleotide sequence within a gene that is removed to generate the final mature RNA product of a gene.

Locus: The physical location of a gene (or other significant DNA sequence) on a chromosome.

Mutation: A change in a DNA or protein sequence.

National Center for Biotechnology Information (NCBI): Part of the National Library of Medicine at the National Institutes of Health (NIH), the NCBI is a collection of biological information in over 30 cross-referenced databases related to genetics and molecular biology.

Nucleotide BLAST: A BLAST performed with nucleotide sequences.

Nucleotide database: One of the databases at the NCBI which contains nucleotide (DNA and RNA) sequences.

Query sequence: The sequence to which other sequences are compared when performing a BLAST alignment. In the case of genetic testing, the reference sequence is the query sequence, while the patient's sequence is the subject sequence.

Phenotype: An organism's observable characteristics or traits.

Protein BLAST: A BLAST performed with protein sequences.

Protein database: One of the databases at the NCBI which contains protein sequences.

Reference sequence: A sequence that has been chosen for the purpose of comparison. At the National Center for Biotechnology Information (NCBI), reference sequences are chosen because they are of high quality and are thought to accurately represent the sequence from the original organism. In genetic testing, a reference sequence is a known and well-studied DNA or protein sequence that does not contain any mutations or changes that are associated with disease. These sequences are used for comparison with patient sequences during genetic testing.

Subject sequence: The sequence being compared when performing a BLAST alignment. In the case of genetic testing, the patient's sequence is the subject sequence, while the reference sequence is the query sequence.

Substitution [mutation]: A change in a DNA or protein sequence, when one nucleotide or amino acid is changed or substituted for another.

Tumor suppressor: A gene that encodes a protein involved in suppressing tumors, thus protecting the cell from one step in the pathway to cancer. When both copies of this gene are mutated, either by inheritance or during the life of an organism, the cell can progress to cancer, usually in combination with other genetic changes.

Resources

More information about how *BRCA1* functions as a tumor suppressor gene, and why some people refer to *BRCA1* inheritance as dominant instead of recessive can be found at the end of the lesson in the Teacher Background section and in the *Appendix* section "*BRCA1*: Is it Dominant or Recessive?" An extensive Question & Answer section, "*BRCA1* and *BRCA2*: Cancer Risk and Genetic Testing," developed by the National Cancer Institute, is also in the *Appendix*.

Additional information about BLAST, as well as amino acid abbreviations and chemistry, can also be found in the *Appendix*.

Credit

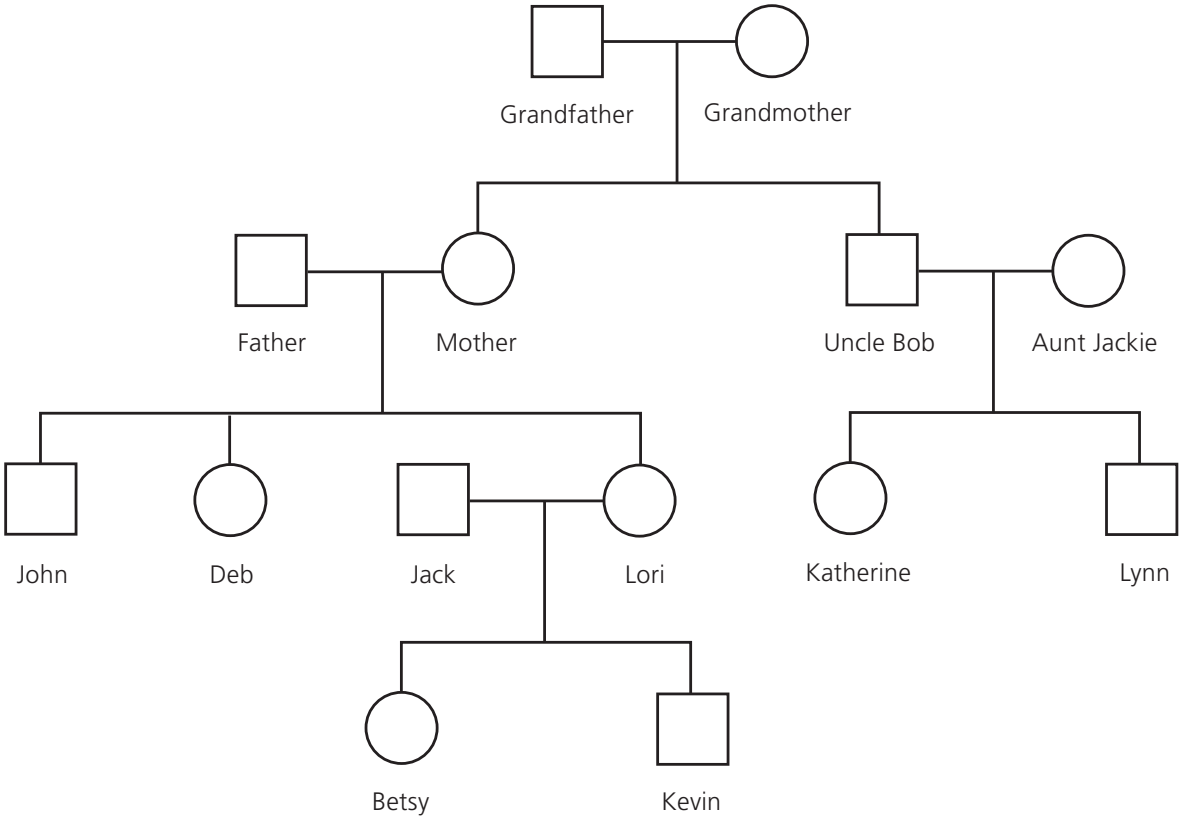
Kraft, Zane. Personal Interview. 30 September 2010.

National Center for Biotechnology (NCBI). Basic Local Alignment Search Tool (BLAST). http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Name _____ Date _____ Period _____

4 Lawler Family Phenotype Pedigree: Who has Breast Cancer?

Part I: Use the case study from Student Handout—*Case Study: A BRCA Genetic Testing Dilemma* (from *Lesson Three*) to fill out the pedigree and Punnett squares. Record as much information about a person as possible. Color in the square or circle if that person has received a diagnosis of breast cancer.



Pedigree Legend

- Female, unaffected
- Male, unaffected
- Female, affected
- Male, affected
- Female, unaffected, deceased
- Male, unaffected, deceased

"Affected" means the individual has received a diagnosis of breast cancer (phenotype). Until tested, the BRCA status (genotype) is unknown.

Part II: Use the pedigree chart on the previous page and the case study on Student Handout—*Case Study: A BRCA Genetic Testing Dilemma* (from *Lesson Three*) to answer the following questions:

1. If someone inherits one copy of *BRCA1* with a mutation from a parent, will they get breast cancer? Why or why not?

2. Which members of the Lawler family might benefit from testing for mutations in the *BRCA1* gene? Why?

3. Do any of the bioethical principles apply to the answer to Question #2? The bioethical principles are: Respect for Persons, Maximize Benefits/Minimize Harms, and Justice.

4. Are there any special considerations one should think about when testing children for a genetic disease? Do any of the bioethical principles speak to this?

5. Any individual having a genetic test would need to sign a consent form for this test. Who, if any, of the individuals on the pedigree chart would likely refuse to sign the form?

Part III: What are the chances of passing on the *BRCA1* mutation to one's children?

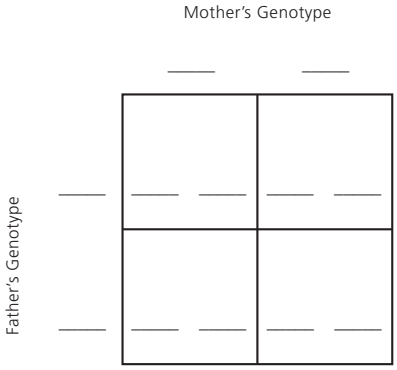
BRCA1-associated cancer involves inheritance of a normal copy of *BRCA1* (which we call BN) and a copy of *BRCA1* containing a mutation. Remember, a person inherits one allele from the mother and one allele from the father. Because there are many different *BRCA1* mutations that can cause cancer, we can use different numbers for each form of the gene (B_1, B_2, B_3). Only one type of mutation tends to affect each family. ***For the Lawler family, we will call this mutated form B_1.*** We would say that someone is ***heterozygous for the mutation*** if they inherited one normal copy of *BRCA1* and one mutated copy of *BRCA1*. Embryos that inherit two mutated copies of *BRCA1* cannot develop. If there is a chance a person is heterozygous, they should consider genetic testing for *BRCA1* mutations. Punnett squares help genetic counselors and patients decide who may benefit from genetic testing.

Let "B_1" denote an allele of the *BRCA1* gene that *can* cause increased risk of cancer

Let "B_N" denote an allele of the *BRCA1* gene that *does not* cause cancer (*N=No cancer*)

6. Fill in the following Punnett squares to show possible different genetic combinations:

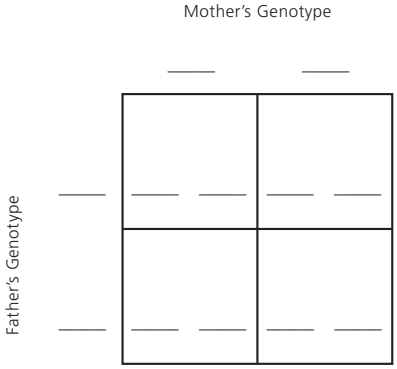
a. Both mother and father are heterozygous for the mutation:



What is the percent chance children will inherit:

- At least one allele for the mutation: _____
- No alleles with the mutation: _____

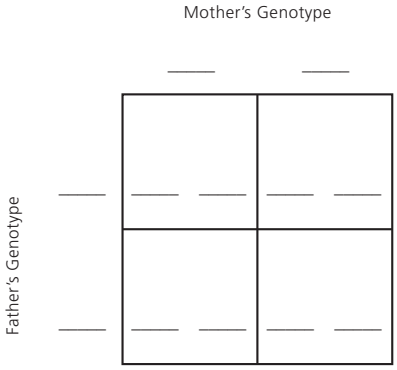
b. Mother has one allele that has the *BRCA1* mutation but the father has none:



What is the percent chance children will inherit:

- At least one allele for the mutation: _____
- No alleles with the mutation: _____

c. Neither mother nor father has an allele with the mutation:



What is the percent chance children will inherit:

- At least one allele for the mutation: _____
- No alleles with the mutation: _____

7. Which scenario (a, b, or c) do you think is the most likely for the Lawler family? Why?

4 Instructions for Aligning Sequences with BLAST

Aim: To understand how genetic tests are performed by comparing the DNA and protein sequences of a family to determine who is carrying the *BRCA1* mutation.

BLAST — Basic Local Alignment Search Tool

Background on *BRCA1*

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 somehow becomes damaged when exposed 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 abolish the BRCA1 protein's normal function, thus allowing cancer to develop.

Instructions: Write the answers to your questions on the Student Handout—*Aligning Sequences with BLAST Worksheet* in your lab notebook or on a separate sheet of paper, as instructed by your teacher.

PART I: Aligning DNA Sequences to a Reference Sequence

1. Access the DNA sequence file your teacher has given you. It contains the *BRCA1* reference DNA sequence and six individual Lawler family sequences.

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

3. Select "nucleotide blast," as shown in ***Figure 1***, since we will be comparing a DNA sequence (sequence of nucleotides) to a DNA sequence (sequence of nucleotides). Note that there are options for comparing protein sequences to protein sequences, and others.

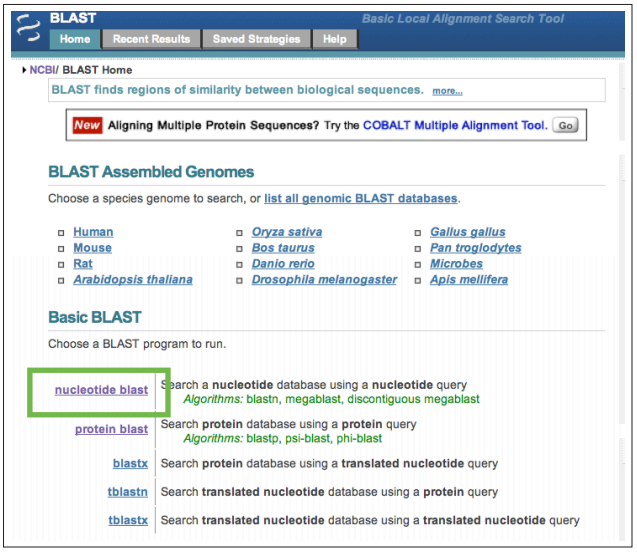


Figure 1: Select "Nucleotide BLAST."
Credit: NCBI BLAST.

4. From the nucleotide blast page, click the box to choose the option to "Align two or more sequences" (see ***Figure 2***).

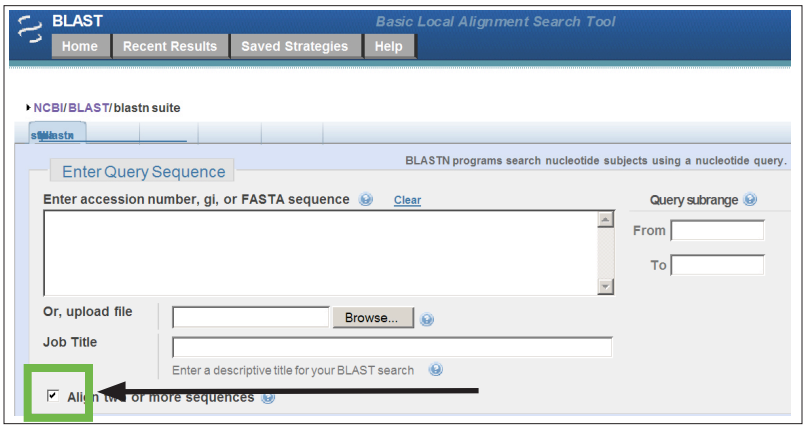


Figure 2: Align two or more sequences. Credit: NCBI BLAST.

5. A second text box will appear.

6. Copy the ***reference sequence*** for *BRCA1* from the file, ***including the ">" ("caret") symbol and the name***, and paste it into the top text box (see ***Figure 3***).

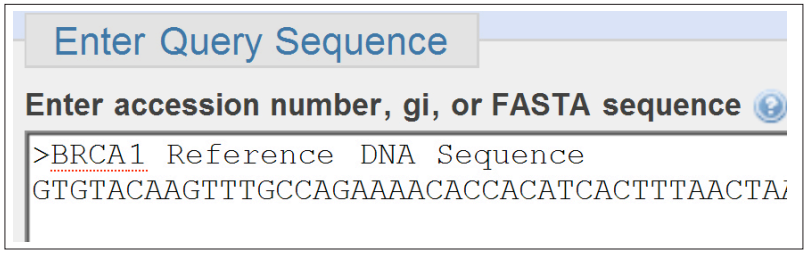


Figure 3: Copy the *BRCA1* reference sequence. Credit: NCBI BLAST.

7. Copy the ***DNA sequence*** from the person you are testing (Deb, Lori, Katherine, Mother, Father, or Uncle) and paste it into the ***bottom*** text box. ***Again, include the ">" symbol and the name***.

8. Click "BLAST."

9. When your search is complete, you will see a window with the BLAST results, showing an alignment of the two DNA sequences you entered above.

10. Click the "Formatting Options" link located near the top of the page (see ***Figure 4***).

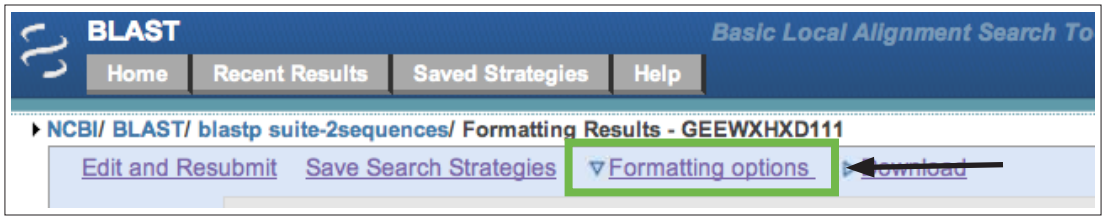


Figure 4: Click the "Formatting Options" link. Credit: NCBI BLAST.

11. Find the Alignment View and use the drop-down menu to choose "Query-anchored with dots for identities." The ***query*** is the reference sequence. The query-anchored 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). ***Dots are used to show nucleotides that are identical and letters are used to show nucleotides that differ.***

12. Click the "Reformat" button (see ***Figure 5***).

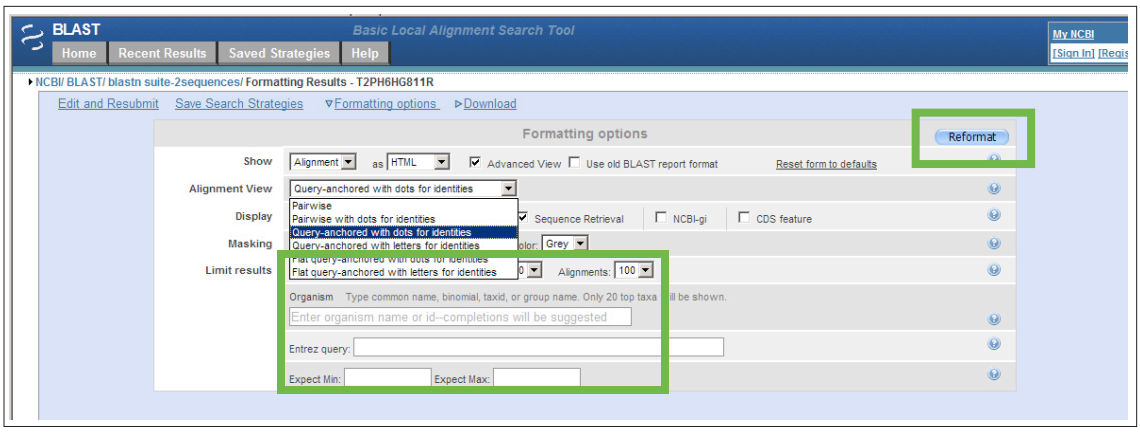


Figure 5: Click the "Reformat" button.
Credit: NCBI BLAST.

13. 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's been a change in the nucleotide at that position. Note the numbers at the ends of the lines refer to the position of the nucleotide (see ***Figure 6***).

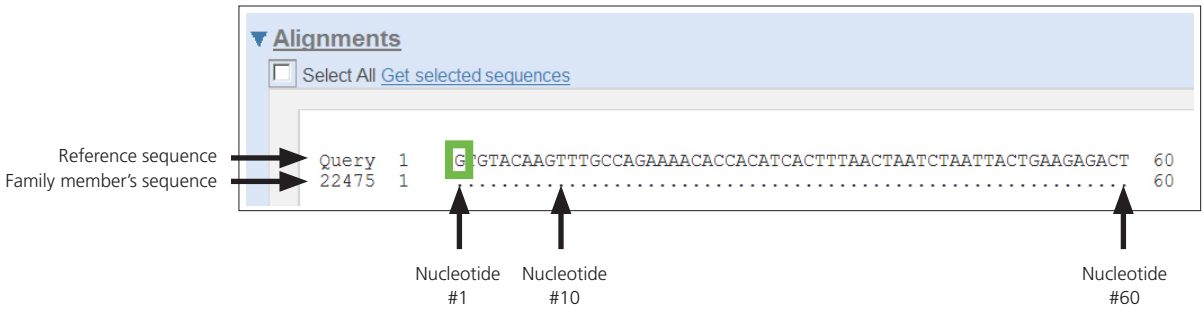


Figure 6: Comparing the query and subject sequences.
Credit: NCBI BLAST.

14. Explain why you think we need to compare DNA sequences and protein sequences of people in this family to a "reference sequence."

15. Are there any differences between the reference sequence (the top sequence marked "query") and the sequence you entered? What do you think this means?

16. BLAST is a powerful tool that can align more than one sequence at a time. Scroll to the top of the page and click the "Home" button (see ***Figure 7***).

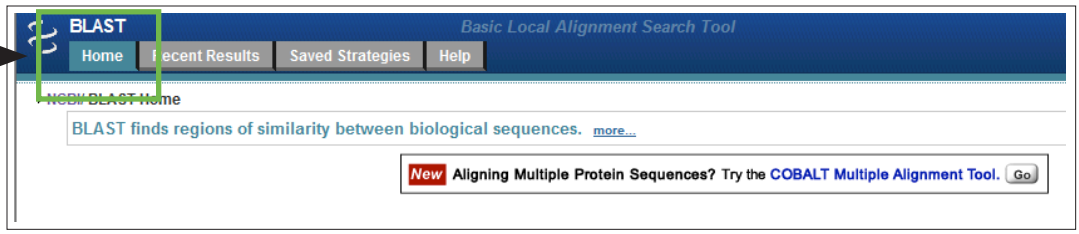


Figure 7: Click the "Home" button.
Credit: NCBI BLAST.

17. Go back to Step #2 to start a new BLAST alignment. This new alignment will include sequences from the entire Lawler family.

18. Copy the ***reference sequence*** for *BRCA1* from the file, including the ">" symbol and the name, and paste it into the ***top*** text box.

19. Copy the ***DNA sequences*** from all six individuals (Deb, Lori, Katherine, Mother, Father, and Uncle) and paste them into the ***bottom*** text box. Again, include the ">" symbols and the names. This can be done in one copy and paste function from the DNA Sequence File.

20. Click "BLAST."

21. Scroll down the page to see if there are positions where the query (reference) sequence differs from the subject (family member) sequence. Note that the numbers at the ends of the lines that refer to the position of the nucleotides, as shown in ***Figure 8***.

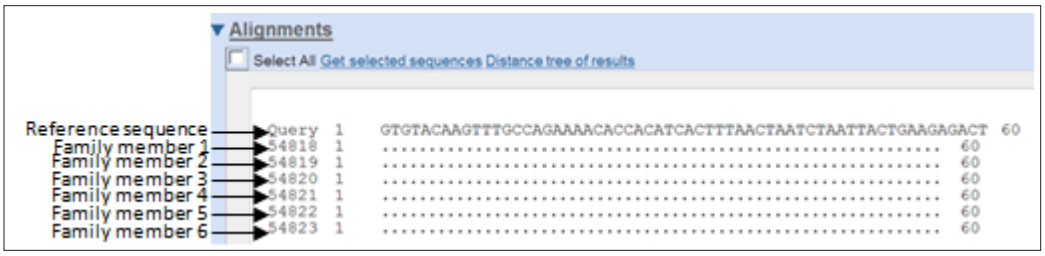
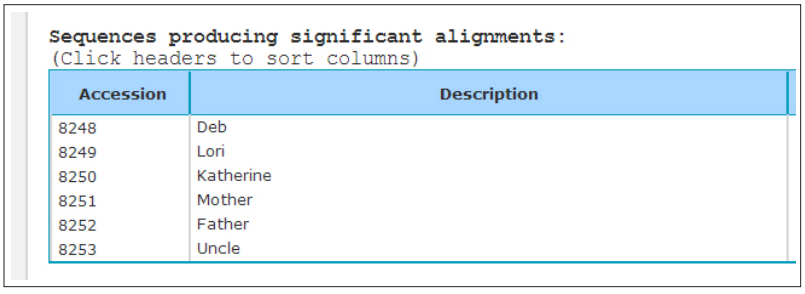


Figure 8: Note the numbers that refer to the positions of the nucleotides.
Credit: NCBI BLAST.

22. In the box above the alignment, you can see the legend for the sequence ID and the name of the subjects, as shown in ***Figure 9***.



Sequences producing significant alignments:
(Click headers to sort columns)

Accession	Description
8248	Deb
8249	Lori
8250	Katherine
8251	Mother
8252	Father
8253	Uncle

Figure 9: Sequence ID and subject names.
Credit: NCBI BLAST.

23. Do all of the family members have the same mutation? What is the location of the nucleotide that differs in some of the family members? (You will need to look at the numbers on the side of the alignment.)

24. On Student Handout—*Aligning Sequences with BLAST Worksheet*, circle the names of the Lawler family members who have this mutation, or list the names in your lab notebook or on your homework paper.

25. These differences, or changes to the DNA sequence, represent a mutation to the *BRCA1* gene. However, we need more information to determine whether this mutation results in a change in the amino acid found in the BRCA1 protein. Amino acids are encoded by three bases, called a codon. On Student Handout—*Aligning Sequences with BLAST Worksheet*, complete the table, including the codons and resulting amino acids (as represented by a one-letter abbreviation), or create a similar table in your lab notebook or on your homework paper. *See the codon table as instructed by your teacher*.

Lawler Family Sequence Analysis

	Reference Sequence	Mutated Sequence
DNA Coding Strand	ATG	?
DNA Template Strand	TAC	?
mRNA Codon	AUG	?
Amino Acid	?	?

26. What does it mean for the individual if that person has the mutation?

27. What does it mean for the individual if that person is free from the mutation?

28. Record your results from the nucleotide BLAST alignment in your **BLAST Results Document** by capturing an image from your computer screen using the following instructions:

a. Open up a new Word® document and label the document with your LASTNAME_BRCA1_NCBI. Type your name, class period, and date at the top of the blank page and add the title "**BLAST Results Document**."

b. Return to the BLAST results page.

c. Scroll down until the sequence showing the *BRCA1* mutation is centered on the computer screen.

i. **For PC users:** Hit the **Prnt Scrn** button on your keyboard. This is often on the top right of the keyboard, to the right of the F12 button.

ii. **For Mac users:** Press the keys: **Command + Shift + 4** at the same time. The image will be saved on your desktop.

d. Return to your Word® document.

i. **For PC users:** Use the paste function to transfer the captured image (**Crtl + V**).

ii. **For Mac users:** Open the **Insert** menu, choose **Picture** from file, and choose the image that you captured from the computer screen.

e. Save this document. Transfer it to a thumb drive or email it to yourself if you will not have access to this computer in the future.

PART II: Aligning Protein Sequences to a Reference Sequence

29. Access the Protein sequence file your teacher has given you. It contains the BRCA1 reference protein sequence and six individual Lawler family sequences.

30. Go back to the NCBI blast website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

31. Select "protein blast" (as shown in ***Figure 10***) since we will be comparing a protein sequence (sequence of amino acids) to a protein sequence (sequence of amino acids).

32. From the protein blast page, click the box to choose the option to "Align two or more sequences," as shown in ***Figure 11***.

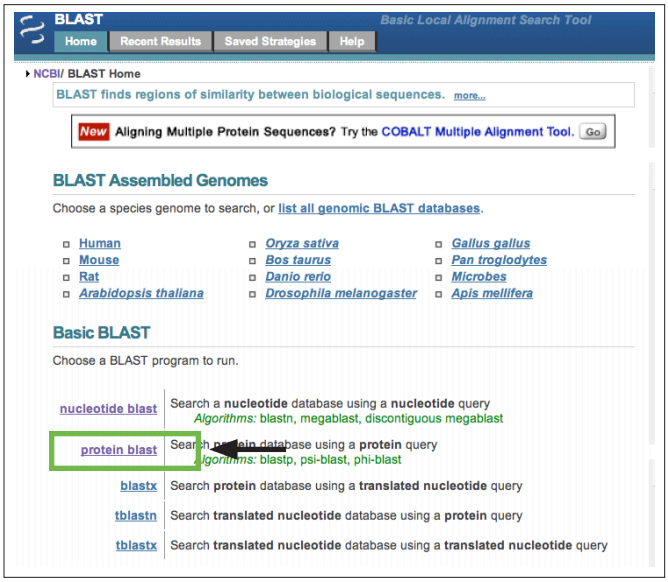


Figure 10: Select "Protein BLAST."
Credit: NCBI BLAST.

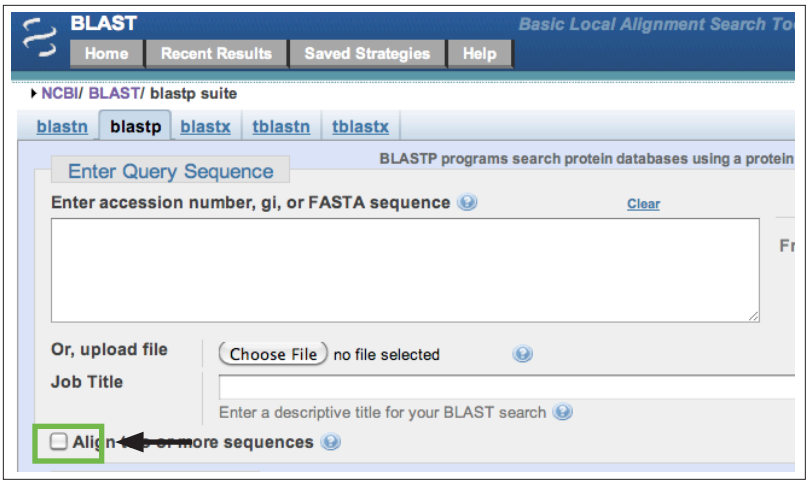


Figure 11: Select "Align two or more sequences."
Credit: NCBI BLAST.

33. A second text box will appear.

34. Copy the **reference sequence** for BRCA1 from the file, including the ">" symbol and the name, and paste it into the **top** text box.

35. Copy all six **protein sequences** from the entire Lawler family (Deb, Lori, Katherine, Mother, Father, and Uncle) and paste them into the **bottom** text box. Again, include the ">" symbol and the name.

36. Click "BLAST."

37. When your search is complete, you will see a window with the BLAST results, an alignment of all the protein sequences you entered above.

38. Click the "Formatting options" link located near the top of the page, as shown in ***Figure 12***.

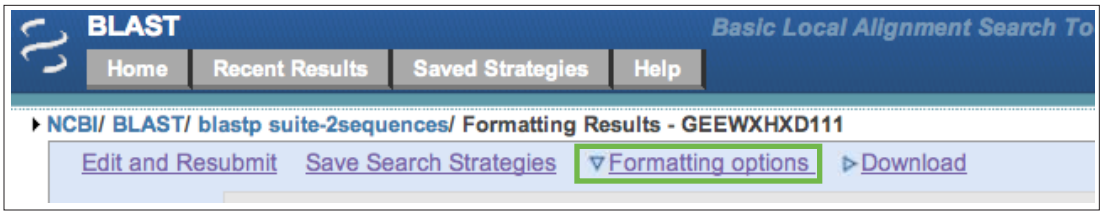


Figure 12: Click "Formatting options."
Credit: NCBI BLAST.

39. Find the Alignment View (see ***Figure 13***) and use the drop-down menu to choose "Query-anchored with dots for identities." ***Dots are used to show amino acids that are identical and letters are used to show the amino acids that differ.***

40. Click the "Reformat" button as shown in ***Figure 13***.

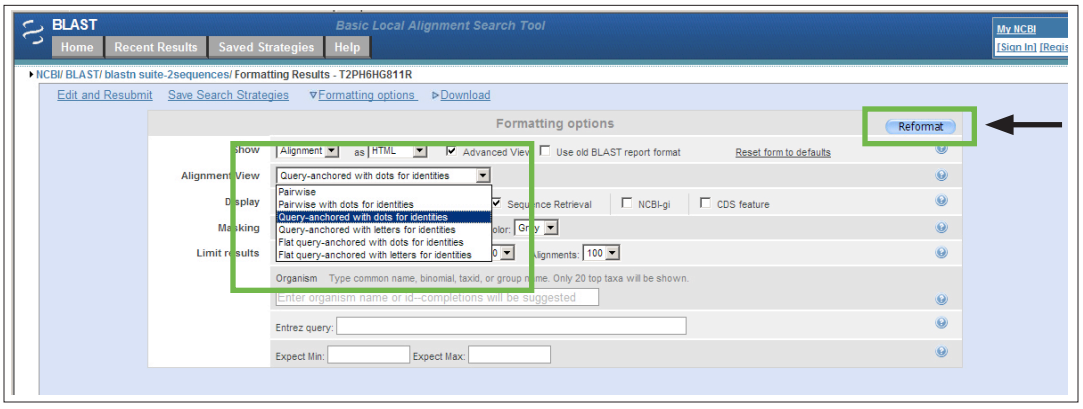


Figure 13: Locate "Alignment View" and the "Reformat" button.
Credit: NCBI BLAST.

41. 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 is there 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 ends of the lines refer to the position of the amino acid, as shown in ***Figure 14***.

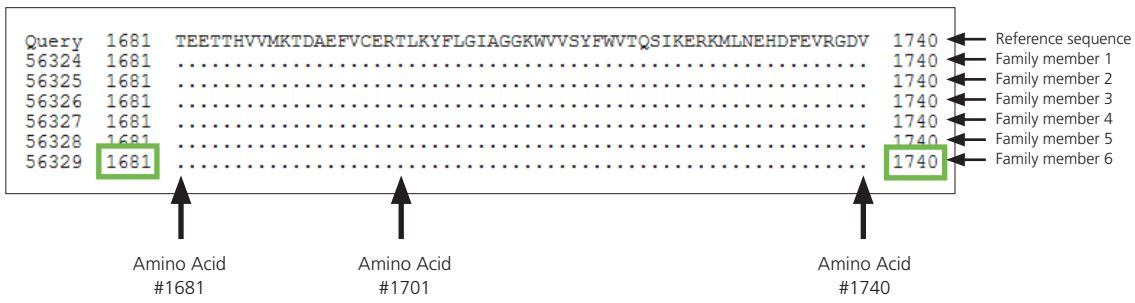


Figure 14: Compare the reference and subject sequences.
Credit: NCBI BLAST.

42. Are there any differences between the reference (query) sequence and the family member sequences you entered? If so, do all of the family members have the same mutation? What was it?

43. These differences, or changes to the amino acid sequence, are a result of the mutation in the *BRCA1* gene. **Answer the following questions:**

    a. What is the amino acid in the reference sequence (as represented by a one-letter abbreviation)?

    b. What is the amino acid in the sequences containing the mutation?

    c. Is this the amino acid that you expected based on your DNA analysis in Part I, in the table "Lawler Family Sequence Analysis?"

    d. Where is the location of the change? (Count carefully using the reference numbers at the end of the row.)

44. Which individuals in the Lawler family have the change in their amino acids?

45. Are your answers to Question #44 the same as your answers from your DNA analysis (Part I, Question #24)? Is this what you would expect? Why or why not?

46. Record your results from the protein BLAST alignment by capturing an image from your computer and saving it in your **BLAST Results Document** from Step #28. Refer to the image capturing instructions in Step #28 if you need help with this step.

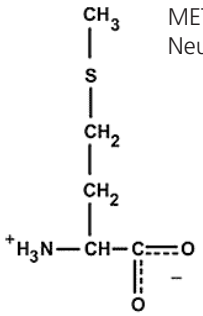
47. Now that you have some test results, return to Student Handout—*Lawler Family Pedigree*.

    i. Fill out as much additional information as you can for the pedigree.

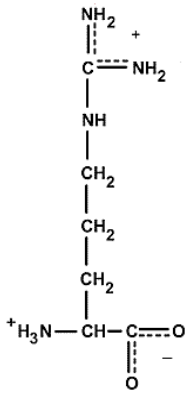
    ii. Which Punnett square most accurately represents the Lawler family? Why?

PART III: Extension Questions

48. ***M*** is the one-letter code for the amino acid methionine. ***R*** is the one-letter code for the amino acid Arginine. Look at the structures below.



METHIONINE (M)
Neutral, Non-Polar



ARGININE (R)
Positive Charge, Polar

49. Describe at least three ways in which methionine and arginine are different (i.e., polarity, size, shape, composition).

50. How might this mutation affect the protein?

Name _____ Date _____ Period _____

4 Aligning Sequences with BLAST Worksheet

BLAST — Basic **L**ocal **A**lignment **S**earch **T**ool

Aim: To understand how genetic tests are performed by comparing the DNA and protein sequences of a family to determine who is carrying the *BRCA1* mutation.

Instructions: Use Student Handout—*Instructions for Aligning Sequences with BLAST* to complete this handout.

PART I: Aligning DNA Sequences to a Reference Sequence

14. Explain why you think we need to compare DNA sequences and protein sequences of people in this family to a "reference sequence."

15. Are there any differences between the reference sequence (the top sequence marked "query") and the subject sequence you entered? What do you think this means?

23. Do all of the family members have the same mutation? _____

    What is the location of the nucleotide that differs in some of the family members? _____

24. Circle the individuals in the Lawler family who have this DNA mutation:

    Deb Lori Katherine Mother Father Uncle

25. These differences, or changes to the DNA sequence, represent a mutation to the *BRCA1* gene. However, we need more information to determine whether this mutation results in a change in the amino acid found in the BRCA1 protein. Complete the table below, including the codons and resulting amino acids.

Lawler Family Sequence Analysis

	Reference Sequence	Mutated Sequence
DNA Sense Strand	ATG	
DNA Template Strand	TAC	
mRNA Codon	AUG	
Amino Acid		

26. What does it mean for the individual if that person ***has*** the mutation?

27. What does it mean for the individual if that person is ***free*** from the mutation?

PART II: Aligning Protein Sequences to a Reference Sequence

42. Are there any differences between the reference (query) sequence and the family member sequences you entered? If so, do all of the family members have the same mutation? What was it?

43. These differences, or changes to the amino acid sequence, are a result of the mutation in the *BRCA1* gene.

    a. What is the amino acid in the reference sequence (as represented by a one-letter abbreviation)? _____

    b. What is the amino acid in the sequences containing the mutation? _____

    c. Is this the amino acid that you expected based on your DNA analysis in Part I, in the table "Lawler Family Sequence Analysis"?

    d. Where is the location of the change? (Count carefully using the reference numbers at the end of the row.)

44. Circle the individuals in the Lawler family who have the change in their amino acids:

    Deb Lori Katherine Mother Father Uncle

45. Are your answers to the question above the same as your answers from the DNA analysis (Question #24)? Is this what you would expect? Why or why not?

PART III: Extension Questions

49. Describe at least three ways in which methionine and arginine are different (i.e., polarity, size, shape, composition).

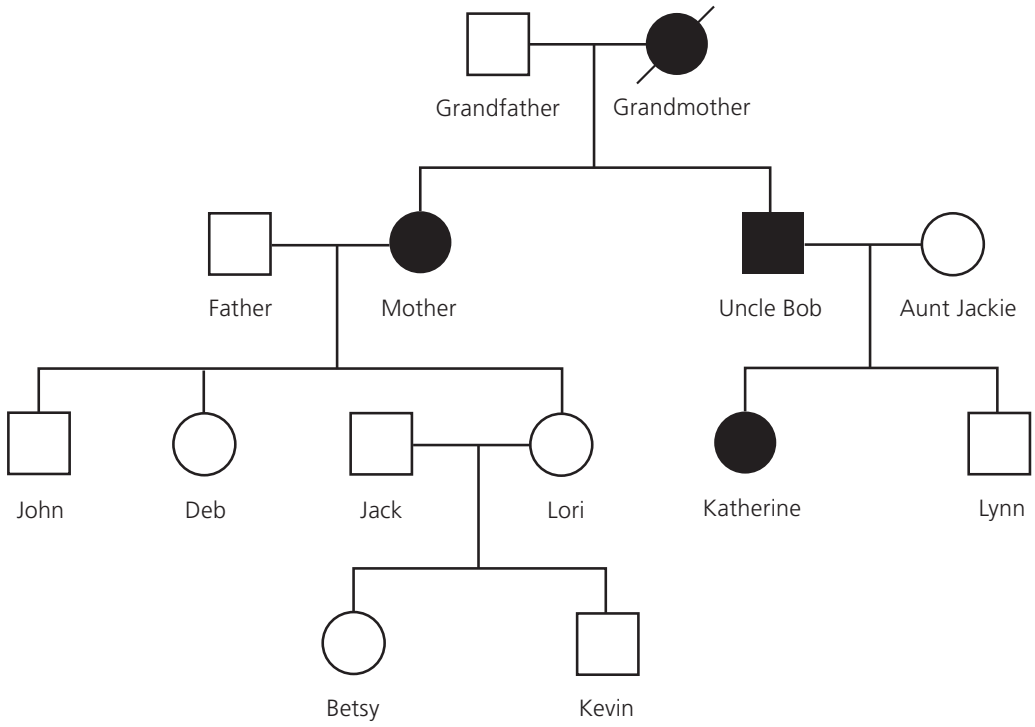
50. How might this mutation affect the protein?

4 Lawler Family Phenotype Pedigree: Who has Breast Cancer? Teacher Answer Key

[**Note:** Suggested point values are included for each question/task, and are intended to provide general guidelines for the weight each question/task could be given. Using these suggested point values, the total value for this worksheet is **16 points** (Part 1: 5 points; Part 2: 7 points; Part 3: 8 points).]

PART I: Use the case study from Student Handout—*Case Study: A BRCA Genetic Testing Dilemma* (from *Lesson Three*) to fill out the pedigree and Punnett squares. Record as much information about a person as possible. Color in the square or circle if that person has received a diagnosis of breast cancer.

(+1 point for each correctly filled-in shape. +1 for grandmother deceased. -1 for each incorrect designation.)



Pedigree Legend

- Female, unaffected
- Male, unaffected
- Female, affected
- Male, affected
- Female, unaffected, deceased
- Male, unaffected, deceased

"Affected" means the individual has received a diagnosis of breast cancer (phenotype). Until tested, the BRCA status (genotype) is unknown.

PART II: Use the pedigree chart on the previous page and the case study on Student Handout—*Case Study: A BRCA Genetic Testing Dilemma* (from *Lesson Three*) to answer the following questions:

1. If someone inherits one copy of *BRCA1* with a mutation from a parent, will they get breast cancer? Why or why not?

No. They may develop cancer. Inheritance of a *BRCA1* mutation brings with it an increased risk for breast and ovarian cancer. They may or may not actually develop cancer during their lifetime.

(+ 1 for answering 'no' with accurate explanation.)

2. Which members of the Lawler family should be tested for mutations to the *BRCA1* gene? Why?

Answers may vary. Deb should be tested because she appears to have the strongest interest in knowing the outcome. Deb should also be tested because if she tests positive for the mutation, she likely inherited the mutation from her mother. This means that Deb's sister and brother have a 50:50 chance of having inherited the mutation. If Deb's grandmother could be tested, her test results would have an effect on the entire family. If she is free from the mutation, it is more likely that the next generation is also free from the mutation. Conversely, if she were to have the mutation, it is likely that both of her children inherited it since they have both been affected by breast cancer.

(+1 for listing at least 2 family members; +1 for logical reasons why.)

3. Do any of the bioethical principles apply to the answer to Question #2? The bioethical principles are: Respect for Persons, Maximizing Benefits/Minimizing Harms, and Justice.

The bioethical principle Respect for Persons would support Deb's decision to get tested since she has the right to make her own decisions about her health care and treatment. This would also support Deb's cousin Lynn's decision to **not** get tested. The principle of Maximize Benefits/Minimize Harms also applies to Deb getting tested for the mutation since her results will have an impact on the rest of her family. "Doing good" for Deb (getting the test) must be weighed against the harm it might do to a relative who does not want to know his or her own predisposition to breast cancer, but for whom Deb's results have an implication. For example, Deb's brother doesn't want to know his own status, but a positive test result for Deb means that he has a 50% chance of having inherited the mutated gene.

(+1 for listing bioethical principles that apply.)

4. Are there any special considerations one should think about when testing children for a genetic disease? Do any of the bioethical principles speak to this?

Consenting to genetic testing for children falls to the parent or guardian. It is important to consider the "age of onset" of the genetic disease being tested. If the disease or condition affects children, then the child would most likely be tested if the parents consent. If the genetic disease or condition doesn't present until adulthood and there is no early treatment, then parents would be directed to wait until the child is old enough to make the decision for him or herself. Children are also considered a "vulnerable population" and must be respected as such. Respect for Persons addresses this.

(+ 1 for listing a special consideration; +1 for the bioethical principle 'respect for persons'.)

5. Any individual having a genetic test would need to sign a consent form for this test. Who, if any, of the individuals on the pedigree chart would likely refuse to sign the form?

Deb's cousin, Lynn (Katherine's sister), does not seem open to discussing the test and would probably not consent. Deb's brother, John, expressed ambiguity and may not consent to the test. Deb's Aunt Jackie was upset by Deb pursuing genetic testing, and would likely not consent to the test.

(+1 for choosing Lynn, Aunt Jackie, and John.)

PART III: What are the chances of passing on the *BRCA1* mutation to one's children?

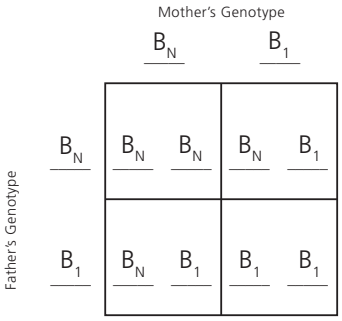
BRCA1-associated cancer involves inheritance of a normal copy of *BRCA1* (which we call BN) and a copy of *BRCA1* containing a mutation. Remember, a person inherits one allele from the mother and one allele from the father. Because there are many different *BRCA1* mutations that can cause cancer, we can use different numbers for each form of the gene (B_1, B_2, B_3). Only one type of mutation tends to affect each family. ***For the Lawler family, we will call this mutated form B_1.*** We would say that someone is ***heterozygous for the mutation*** if they inherited one normal copy of *BRCA1* and one mutated copy of *BRCA1*. Embryos that inherit two mutated copies of *BRCA1* cannot develop. If there is a chance a person is heterozygous, they should consider genetic testing for *BRCA1* mutations. Punnett squares help genetic counselors and patients decide who may benefit from genetic testing.

Let "B_1" denote an allele of the *BRCA1* gene that *can* cause increased risk of cancer

Let "B_N" denote an allele of the *BRCA1* gene that *does not* cause cancer (*N=No cancer*)

6. Fill in the following Punnett squares to show possible different genetic combinations:

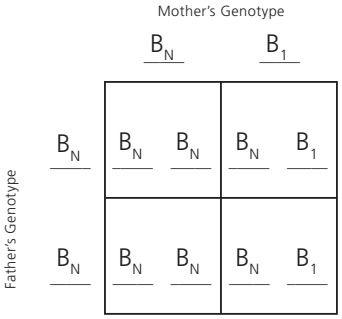
a) Both mother and father are heterozygous for the mutation:



What is the percent chance children will inherit:

- At least one allele for the mutation: 75%
- No alleles with the mutation: 25%

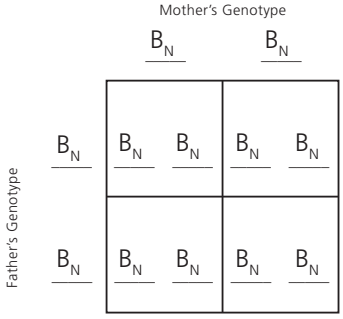
b) Mother has one allele that has the *BRCA1* mutation but the father has none:



What is the percent chance children will inherit:

- At least one allele for the mutation: 50%
- No alleles with the mutation: 50%

c) Neither mother nor father has an allele with the mutation:



What is the percent chance children will inherit:

- At least one allele for the mutation: 0%
- No alleles with the mutation: 100%

Let students know that if both parents passed on mutated copies of *BRCA1* (B1B1), the condition would be so harmful that the embryo would not develop. (+1 for each correctly filled in Punnett square; +0.5 for each correct percentage.)

7. Which scenario (a, b, or c) do you think is the most likely for the Lawler family? Why?

Answer: b. The mother has breast cancer and tested positive for a mutation in *BRCA1*. The father is not a carrier. (+1 for answering 'b'; +1 for explanation.)

4 Aligning Sequences with BLAST Worksheet
Teacher Answer Key

[**Note:** Suggested point values are included after each question, and are intended to provide general guidelines for the weight each question could be given. Using these suggested point values, the total value for this worksheet is **20 points, plus 5 points for the optional extension questions**.]

PART I: Aligning DNA Sequences to a Reference Sequence

14. Explain why you think we need to compare DNA sequences and protein sequences of people in this family to a "reference sequence."

Reference sequences are sequences that are known to be free of any cancer-causing mutations and are used to determine if a patient's sequence contains any mutations, relative to the reference sequence.

(+1 for including bolded portion.)

15. Are there any differences between the reference sequence (the top sequence marked "query") and the sequence you entered? What do you think this means?

This change indicates that the patient/family member has inherited a mutation in *BRCA1*. For a list of family members whose sequences contain the mutation, see Question #24 below.

(+1 for indicating there are changes; +1 for explaining that there is a mutation.)

23. Do all of the family members have the same mutation? Yes

What is the location of the nucleotide that differs in some of the family members?

Base #332 (+1 for 'yes' and correct location.)

[**Note:** This is base #332 in this DNA sequence; the full *BRCA1* gene is much longer.]

24. Circle the individuals in the Lawler family who have this DNA mutation:

Deb Lori Katherine Mother Father Uncle

(+2 for circling all correct family members, +0.5 pts each.)

25. These differences, or changes to the DNA sequence, represent a mutation to the *BRCA1* gene. However, we need more information to determine whether this mutation results in a change in the amino acid found in the BRCA1 protein. Complete the table below, including the codons and resulting amino acids.

Lawler Family Sequence Analysis

	Reference Sequence	Mutated Sequence
DNA Sense Strand	ATG	***AGG***
DNA Template Strand	TAC	***TCC***
mRNA Codon	AUG	***AGG***
Amino Acid	***methionine***	***arginine***

(+1 for correct amino acids; +1 for correct mutated sequence, +1 for correct transcription of mutated strand. 3 points total.)

26. What does it mean for the individual if that person ***has*** the mutation?

A person with this type of mutation has a much higher chance of developing breast cancer in his or her lifetime. A cancer-causing mutation in the *BRCA1* or *BRCA2* gene could increase a woman's chance of breast cancer from about 12% (the average lifetime risk for all women) to anywhere between 50-85%. A mutation in the BRCA genes also increases her risk of ovarian cancer. A cancer-causing mutation in the *BRCA1* or *BRCA2* gene also increase a man's risk of getting breast cancer.

(+1 for higher chance of developing breast cancer.)

27. What does it mean for the individual if that person is ***free*** from the mutation?

The chance of developing breast or ovarian cancer is no higher than for the general population.

(+1 for same chance of developing breast or ovarian cancer.)

PART II: Aligning Protein Sequences to a Reference Sequence

42. Are there any differences between the reference (query) sequence and the family member sequences you entered? If so, do all of the family members have the same mutation? What was it?

All of the family members noted above in Question #24 should have the same mutation (Deb, Katherine, Mother, Uncle; methionine to arginine).

(+1 for 'yes' and +1 for methionine to arginine.)

43. These differences, or changes to the amino acid sequence, are a result of the mutation in the *BRCA1* gene.

a. What is the amino acid in the reference sequence (as represented by a one-letter abbreviation)?

M (Methionine). (+1.)

b. What is the amino acid in the sequences containing the mutation?

R (Arginine). (+1.)

c. Is that what you expected based on your DNA analysis in Part I, in the table "Lawler Family Sequence Analysis"?

Yes, this is what was predicted based on the codons seen during the DNA analysis. (+1.)

d. Where is the location of the change? (Count carefully using the reference numbers at the end of the row.)

Amino acid #1775. (+1 for correct number.)

44. Circle the individuals in the Lawler family who have the change in their amino acids:

Deb Lori Katherine Mother Father Uncle

(+2 for circling all correct family members, +0.5 pts each.)

45. Are your answers to the question above the same as your answers from the DNA analysis (Question #24)? Is this what you would expect? Why or why not?

Yes, these are the same. This is what we would expect, as the DNA encodes the protein we are now analyzing.

(+1 for answering 'yes'; +1 for explaining that DNA codes for the protein.)

PART III: Extension Questions

49. Describe at least three ways in which methionine and arginine are different (i.e., polarity, size, shape, composition).

- Methionine is a neutral molecule, arginine is positively charged.
- Methionine is a non-polar molecule, arginine is polar.
- Methionine is a smaller molecule than arginine.
- Students might know that methionine is hydrophobic, and arginine is hydrophilic based on their polarity.

(+1 for each difference up to +3.)

50. How might this mutation affect the protein?

It might not work correctly if one amino acid is substituted for another. The larger amino acid might take up too much space, changing the shape of the structure. The change in the charge and polarity of the amino acid if M is mutated to R might also affect how the protein works.

Students may know that hydrophobic (non-polar) amino acids fold into the inside of the protein structure, while hydrophilic (polar) amino acids tend to rotate to the outside of the protein structure. Substituting a polar amino acid for a non-polar amino acid may change the shape of the protein significantly.

(+1 for each reasonable possible effect.)