



Credits

Credits/Funding Source

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Overview

Unit Overview

The curriculum unit explores how bioinformatics is applied to genetic testing. Specifically, the bioinformatics tools of BLAST and Cn3D are used to investigate the genetic and molecular consequences of a mutation to the Breast Cancer Susceptibility 1 (*BRCA1*) gene. Students are also introduced to principles-based bioethics in order to support their thoughtful consideration of the many social and ethical implications of genetic testing. Throughout the unit, students are presented with a number of career options in which the tools of bioinformatics are used.

Essential Understandings

1. Biological molecules store and process information.
2. The structure of molecules is closely related to their function. Changes in structure can often impact function.
3. Acquisition of biological information has many societal and ethical implications; students need tools to evaluate and decide how information should be used.
4. Technology influences how science is done; bioinformatics gives us new tools to understand biological information.
5. Bioinformatics is used in many areas of life sciences and related fields.

Unit Objectives

1. Students will be able to explain how bioinformatics tools are useful in analyzing biological sequence and structure information.
2. Students will be able to apply sequence analysis and protein visualization tools to explore genetic disorders.
3. Students will be able to identify and critically evaluate the ethical implications of genetic testing for individuals and their families, and society at large.
4. Students will evaluate the use of bioinformatics in the life sciences and describe how bioinformatics tools could be used in various careers.

Instructional Components

The Curriculum: The *Using Bioinformatics: Genetic Testing* curriculum consists of six sequential lessons, a seventh lesson which focuses on careers that use the tools of bioinformatics, and an assessment activity.

Throughout this curriculum, a variety of resources are provided. These materials include:

- Student “Handouts” that are designed to be printed or copied and given to each student as a “worksheet.” Answers to lesson activity and/or homework questions made be completed on the handouts, on separate sheets of paper, or in lab notebooks, as desired by the teacher.

- “Class Sets” that contain lesson activity instructions for students and are designed to be printed or copied and re-used as class sets. Questions that students should answer on their handout, piece of paper, or lab notebook are indicated with an icon.



- Teacher “Resources” that include teacher demonstrations and additional information.
- Teacher Answer “Keys” that provide suggested answers and scoring information.

Time Commitment: Each lesson requires a minimum of one hour of class time. Some lessons require two class sessions, and some lessons include homework assignments. The entire unit (eight lessons) is expected to take 10–11 class periods of 50 minutes each.

Prior Knowledge Needed: This curriculum is not designed to introduce students to the “Central Dogma” of biology (that information in DNA is transcribed into mRNA and then translated into protein), but to reinforce that concept. Students should have already been exposed to DNA replication, transcription, and translation. Student understanding of these processes should be deepened through the use of this curriculum.

Career Component

Each lesson in the curriculum is accompanied by a PowerPoint slide highlighting a person in a career that uses bioinformatics, followed by a slide providing job information about that career. Student Handout—*Careers in the Spotlight* is given to students during the first lesson. Students are expected to take daily notes on this handout at the beginning of class for the duration of the unit.

Lesson Seven focuses entirely on careers that use bioinformatics. Students use interview transcripts (from professionals working in careers that use bioinformatics) and internet research to augment the information they have already constructed for Student Handout—*Careers in the Spotlight*.

Although bioinformatics is a career choice in itself, there are a wide variety of careers that use the tools of bioinformatics. This curriculum highlights a broad range of career paths, even if the use of bioinformatics within that career is not central.

Technology Requirements

For each lesson, teachers will need to be able to project a PowerPoint slide for the class to see. If this is not possible, teachers can describe the careers that begin each lesson to students based on the PowerPoint slides provided, or copy the PowerPoint slides to transparencies and project them for students using an overhead projector.

Lesson One requires the capability to show an online streaming video to the class. Alternatively, a DVD player and screen can be used if the teacher has a DVD copy of the NOVA video *Cracking the Code of Life*.

Lessons Two, Four, Five, Seven, and Eight require that students have access to both the internet and word processing software.

Lesson Five requires that the teacher project images from a computer with an internet connection. This would also be helpful for *Lessons Two, Four, and Seven*.

Lesson Five includes an animation that can be projected from the teacher’s computer. This lesson also requires the Cn3D program to be downloaded and installed on all student computers. The program can be downloaded from: <http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml>.

Before Beginning the Unit

Set classroom discussion norms. It is especially important to foster a safe classroom atmosphere when discussing ethical issues about genetic testing that may involve conflicting moral choices. Please review or create classroom discussion ground rules (norms) before proceeding. Instructions for doing this can be found in the Appendix.

Notify students that the class will be discussing genetics and genetic diseases, including cancer. Everybody in the classroom has been, or will be, touched in some way by this topic. Before the unit begins, give students a chance to alert the teacher to any genetic conditions or diseases that may be affecting them or their family, if the student would like to do so. Students will have different levels of comfort for discussing these issues publically.

Prepare for the Meet the Gene Machine play acted out in Lesson One. Assign the parts of Scientist and TV Talk Show Host to two willing students. Teachers may also assign the role of stage manager/director to a student who can create the minimal set and help produce the play.

Install Cn3D on all computers. Contact your school administrator or IT support staff to be sure the Cn3D program has been downloaded and installed on all student computers for Lesson Five. The program can be downloaded from: <http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml>.

Additional Resources

Breast Cancer and BRCA: Background information about the structure, function, and risks associated with mutations to the *BRCA1* or *BRCA2* genes can be found in the *Appendix*.

DNA Structure: *Exploring DNA Structure* by Dr. Sandra Porter contains information on the discovery and structure of DNA along with hands-on activities that students can use to explore the structure of DNA first-hand. Students determine where molecules bind to DNA, investigate base-pairing, examine the phosphodiester backbone, and study the interaction between DNA strands. *Exploring DNA Structure* is also available on a CD together with 76 DNA structures, Cn3D, and the textbook. For more information, see www.digital-world-biology.com.

Ethics: Additional information about ethical theories and perspectives can be found in *An Ethics Primer: Lesson Ideas and Ethics Background* by Jeanne Ting Chowning and Paula Fraser, produced through the Northwest Association for Biomedical Research. NWABR's *Bioethics 101 Curriculum* provides a systematic, five-lesson introductory course to support educators in incorporating bioethics into the classroom through the use of sequential, day-to-day lesson plans. This curriculum is designed to help science teachers in guiding their students to analyze issues using scientific facts, ethical principles, and reasoned judgment. The complete *Ethics Primer* and *Bioethics 101* are available free for download from <http://www.NWABR.org>.

Molecular Structures: Have you ever wanted to find molecular structures that you can use as class examples? *A Beginner's Guide to Molecular Structure*, by Dr. Sandra Porter, navigates through the NCBI databases to help teachers determine if structures come from normal or mutant proteins, and to identify the parts of the protein that are found in the structure. Activities include superimposing influenza structures to see why one strain could become resistant to Tamiflu, working with green fluorescent protein, and more. For more information, see <http://www.digital-world-biology.com>.

Lesson Overview

Lesson One: Bioinformatics and Genetic Testing

A short topical play introduces students to the fields of bioinformatics, genetic testing, direct-to-consumer genetic testing, and ethical considerations. Students discuss some of the broad implications and ethical questions raised from gaining information through genetic testing. Students then consider a number of genetic tests and their potential usefulness and value and, as a class, explore the website of 23andMe, a company that offers direct-to-consumer genetic tests. The lesson wraps up as it began—by engaging students in a story. Through a short video, students are introduced to a family impacted by breast cancer. In Lesson One, students also learn how **bioengineers** might use bioinformatics tools in their career.

Lesson Two: Navigating the NCBI

Students navigate parts of the National Center for Biotechnology Information (NCBI) website and work independently to explore databases, focusing on the *BRCA1* gene and the bioinformatics tool Map Viewer. Through an analogy that compares two collections of databases (iTunes® and the NCBI), students connect with their own prior knowledge to better understand database structure and function. In *Lesson Two*, students learn how **veterinarians** might use bioinformatics tools in their career.

Lesson Three: Exploring Genetic Testing: A Case Study

In this lesson, students engage in a case study about a family with a history of breast cancer. Students consider ethical issues surrounding genetic testing as they decide whether or not family members should get tested for *BRCA1* or *BRCA2* mutations. Students then evaluate the case through the principles-based bioethics concepts of: Respect for Persons, Maximize Benefits/Minimize Harms, and Justice. Students apply the principles to help them reason through their decision as they participate in a Structured Academic Controversy. In *Lesson Three*, students learn how **genetic counselors** might use bioinformatics tools in their career.

Lesson Four: Understanding Genetic Tests to Detect *BRCA1* Mutations

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 as to 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.

Lesson Five: Learning to Use Cn3D: A Bioinformatics Tool

Up to this point, students have seen the *BRCA1* protein represented in a linear, sequential form. In this lesson, students are introduced to the high importance of a protein's three-dimensional structure. Students first engage in a short activity in which they use a pipe cleaner to perform a simple function, as an analogy for the relationship between a protein's structure and function. Students then learn to navigate between linear protein sequences and three-dimensional structures by using the bioinformatics tool Cn3D. Students begin by viewing and manipulating DNA—a familiar molecule to students—using Cn3D. When students are familiar with the program, students visualize parts of the *BRCA1* protein to show how a specific mutation in the *BRCA1* gene ultimately changes or destroys the protein's function. In *Lesson Five*, students learn how **3D animators** might use bioinformatics tools in their career.

Lesson Six: Evaluating Genetic Tests: A Socratic Seminar Discussion

In this lesson, students apply the ethical skills and scientific knowledge they have acquired over the previous lessons to determine (1) whether *BRCA1* testing meets the standards of a useful genetic test, or (2) whether direct-to-consumer genetic testing should include genetic counseling of clients. Students or teachers may choose from one of two readings, after which students participate in a Socratic Seminar in order to deepen their understanding about genetic testing. Through the seminar discussion of the first reading, students become familiar with a framework for considering genetic tests in terms of their clinical validity and the availability of effective treatment. Through the seminar discussion of the second reading, students become familiar with issues and preliminary data regarding the effects of direct-to-consumer genome-wide screening. After the seminar, students are supported in coming to an individual position about genetic testing through the integration of scientific facts, stakeholder viewpoints, and ethical considerations. In *Lesson Six*, students learn how **bioethicists** might use bioinformatics tools in their career.

Lesson Seven: An Introduction to Bioinformatics Careers

In this lesson, students explore more deeply the information they have learned throughout the unit about people in various careers that use bioinformatics. Students choose one career they would like to learn more about. They further explore that career by reading a series of in-depth questions asked of the person highlighted in that career, as well as provided internet resources. Students then respond to a job posting for a summer internship in their chosen field, developing a resume for that position. Optional activities include peer-editing of resumes and socializing in a professional environment.

Lesson Eight: Genetic Testing Unit Assessment: *ALAD* and *SOD1*

As an assessment of the unit, students revisit some of the bioinformatics tools they have used in prior lessons in order to locate a mutation in a protein associated with a genetic condition. Students also evaluate current genetic tests for the condition using the criteria of clinical validity and treatment options. Two conditions and their tests are presented: porphyria and amyotrophic lateral sclerosis (ALS).

National Science Education Standards

	Lesson 1	Lesson 2	Lesson 3	Lesson 4	Lesson 5	Lesson 6	Lesson 7	Lesson 8
Science as Inquiry								
Abilities necessary to do scientific inquiry		•		•	•		•	•
Understandings about scientific inquiry	•			•	•	•	•	•
Science and Technology								
Abilities of technological design	•	•		•	•	•	•	•
Understandings about science and technology	•	•	•	•	•	•	•	•
Science in Personal and Social Perspectives								
Personal health and community health	•	•	•	•	•	•	•	•
Science and technology in local, national, and global challenges	•	•	•	•	•	•	•	•
History and Nature of Science								
Science as a human endeavor	•	•	•	•	•	•	•	•
Nature of scientific knowledge	•	•	•			•	•	•

Source: National Research Council. (1995). *National Science Education Standards*. Washington D.C.: National Academies Press.

Next Generation Science Education Standards

	Lesson 1	Lesson 2	Lesson 3	Lesson 4	Lesson 5	Lesson 6	Lesson 7	Lesson 8
Scientific Practices								
1. Asking Questions	•	•	•	•	•	•		•
2. Developing and Using Models								
3. Planning and Carrying Out Investigations		•		•	•			•
4. Analyzing and Interpreting Data		•		•	•			•
5. Using Mathematics, Information and Computer Technology, and Computational Thinking		•		•	•			•
6. Constructing Explanations	•	•	•	•	•	•		•
7. Engaging in Argument from Evidence			•			•		•
8. Obtaining, Evaluating, and Communicating Information	•	•	•	•	•	•	•	•
Crosscutting Concepts								
Patterns								
Cause and Effect: Mechanisms and Explanation		•		•	•			•
Scale, Proportion, and Quantity								
Systems and System Models								
Energy and Matter: Flows, Cycles, and Conservation								
Structure and Function				•	•			•
Stability and Change								
Core Ideas: Life Sciences								
LS 1: From Molecules to Organisms: Structures and Processes								
LS 2: Ecosystems: Interactions, Energy, and Dynamics								
LS 3: Heredity: Inheritance and Variation of Traits		•	•	•	•			•
LS 4: Biological Evolution: Unity and Diversity		•						

Source: Committee on Conceptual Framework for the New K-12 Science Education Standards, National Research Council. (2011). A Framework for K-12 Science Education: Practices, Crosscutting Concepts, and Core Ideas. Washington D.C.: National Academies Press.

1 Bioinformatics and Genetic Testing

Introduction

Through a short topical play, students are introduced to the fields of **bioinformatics**, **genetic testing**, **direct-to-consumer genetic testing**, and ethical considerations. Students discuss some of the broad implications and ethical questions raised by gaining information through genetic testing. Students then consider a number of genetic tests and their potential usefulness and value and, as a class, explore the website of 23andMe, a company that offers direct-to-consumer genetic tests. The lesson wraps up as it began—by engaging students in a story. Through a short video, students are introduced to a family impacted by breast cancer. In *Lesson One*, students also learn how **bioengineers** might use bioinformatics tools in their career.

Learning Objectives

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

- Genetic tests are available from commercial companies that market directly to consumers (direct-to-consumer genetic testing).
- Genetic tests are not available for all conditions or abilities, and vary in their usefulness and clinical validity.
- Genetic tests can have social and ethical implications.
- Bioinformatics tools are used by people in many career fields, including bioengineers.

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

- Recognize the social and ethical implications of genetic testing.
- Weigh some of the harms and benefits of direct-to-consumer genetic testing.
- Give examples of direct-to-consumer genetic tests.

Key Concepts

- The results of genetic tests can have social and ethical implications.
- A variety of genetic tests are available through companies that offer direct-to-consumer genetic testing, and the tests can vary greatly in their ability to predict disease.
- The field of bioinformatics uses computers to search biological databases, compare sequences, and represent protein structures.
- Bioinformatics gives us the tools to design and validate genetic tests.
- Bioinformatics tools are used by people in many careers, including bioengineers.

Class Time

Two class periods of 50 minutes each, 100 minutes total. One class period of 50 minutes may be sufficient if student discussion time is limited to 5–10 minutes for each activity, or if portions of the lesson are assigned as homework (see *Homework* section).

Prior Knowledge Needed

- An understanding of the Central Dogma of Molecular Biology (the flow of genetic information from DNA to RNA to protein).

Common Misconceptions

- A positive result from a genetic test means a person will certainly get that disease.
- Scientists and doctors understand the functions of all the genes in the human genome.
- Only some people have BRCA genes (i.e., people who have BRCA-related cancer).

Materials

Materials	Quantity
Copies of Student Handout— <i>Careers in the Spotlight</i>	1 per student
Copies of Student Handout— <i>Meet the Gene Machine</i>	2
Class set of Student Handout— <i>Direct-to-Consumer Genetic Testing Homework: 23andMe</i> <i>Optional: See Homework section</i>	1 per student (class set) (Optional)
Class set of Student Handout— <i>Understanding Genetics and SNPs</i> <i>Optional: See Teacher Resource—Direct-to-Consumer Genetic Testing Website Exploration</i>	1 per student (class set) (Optional)
Teacher Resource— <i>Discussion Questions for Meet the Gene Machine</i>	1
Teacher Resource— <i>Direct-to-Consumer Genetic Testing Website Exploration</i>	1
Teacher Answer Key— <i>Answers for Direct-to-Consumer Genetic Testing Homework: 23andMe</i> <i>Optional: See Homework section</i>	1 (Optional)
Teacher Answer Key— <i>Understanding Genetics and SNPs</i> <i>Optional: See Teacher Resource—Direct-to-Consumer Genetic Testing Website Exploration</i>	1 (Optional)
Cotton swab (prop for <i>Meet the Gene Machine</i>)	1
Large printout of paper either in a roll or sheet form (prop for <i>Meet the Gene Machine</i>)	1
A 'Gene Machine' (box or small lab machine) under a cover	1

Computer Equipment, Files, Software, and Media

Computer with internet access and projector to display PowerPoint slides, teacher-directed website exploration detailed in Teacher Resource—*Direct-to-Consumer Genetic Testing Website Exploration*, and the NOVA video segment, "A Family Disease."

Alternative: Print PowerPoint slides onto transparencies and display with overhead projector. The NOVA segment is available on DVD if internet access is not available.

Lesson One PowerPoint Slides—Bioinformatics and Genetic Testing.

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>

Video: The eight-minute NOVA video segment "A Family Disease" from *Cracking the Code of Life* can be found streaming online at: http://www.pbs.org/wgbh/nova/genome/media/2809_q056_14.html. Clicking on the "Advanced Options" button leads to instructions for full-screen viewing. Purchasing information for the NOVA video can be found in the *Credit* section at the end of this lesson plan.

Teacher Preparation

- Load the classroom computer with the *Lesson One PowerPoint Slides* and the video segment "A Family Disease" (DVD or online streaming video).
- Make copies of the Student Handouts, one per student. Student Handout—*Careers in the Spotlight* will be used by students throughout the unit. Student Handout—*Direct-to-Consumer Genetic Testing Homework: 23andMe* is an optional homework assignment (see Homework section of this lesson plan), and is designed to be re-used as a class set.
- Set up two chairs at the front of the room, as for a talk show.
- Collect the props needed for the *Meet the Gene Machine* play.
- *Optional:* Assign two students or recruit two student or teacher volunteers to read the *Meet the Gene Machine* play before the in-class performance (for the roles of TV Talk Show Host and Scientist).

Procedure

Day 1

WARM UP

1. Explain to students the **aims of this lesson**. Some teachers may find it useful to write the aims on the board.
 - a. **Lesson Aim:** Introduce students to the field of bioinformatics.
 - b. **Lesson Aim:** Understand what type of genetic information a for-profit company (23andMe) is selling to the general public.
 - c. **Lesson Aim:** Introduce students to the *BRCA1* gene, which is involved in breast and ovarian cancer.

Teachers may also wish to discuss the *Learning Objectives* of the lesson, which are listed at the beginning of this lesson plan.

2. Tell students that the class will begin a unit of study about genetic testing and bioinformatics, and briefly define each term:

- **Genetic Testing** is the analysis of a person's DNA.

It is usually done to determine whether that individual carries changes (mutations) to genes that make them more susceptible to a disease. It is also popularly used to find out about ancestry and paternity.

- **Bioinformatics** is the application of computer science and information technology to biology and medicine.

Let students know that the focus of today's lesson will be on genetic testing, and that they will learn more about bioinformatics in *Lesson Two*. The topic of genetic testing can lead to some interesting social and ethical questions, as they will see in the play *Meet the Gene Machine*.

3. Show the PowerPoint for *Lesson One*, beginning with **Slide #1**. This slide highlights bioengineer Adrienne Minerick, PhD.



BIOENGINEER
ADRIENNE R. MINERICK, PhD

Place of Employment:
Michigan Technological University

Type of Research:
Biomedical Microdevices
For example, tiny devices to measure and manipulate living cells

"If a person is determined to learn, there will always be opportunities or resources for that person to pursue an education in science and engineering... There is a **real** demand for scientists and engineers whose contributions advance knowledge, technology, and the economic foundation of our society. I chose my career because I wanted to be a part of advancing knowledge and facilitating others to gain knowledge."

Bioinformatics & Genetic Testing: **Slide #1**

4. Give each student a copy of Student Handout—*Careers in the Spotlight*.
5. Students should think about, and write down, what kind of work a bioengineer might do (Bioengineer Question #1). This will be revisited at the end of the lesson.
6. Tell students to keep their *Careers in the Spotlight* handout available for future lessons.

PART I: Meet the Gene Machine

7. Introduce students to genetic testing and bioinformatics through a short play entitled *Meet the Gene Machine*. This comical play raises some of the broad implications of genetic testing.
8. Have the volunteer performers for the *Meet the Gene Machine* play come to the front of the room. Introduce one performer as Chris, a scientist, and the other as a popular TV talk show host.
9. Have students perform the play for the class.
10. After the play, use Teacher Resource—*Discussion Questions for Meet the Gene Machine* to lead a discussion about the play. Questions include:
 - What genetic traits are mentioned in the play?
 - Are genetic tests for all the traits equally valuable? Are tests for some traits more important than others? Why?
 - Would you want to know the results from a genetic test if you knew the contribution to the disease was about 50% genetic and 50% environmental? Why or why not?
 - How might bioethics play a role in this scenario?
 - How might bioinformatics play a role in this scenario?
 - What are some harms that could come from genetic testing?
 - What are some benefits that could come from genetic testing?
 - How is this skit realistic?
 - How is this skit not realistic?
 - Do you think that gene machines currently exist?

Possible answers can be found on Teacher Resource—*Discussion Questions for Meet the Gene Machine*.

Day 2

PART II: Direct-to-Consumer Genetic Testing

12. Ask students, “Do you think genetic tests are currently available for each of the traits mentioned in the *Meet the Gene Machine* play?”
13. Go through the list of genetic characteristics one by one and let students vote informally (thumbs up/thumbs down) as to whether they think genetic tests are currently available for each genetic trait mentioned in the play. The characteristics are:
 - Hair color
 - Musical ability

- Red blood cell count
- Height
- Carrier status for cystic fibrosis
- Predisposition towards breast cancer
- Predisposition towards alcoholism

14. Then, ask students to vote (thumbs up/thumbs down) as to whether they think information about the following additional traits is available through genetic testing:

- Sickle-cell anemia
- Earwax type
- Prostate cancer susceptibility
- Eye color
- Restless leg syndrome
- Mitral valve disease in dogs
- Resistance to HIV/AIDS

15. Tell students that they can find the answers to the above questions by visiting a number of websites. Today, the class will explore the website 23andMe, which offers **direct-to-consumer genetic testing**.

16. Using Teacher Resource—*Direct-to-Consumer Genetic Testing Website Exploration* as a guide, project the website for 23andMe (<http://www.23andme.com>) and explore it together as a class.

17. Teacher Resource—*Direct-to-Consumer Genetic Testing Website Exploration* has four parts:

- **Part A** exposes students to the variety of genetic tests available directly to the consumer.
- **Part B** distinguishes between Clinical Reports and Research Reports and asks if each test has equal **clinical validity** (these terms are defined in the Teacher Resource).
- **Part C** introduces the Breast Cancer Susceptibility Genes (BRCA) to students as **tumor suppressor genes** that normally prevent cancer. Mutations in these genes can lead to hereditary breast cancer and ovarian cancer when the normal functions of the proteins the genes encode are lost.
- **Part D** is recommended as homework if student have access to the internet at home. This section gives students background on how genetic testing works and contains a number of helpful animations.

PART III: Video Segment—A Family Disease

18. After exploring 23andMe as a class, remind students that genetic conditions touch the lives of people every day, including students in this classroom. Toward the end the lesson, ground students in a family's story about the repercussions of an inherited genetic disease and the options for genetic testing by sharing a video segment.

19. Show the eight-minute video segment "A Family Disease," Scene #14, from the NOVA video *Cracking the Code of Life*.

Direct-to-consumer genetic tests:

Direct-to-consumer genetic testing means an individual can receive information about his or her genetic condition without the use (or support) of a doctor or genetic counselor by submitting a DNA sample directly to the genetic testing company.

Clinical validity: How accurately a test predicts whether or not a person will get a particular disease or symptom (known as the "clinical outcome").

Tumor suppressor genes: Genes that encode proteins that help protect the cell from one step on the path to cancer. When both copies of this gene are mutated to cause a loss or reduction to their function, the cell can progress to cancer.


BRCA1: BReast **CA**ncer Susceptibility Gene **1**. *BRCA1* codes for the BRCA1 protein, which helps repair DNA damage and functions as a tumor suppressor.

20. After the video, tell students that they will be applying what they are learning about bioinformatics and genetic testing to a specific disease during the rest of the unit: inheritable breast cancer caused by mutations in one of the Breast Cancer Susceptibility genes (BRCA genes), specifically **BRCA1**.

Closure: *Careers in the Spotlight*

21. Close the lesson by returning to the picture of the bioengineer from the Lesson One PowerPoint Slides (**Slide #1**).

Bioinformatics & Genetic Testing: **Slide #1**



BIOENGINEER

ADRIENNE R. MINERICK, PhD

Place of Employment:
Michigan Technological University

Type of Research:
Biomedical Microdevices
For example, tiny devices to measure and manipulate living cells

*"If a person is determined to learn, there will always be opportunities or resources for that person to pursue an education in science and engineering... There is a **real** demand for scientists and engineers whose contributions advance knowledge, technology, and the economic foundation of our society. I chose my career because I wanted to be a part of advancing knowledge and facilitating others to gain knowledge."*

22. Show **Slide #2**, which provides job information for a bioengineer. Review this information with students.

Bioinformatics & Genetic Testing: **Slide #2**

CAREERS IN SPOTLIGHT: BIOENGINEER

What do they do?
A bioengineer uses the principles and tools of engineering to address problems in biology and medicine, creating usable products. These include designing medical devices, diagnostic equipment, renewable bioenergy, and genetically modified organisms.

What kind of training is involved?
A bachelor's degree in engineering is required for almost all entry-level engineering jobs. Sometimes a graduate degree is also required, such as a Master's degree or PhD

What is a typical salary for a Bioengineer?
The average salary is about \$55,000/year (\$26/hour), with a range from \$45,000/year (\$22/hour) to more than \$120,000/year (\$58/hour).

23. Tell students that today they were introduced to genetic testing and bioinformatics through the play *Meet the Gene Machine*, and by exploring the direct-to-consumer genetic testing company 23andMe. Bioengineers like Dr. Minerick are instrumental to gene testing in many ways, including:

- Much of our understanding about the functions of genes and their associations with diseases is made possible by laboratory equipment designed by bioengineers. This includes machines to help us purify sequences and analyze DNA, which are both necessary steps when performing genetic tests.

- If we someday have a “Gene Machine,” it will likely be a bioengineer who designs it and makes it!
24. Genetic testing today requires much more time to purify and analyze the patient’s DNA than shown in *Meet the Gene Machine*. In addition, one cannot simply look at a stretch of A’s, T’s, C’s, and G’s and know that it codes for a specific trait. This is why scientists use bioinformatics to help make sense of this vast amount of data.
 25. Ask students to answer Bioengineer Question #2 on their *Careers in the Spotlight* handout, which has students explain how this lesson has changed their understanding about the kind of work a bioengineer does.
 26. Ask students to also answer Bioengineer Question #3 on their *Careers in the Spotlight* handout, which has students explain how a bioengineer might use bioinformatics in his or her work.
 27. 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:

- A. Ask students to write about the activities they learned in *Lesson One* in their lab notebooks, on another sheet of paper, or in a word processing program like Notepad or Microsoft Word® 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...
- B. For students with internet access at home, Part D of Teacher Resource—*Direct-to-Consumer Genetic Testing Website Exploration* can be given as homework. Direct students to explore the “How it Works” section of 23andMe (<http://www.23andMe.com>). Students should click on “Genetics 101” and watch and take notes on all four short animations found in the “Genetics” section. Students can be encouraged to explore other interesting videos and animations on this page as well.
- C. To allow students additional time to explore the 23andMe website, an optional homework assignment, Student Handout—*Direct-to-Consumer Genetic Testing Homework: 23andMe*, may be assigned to be completed in-class or outside class, depending upon computer access and time available.
- D. For students without internet access at home, students can write a paragraph reflecting on the family shown in the video. If the student were in the daughter’s position, what would he or she do? Why?

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

Glossary

Bioinformatics: Bioinformatics is the application of computer science and information technology to biology and medicine. Bioinformatics makes it possible to analyze large amounts of complex biological data and can be used to search biological databases, compare sequences, and draw molecular structures. Bioinformatic techniques are used to design and carry out the computer-based portion of genetic tests.

BRCA1: BReast **CA**ncer Susceptibility Gene **1**. *BRCA1* codes for the BRCA1 protein, which helps repair DNA damage and functions as a tumor suppressor.

Clinical validity: How accurately a test predicts whether or not a person will get a particular disease or symptom (known as the “clinical outcome”).

Direct-to-consumer genetic tests: Direct-to-consumer genetic testing means an individual can receive information about his or her genetic condition without the use (or support) of a doctor or genetic counselor by submitting a DNA sample directly to the genetic testing company.

Established research report: Established Research Reports from 23andMe provide information about conditions and traits for which there are genetic associations supported by multiple, large, peer-reviewed studies. Because these associations are widely regarded as reliable, 23andMe uses them to develop quantitative estimates and explanations of what they mean for individuals receiving direct-to-consumer genetic tests.

Genetic testing: The analysis of a person’s DNA. It is usually done to determine whether that individual carries changes (mutations) to genes that make them more susceptible to a disease. It is also popularly used to find out about ancestry and paternity.

Preliminary research reports: Preliminary Research Reports from 23andMe are based on peer-reviewed, published research for which the findings still need to be confirmed by the scientific community. They also include topics where there may be contradictory evidence. Topics may move from Preliminary Research to Established Research when and if sufficient follow-up studies are performed.

Tumor suppressor gene: Gene that encodes a protein that helps protect the cell from one step on the path to cancer. When both copies of this gene are mutated to cause a loss or reduction to their function, the cell can progress to cancer.

Resources

For more information about polymerase chain reaction, DNA sequencing, and microarray technologies, the Howard Hughes Medical Institute (HHMI) “Bioactive” website has a large collection of useful videos and animations on a number of topics. These can be freely accessed online at: <http://www.hhmi.org/biointeractive/video/index.html>.

Credit

Minerick, Adrienne Robyn. Personal Interview. 29 September 2010.

“Meet the Gene Machine” was written by Laura Streith, Karen Bultitude, Frank Burnet, and Claire Wilkinson at the Science Communication Unit at the University of the West of England and was funded by Wellcome Trust, United Kingdom. Adapted and used with permission.

The two-hour NOVA video *Cracking the Code of Life* can be purchased at Amazon.com or through the PBS website at: <http://www.shoppbs.org/product/index.jsp?productId=2980750>. Although many genetic advances have occurred since the video’s 2001 release date, many of the video segments are relevant to this curriculum.

The NCBI resource on genetic tests called “Gene Tests” can be found at: <http://www.ncbi.nlm.nih.gov/sites/GeneTests/>.

Name _____ Date _____ Period _____

1

Careers in the Spotlight

What is bioinformatics? Bioinformatics is the application of computer science and information technology to biology and medicine.



<p>Lesson One Career: Bioengineer Adrienne R. Minerick, PhD</p> <p>1. What kind of work do you think a bioengineer does?</p>	<p>2. How has this lesson changed your understanding about the kind of work a bioengineer does?</p> <p>3. How do you think a bioengineer might use or benefit from bioinformatics?</p>
<p>Lesson Two Career: Veterinarian Deborah Tegarden, DVM</p> <p>1. What kind of work do you think a veterinarian does?</p>	<p>2. How has this lesson changed your understanding about the kind of work a veterinarian does?</p> <p>3. How do you think a veterinarian might use or benefit from bioinformatics?</p>
<p>Lesson Three Career: Genetic Counselor Robin Bennett, MS</p> <p>1. What kind of work do you think a genetic counselor does?</p>	<p>2. How has this lesson changed your understanding about the kind of work a genetic counselor does?</p> <p>3. How do you think a genetic counselor might use or benefit from bioinformatics?</p>

LESSON 1

HANDOUT



Lesson Four Career: Laboratory Technician

Zane Kraft, MS

1. What kind of work do you think a **laboratory technician** does?

2. How has this lesson changed your understanding about the kind of work a **laboratory technician** does?

3. How do you think a **laboratory technician** might use or benefit from bioinformatics?

Lesson Five Career: 3D Animator

Beth Anderson

1. What kind of work do you think a **3D animator** does?

2. How has this lesson changed your understanding about the kind of work a **3D animator** does?

3. How do you think a **3D animator** might use or benefit from bioinformatics?

Lesson Six Career: Bioethicist

Kelly Edwards, PhD

1. What kind of work do you think a **bioethicist** does?

2. How has this lesson changed your understanding about the kind of work a **bioethicist** does?

3. How do you think a **bioethicist** might use or benefit from bioinformatics?

1

Meet the Gene Machine

Modified from “Meet the Gene Machine,” written by **Laura Strieth**, **Karen Bultitude**, **Frank Burnet**, and **Clare Wilkinson**. “Meet the Gene Machine” was devised by the Science Communication Unit at the University of the West of England, Bristol, and is funded by the Wellcome Trust (adapted with permission).

A daytime tv talk show set. Sofa, coffee table, vase of flowers, gene machine (covered). Host and scientist are preparing to go live. Host is standing at the sofa, checking makeup and hair, which requires more concentration than talking to the scientist. Host is stressed and short-tempered at first, but becomes relaxed and happy when on air, brimming with confidence. Scientist is nervous, but excited. They are not yet on the air.

p>Host Come on you guys; two minutes is all!! Are these flowers for real? Move that boom, it’s right in my line of sight, and has anybody seen my next guest?!!
p>Chris Hello, I am Dr. Chris Taylor, I’m a scientist from...
p>Host Great, (INDICATING SOFA) you sit at that end. You know who I am of course. Have you done much TV?
p>Chris No, this is my first time, actually I’m feeling a bit...
p>Host Great, it’s a piece of cake. You’ll love it. Did you bring the gizmo?
p>Chris Yes, it’s here. Should I get it out?
p>Host We have a saying in TV; don’t tell me, show me. Better make it quick, we’ve got two minutes for the break then we’re back with you as first item. What do you guys call that thing?
p>Chris Oh, it’s called the *Microarray and Microassay Hyper Channel Optimizing Genetic Analysis with Real Time Interpretive Functionality Unit*.
p>Host OK... the Gene Machine and what does it do?
p>Chris We’ll take a sample of DNA, insert it in here, and the Microarray and...
p>Host Gene Machine.
p>Chris ...and the Gene Machine analyzes it and prints out a complete genetic profile, which I’ll help to interpret.
p>Host Interpret? What’s it speak, German?
p>Chris No, but the results can be confusing. Very few things in genetics are black and white and most people need help figuring out what the test results mean to them.
p>Host Do you think I got my own talk show by being “most people”? No, I got here by having the newest, most exciting gadgets on my show. Did you see Jerry has a lie detector on his show? Hah, we got genetic tests – how cool is that?

LESSON 1

HANDOUT

Chris It's not a toy.

Host Is a lie detector a toy? This is reality, not a game show. One minute left – how does it work?

Chris It looks at the 25,000 genes that play a vital role in making us who we are; things like hair color, height, aspects of personality all have some genetic basis.

Host So if we've all got these genes that control everything, how do we end up so different?

Chris There are many, many different versions of genes out there and we all inherit different combinations from our parents. Only identical twins have the same genes.

Host Is that why they always dress the same?

Chris When they get the choice they don't. Identical twins may seem similar, but actually, there are lots of differences between them.

Host So how does that happen?

Chris Our genes are only part of the story. What happens to us in our lives also makes a difference to us.

Host Kind of "Inheritance vs. Circumstance," no, how about "Biology vs. Experience?"

Chris It's usually called "Nature vs. Nurture."

Host Hey, that's good – are you looking for a job?

Chris I have one.

Host And now we have the Gene Machine. This is great. So, where do you get the DNA?

Chris That's the easy part; I just take a sample with this cotton swab.

Host "The Cotton Swab of Destiny." Go for it.

Chris What?

Host Fifteen seconds, grab the DNA.

Chris From you?

Host Who else? This is my show.

Chris I need you to sign a consent form.

Host I don't sign anything without a lawyer.

Chris Are you sure about this?

Host Didn't I say so?

Chris Well...

Host Five seconds.

Scientist takes a swab of the inside of host's cheek.

Host Places everyone! (TO SCIENTIST) Do something with that hair.

Show prepares to go live. 3 ... 2 ... 1 ... We are live. Host turns casually from Chris delighted to see the audience back for more. Host is relaxed and confident. Chris is flustered and adjusting hair.

Host Welcome back; so good to see you all again. Now, stop what you're doing; sit down and pay attention, because we are about to make television history. Ladies and Gentlemen, please meet the Gene Machine.... (LEADS THE APPLAUSE)

Applause

Host And with me today is scientist, Dr. Chris Taylor.

Chris Hello.

Host Chris, we are all just so excited about having you and the Gene Machine here with us today.

Chris (STILL HOLDING THE COTTON SWAB) It's great to be here.

Host Now Chris and other scientists have invented a fantastic new device that can read all your genes.

Chris Yes, It's called the Microarray...

Host The Gene Machine, that's right Chris. Now as I understand it Chris, we all have genes inherited from our parents.

Chris Yes, we actually have two full sets of genes, one from each parent. One from the sperm and one from the egg.

Host OK, OK, keep it clean doctor, this is a family show. Now, these genes can tell us a lot of things, can't they?

Chris Yes, our genes determine many things about us, many of our key characteristics.

Host So the Gene Machine can tell us much more than, for instance, a lie detector?

Chris Oh yes, a lie detector will only tell you if somebody is lying.

Host Whereas we will know the truth.

Chris (SEES DISASTER LOOMING) The output from the machine is true, yes. But it can only tell us about a person's genes....

Host You bet. In fact, this will change the whole future of reality television. With this new machine we can get closer to the truth than ever before.

Chris Errm, it's not just for television.

Host (WORRIED) Oh? Who else has a Gene Machine?

Chris Well, medicine obviously.

Host Really?

Chris Yes, many common diseases have genetic components. Identifying those genes will mean doctors can treat people more effectively.

Host Isn't that great!

Chris Obviously the police and others would like to use the machine to assist in identifying people...

LESSON 1

HANDOUT

Host But the biggest impact will be here on TV. And guess who will be the very first celebrity to Meet the Gene Machine...

Chris You?

Host (LAUGHS) As we like to say in television "Here's one I prepared earlier." (INDICATES MACHINE)

Chris Oh yes, we did. I have it here.

Operates machine. The swab goes in one side and a large printout comes out the other side.

Host Wow, that's a lot of paper. More is always better, right?

Chris (TRYING TO HOLD THE PRINTOUT) Well, we all have the same amount of genetic information, but there is a lot of it. Even if genetic tests only look a small part of each gene, that is still quite a bit of information.

Host So what does it say?

Chris Well first of all, it confirms that that is your natural hair color, and will be until you are in your 50s. However long that may be.

Host Does it tell you my age?

Chris No, your genes stay more or less the same all through your life.

Host So I have the genes of a teenager? (CHUCKLE) What else can you see there?

Chris (JUGGLING THE PAPERS) Ah, you have a number of gene variants associated with being musical.

Host I knew it!! I always said I had talent. My mom said I was too lazy to have lessons.

Chris You have some versions of some genes that are associated with musical ability. But, yes, hard work and practice are still very important.

Host I could have been a superstar – wait a minute, I AM.

Chris (TRIES TO ENJOY THE JOKE)

Host OK, so what else have you got there?

Chris Well, it says here that you are likely to be quite tall.

Host (BECOMING IMPATIENT) I can see that for myself; let me have a look. (TAKES THE PRINTOUT)

Host What's this?

Chris That says that you produce a lot of red blood cells.

Host So?

Chris So you should have high levels of fitness and stamina.

Host OK. Tell me something I don't know. What's this?

Chris Oh, that's quite common.

Host So what does it do?

Chris We can talk about that after the show.

Host I'm happy to share this with my public.

Chris That says you carry the gene variant that causes cystic fibrosis.

Host Now you're wrong there, I'm as fit as a fiddle, you just said so.

Chris No, it's not like that. I said earlier that genes come in different varieties, and some varieties cause harm. If you had inherited this variety from each parent, then you would have the disease.

Host So I'm OK?

Chris Yes, but you could pass the gene on to your own children, and the disease too, if your partner also has this version of the gene.

Host How would I know?

Chris Ask your partner to use the machine.

Host Oh boy, our lawyers are gonna love you. OK, what does this one mean?

Chris Ah, now that's a really interesting gene.

Host Uh huh.

Chris Well, it's pretty technical.

Host You don't think my audience is stupid, do you?

Chris No, no, no, it's just...

Host Yes?

Chris (DEEP BREATH) That gene suggests you have a predisposition towards breast cancer.

Host What's a predisposition?

Chris Well, it doesn't mean you will get the disease, but you might.

Host Anybody might.

Chris Yes, but you are at greater risk.

Host How much greater?

Chris That would be hard to say.

Host So what's the point in telling me? It's in my genes, so there's nothing I can do

Chris There are lots of things you can do to reduce the risk: diet, exercise, self examination. How often do you examine your breasts?

Host (NO LONGER HAVING FUN) You scientists really are quite blunt aren't you? We've got time for one more result before the break. How about this one?

Chris That one?

Host Yeah.

Chris (INDICATES ANOTHER) This one's interesting.

Host That one.

Chris It's sort of private.

LESSON 1

HANDOUT

Host I have nothing to hide.

Chris Are you sure?

Host It's a disclosure show – get disclosing.

Chris You are at very high risk of alcohol dependence.

Host What is that supposed to mean?

Chris Alcohol dependence is a condition in which a person...

Host (STANDS UP) I know what an alcoholic is.

Chris (STANDING UP) Look, you asked me to tell you.

Host Are you trying to ruin my reputation?

Chris Of course not, in fact I tried to warn you about revealing this information in public.

Host You didn't tell me it would be like this. This is terrible.

Chris The information in our genes is very powerful, and valuable.

Host Yes, to blackmailers and paparazzi.

Chris No, to all of us.

Host You want to do this to everyone?

Chris Of course not, but we can't keep this information locked away forever. Gene testing is here to stay.

Host People have a right to privacy!

Chris People have a right to know!

Host To know how much? To know about who? Who decides?

Chris The American public of course, but do you have the nerve to ask them what they think?

Host Yes, I do.

1

Direct-to-Consumer Genetic Testing Homework: 23andMe

Aim: To understand what type of genetic information a for-profit company is selling to the general public and determine the accuracy of that information.



Instructions: Go to the complete list of health reports at the 23andMe genetic testing website at <https://www.23andme.com/health/all/>. Answer these questions on a separate sheet of paper and turn in your answers to your teacher.

1. In your own words, describe an Established Research Report.
2. Find a condition that has an Established Research Report (there is a * after the name). Write that condition's name on your paper, and click the link to the report for that condition.
3. Using the information under the "Example Data" tab, in your own words, summarize the description paragraph of the disease or condition.
4. Look at the "Genes vs. Environment" section of the report – what does it say about the heritability of your condition and how much genetics might play a part in it?
5. Often, the Example Genetic Data is given in terms of % men of European ethnicity. However, the condition may actually affect other populations more. Read the **Description** and **Genes vs. Environment** sections to find if it is currently known that your condition affects certain populations more than others. If so, explain which populations.
6. Go back to the "Health Reports: Complete List." In your own words, describe a Preliminary Research Report.
7. Find a condition that has a Preliminary Research Report. Write down the name of that condition and click the link to the report for that condition.
8. Using the information under the "Example Data" tab, in your own words, summarize the description paragraph about the disease or condition and describe how much genes contribute to the condition.
9. Are Established Research Reports or Preliminary Research Reports more scientifically reliable? Explain.
10. Clinical validity is a term that bioethicists, doctors, and genetic counselors use to describe how accurately a genetic test predicts whether a person will get a particular disease or symptom. Are all the tests offered on 23andMe equally clinically valid? Explain.

1 Understanding Genetics and SNPs

Aim: To use educational animations developed by 23andMe to learn more about genetics and direct-to-consumer genetic testing.



Instructions: Go to the 23andMe website <https://www.23andme.com/>. Follow the instructions below and answer the questions on a separate sheet of paper. Turn your answers in to your teacher.

1. Click the "How it Works" button under the green bar at the top of the page.
 - a. How is this like the Gene Machine?
 - b. How is it different from the Gene Machine?
2. From the "How it Works" section, scroll down to the cartoon picture on the left, under "Genetics 101." Click to begin where it says "Watch an animated guide to your genes, SNPs, phenotype, and more."
 - a. Approximately how many genes does each human cell contain?
 - b. Approximately how similar are human beings to chimpanzees?
 - c. Approximately how similar are human beings to one another?
3. From the column on the left, under "Genetics," click "What are SNPs?" and "What is phenotype?" (See **Figure 1**).

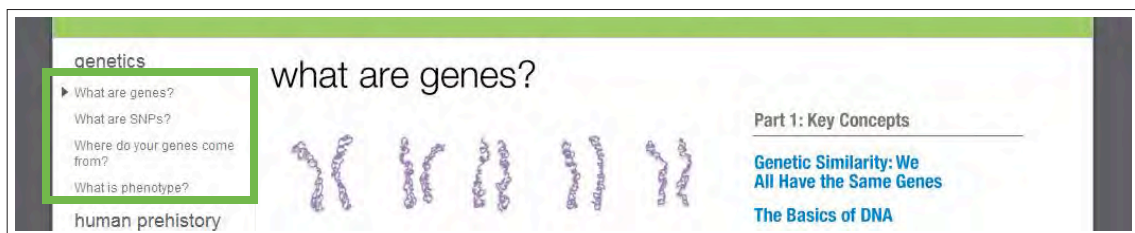


Figure 1: Watch an animated guide. Credit: 23andMe, <https://23andme.com/>.

- a. What is a SNP? Explain not only what the abbreviation stands for, but also what a SNP is.
- b. Most genetic tests offered by 23andMe evaluate SNPs. Do all SNPs result in a change in phenotype? Explain.

1

Discussion Questions for Meet the Gene Machine

1. What genetic traits are mentioned in the play? (List these traits on the board for all to see.)

- Hair color
- Musical ability
- Red blood cell count (leading to high levels of fitness and stamina)
- Height
- Carrier status for cystic fibrosis
- Predisposition towards breast cancer and alcoholism

2. Are genetic tests for all of the traits mentioned in the play equally valuable? Are tests for some traits more important than others? Why?

Example: A genetic test to determine hair color is not very useful if hair color can be changed and the results do not have an effect on one's health. A test to determine whether or not a person carries the gene variant that causes cystic fibrosis can be important, especially if the disease is known to run in a partner's family.

3. The environment plays a large part in diseases, even genetic diseases. In the play, Chris and the host have this interaction:

Chris (DEEP BREATH) That gene suggests you have a predisposition towards breast cancer.
 Host What's a predisposition?
 Chris Well, it doesn't mean you will get the disease, but you might.
 Host Anybody might.
 Chris Yes, but you are at greater risk.
 Host How much greater?
 Chris That would be hard to say.
 Host So what's the point in telling me? It's in my genes, so there's nothing I can do.
 Chris There are lots of things you can do to reduce the risk: diet, exercise, self examination.

Would you want to know the results from a genetic test if you knew the contribution to the disease was about 50% genetic and 50% environment? Why or why not?

Answers will vary. Some students may choose not to know because they have no control over part of it, and some may choose to know because they do have control over part of it. Some may say it's worth reducing risk through a healthy diet and exercise even if they don't know the results from the genetic test.

4. How might bioethics play a role in this scenario?

Bioethics, and the use of bioethical principles (introduced to students in *Lesson Three*) can help students analyze difficult or conflicting issues in a systematic, rational way. Issues include the right to privacy versus the right for individuals and families to know about their genetic make-up.

LESSON 1

RESOURCE

5. How might bioinformatics play a role in this scenario?

The Gene Machine itself uses tools of bioinformatics. Genetic testing is possible through the use of bioinformatic tools. Students will use these tools in *Lessons Two, Four and Five*.

6. What are some harms that could come from genetic testing?

Answers may vary. For example, a person's genetic information might not remain private, as in the skit. A person might also be anxious, and not live life to the fullest, knowing that he or she has a predisposition to a genetic disease. There can also be harms associated with testing for a disease for which there is no cure.

7. What are some benefits that could come from genetic testing?

Answers may vary. For example, a person who has a predisposition to a disease can make positive health and environmental changes. People considering parenthood can screen for genetic diseases if a serious disease is known to run in the family.

8. How is this skit realistic?

Answers may vary. Many talk shows today show genetic testing, such as for paternity. However, there are challenges in translating knowledge from scientists to the public, and there are risks of over-simplifying genetic diseases. Like the host, many people don't realize how serious the information obtained from a genetic test may be. There are a number of known genetic diseases for which tests are available. According to the NCBI resource *GeneTests*, there are more than 1,500 genetic tests currently available, some of which students will learn more about by exploring 23andMe.

9. How is this skit not realistic?

Answers may vary. It is commonly assumed that scientists and doctors fully understand the functions of all 25,000 genes in the human genome. However, much research still needs to be done. In addition, many characteristics or phenotypes are the result of complex interactions between genes and environment, or the result of the interaction of many genes (called "multifactorial traits"), meaning simple genetic tests would be misleading. The test results were also provided much too quickly. Current sequencing technology would require days to produce this much data. Also, a signed consent form would be required before performing any genetic test. This form would provide information about the risks and benefits of the genetic test.

10. Do you think that gene machines currently exist?

Students may be familiar with biotechnology applications, including polymerase chain reaction and DNA sequencing. Some students may have seen the movie "GATTACA," in which a character provides a hair sample and receives a complete printout of her genome in a matter of minutes. However, there is currently no 'gene machine' like the one in this play. Bioengineers have helped develop many tools and machines that make genetic testing, as well as genetic research, possible. Genetic testing is currently a multi-step process: DNA is extracted using a machine like a centrifuge, the DNA is copied using a thermocycler and polymerase chain reaction (PCR), and then the DNA is sequenced using a DNA sequencing machine. Multiple genetic tests can be performed at the same time on the same sample using a process called DNA microarray, which can be used to detect single nucleotide changes in a given region of DNA. However, all of these types of genetic tests require much more time to perform than the genetic tests in this play. In addition, one cannot simply look at a stretch of A's, T's, C's, and G's and know that it codes for a specific trait. This is why scientists use bioinformatics to help make sense of this vast amount of data.

1 Direct-to-Consumer Genetic Testing Website Exploration

PART I: What Types of Genetic Tests are Available?

1. Go to the 23andMe homepage: <https://www.23andme.com/>. Ask students: **What are the types of DNA tests that 23andMe offers?**

Ancestry Edition and Health Edition.

2. Take a few minutes to become familiar with the site. Ask students: **What do you think 23andMe does? Who is it for?**

23andMe's mission is, "To be the world's trusted source of personal genetic information." They allow individuals to receive genetic information without the use of a doctor or medical professional. Their services are available to anybody who can pay the fee for service.

3. Return to the Welcome page, found on the upper left green bar. Scroll down to find and click the blue link to "see all 201 topics" to take you to the complete list of "Health Reports" (see **Figure 1**). 23andMe is adding new topics all the time, so this number may have increased by the time you view this website.

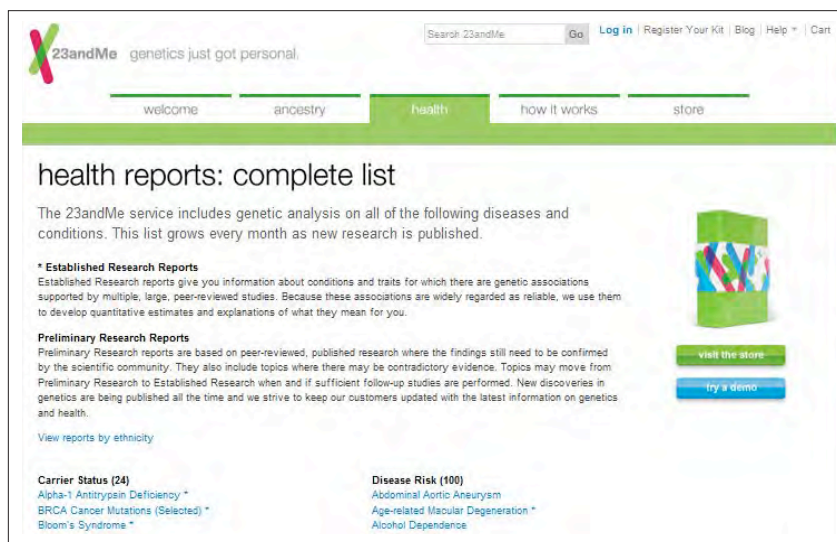


Figure 1: Health Reports.
Credit: 23andMe, <https://www.23andme.com/>.

4. One at a time, scroll through the list of Health Reports to find some of the genetic traits from the list that the class “voted” on earlier, such as Height (under “Traits”) or Sickle-Cell Anemia (under “Carrier Status”).

Does 23andMe have a genetic test for all of those traits?

23andMe tests for all of the diseases and traits mentioned in “Meet the Gene Machine,” except for musical ability, red blood cell count, and mitral valve disease in dogs. Information about mitral valve disease is available to veterinarians, using the tools of bioinformatics.

5. Look at the complete list of reports. **Which traits do students find most interesting?**

Answers will vary depending on students’ interests.

6. Click on some traits or diseases that are interesting to students, and/or that you have studied in class. Explore the tabs at the top of the page (Example Data, How it Works, Technical Report) and the video(s) or picture(s) on the page.

PART II: Established versus Preliminary Research Reports

7. From any sample report page, click on “>>view all sample reports” on the top right of the page to return to the complete list of health and trait topics (see **Figure 2**).

Figure 2: View all sample reports.
Credit: 23andMe, <https://www.23andme.com/>.



8. For any given Carrier Status, Disease Risk, Drug Response, or Trait, there are **Established Research Reports** and **Preliminary Research Reports**. Show students examples of each of these reports in the context of diseases or traits. Those with Established Research Reports are noted with asterisks*.

What is an Established Research Report? Example: Cystic fibrosis

According to 23andMe: “Established Research Reports give you information about conditions and traits for which there are genetic associations supported by multiple, large, peer-reviewed studies. Because these associations are widely regarded as reliable, we use them to develop quantitative estimates and explanations of what they mean for you.” In other words, there is good scientific evidence that the genes described in Established Research Reports are truly associated with a disease or condition.

Clinical Validity: How accurately a test predicts whether or not a person will get a particular disease or symptom (known as the “clinical outcome”).

Established Research Report:

Established Research Reports from 23andMe provide information about conditions and traits for which there are genetic associations supported by multiple, large, peer-reviewed studies. Because these associations are widely regarded as reliable, 23andMe uses them to develop quantitative estimates and explanations of what they mean for individuals receiving direct-to-consumer genetic tests.

What is a Preliminary Research Report? Example: Alcohol dependence

According to 23andMe: “Preliminary Research reports are based on peer-reviewed, published research for which the findings still need to be confirmed by the scientific community. They also include topics where there may be contradictory evidence. Topics may move from Preliminary Research to Established Research when and if sufficient follow-up studies are performed.” In other words, the associations described in Preliminary Research Reports represent initial findings and should be interpreted with caution.

Preliminary Research Reports:

Preliminary Research Reports from 23andMe are based on peer-reviewed, published research for which the findings still need to be confirmed by the scientific community. They also include topics where there may be contradictory evidence. Topics may move from Preliminary Research to Established Research when and if sufficient follow-up studies are performed.

Which type of report is supported with more reliable science?

Established Research Reports. Emphasize the importance of higher **clinical validity**, and information gained from large, peer-reviewed studies which can be supported by more than one study. A test with high clinical validity means that the test is very good at predicting whether someone will get the disease or condition.

Are all the genetic tests offered at 23andMe equally clinically valid?

No. The scientific community has not yet agreed about the level of meaningfulness of these tests. The tests can vary greatly in their ability to predict disease. Because of this variability, results of these tests can be difficult to interpret.

PART III: Breast Cancer Susceptibility Genes

9. Give students some background about the Breast Cancer Susceptibility (BRCA) genes. Tell students that this unit of study focuses on one gene—**BRCA1**—that plays a crucial role in DNA repair. *BRCA1* is a tumor suppressor gene that normally prevents cancer. Mutations in this gene can lead to hereditary breast cancer and ovarian cancer when the normal function is lost. BRCA is sometimes pronounced “BRACK-uh.”
10. Tell students that there are two BRCA genes, *BRCA1* and *BRCA2*. This unit focuses primarily on *BRCA1*.
11. Click on “BRCA Mutations (Selected)” from the complete list of health reports from above (Part B). Read the note in the peach-colored box (see **Figure 3**). **How complete is the BRCA test at 23andMe?**

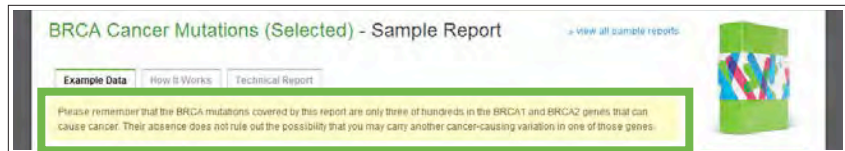
23andMe tests for only three of the hundreds of known mutations in the *BRCA1* and *BRCA2* genes. These *BRCA1* and *BRCA2* genes have been patented by the biopharmaceutical company Myriad Genetics Incorporated. Myriad has also patented the method of testing the genes for mutations, so much of the *BRCA1* and *BRCA2* information is unavailable to 23andMe.

BRCA1: BReast **C**ancer Susceptibility Gene 1. *BRCA1* codes for the BRCA1 protein, which helps repair DNA damage and functions as a tumor suppressor.

LESSON 1

RESOURCE

Figure 3: Read the note in the peach box.
Credit: 23andMe, <https://www.23andme.com/>.



If you suspected mutations to the BRCA1 and BRCA2 genes ran in your family, would you choose to get tested at 23andMe or through Myriad?

Myriad is considered the gold standard for *BRCA1* and *BRCA2* testing since they have a patent on the gene and therefore have unhindered access to the genetic information.

12. Continue scrolling through the BRCA Cancer Mutations Sample Report. As a class, read the “About Breast/Ovarian Cancer” section.

Direct-to-Consumer Genetic Testing (continued)

[**Note:** Omit this question if students have not learned about types of mutations.]

13. Scroll down to the “Example Genetic Data.” ***Drawing on previous knowledge, what are the most common types of mutations?***

Insertions, substitutions, and deletions.

What type of mutation might 185delAG be?

The del refers to a deletion mutation. (See **Figure 4.**)

What else can you tell from the name of the mutation?

At nucleotide #185 in the DNA sequence, there is a deletion of an A and a G—an Adenosine and a Guanine.

Figure 4: The *del* refers to a deletion mutation.
Credit: 23andMe, <https://www.23andme.com/>.



14. Scroll down to look at the other two mutations on the “Example Genetic Data.” **What does 5382insC mean?**

At nucleotide #5382, a Cytosine is inserted.

What does 6174delT mean?

At nucleotide #6174, a Thymine is deleted.

15. Scroll down the page to find the section on “Citations.” **Do these citations look to be from valid, scientific sources?**

Yes. *Science* magazine, *New England Journal of Medicine*, and *American Journal of Human Genetics* are all well-respected, peer-reviewed sources.

16. Point out that the research cited is done with equipment designed by **bioengineers**, our career of interest for the day.

PART IV: How it Works

[Optional: Could Be Assigned for Homework Using the Student Handout—*Understanding Genetics and SNPs*]

17. Click the “How it works” button under the green bar at the top of the page. **How is this like the Gene Machine?**

A genetic sample (saliva) is sent to 23andMe for analysis and results are given to the client.

How is it different from the Gene Machine?

It takes two to four weeks to analyze the sample; the results are not instant, as with the Gene Machine. Although not shown, the process requires a number of steps, not one machine. 23andMe looks at over 200 traits and topics; it is unclear what is (or is not) analyzed with the Gene Machine.

18. Scroll down to the cartoon picture on the left, under “Genetics 101.” Click to begin where it says “Watch an animated guide to your genes, SNPs, phenotype, and more.”

19. From the column on the left, under “Genetics,” click any of the four animation titles. Of specific interest are “What are SNPs?” and “What is phenotype?” (See **Figure 5**.)



Figure 5: Click on the four animation titles.
Credit: 23andMe, <https://www.23andme.com/>.

1

Answers for Direct-to-Consumer Genetic Testing Homework: 23andMe

[**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 **10 points**.]

1. In your own words, describe an Established Research Report.

An Established Research Report contains information from **multiple, large peer-reviewed scientific studies**. A peer-reviewed study has been examined by the scientific community and the research methods and conclusions of the study authors has been found to be appropriate. These reports contain more reliable information than "Preliminary Reports."

(+1 for including a description or for explaining that they are more reliable than preliminary reports.)

2. Find a condition that has an Established Research Report (there is a * after the name). Write that condition's name on your paper, and click the link to the report for that condition.

Student answers will vary.

3. Using the information under the "Example Data" tab, in your own words, summarize the description paragraph of the disease or condition.

Student answers will vary based on their answer to Question #2.

(+1 for an accurate description of the condition.)

4. Look at the "Genes vs. Environment" section of the report – what does it say about the heritability of your condition and how much genetics might play a part in it?

Student answers will vary based on their answer to Question #2.

However, many conditions have both genetic and environmental factors.

(+1 for statement about the heritability of the condition; +1 for further explanation of genetic contribution.)

5. Often, the Example Genetic Data is given in terms of % men of European ethnicity. However, the condition may actually affect other populations more. Read the **Description** and **Genes vs. Environment** sections to find if it is currently known that your condition affects certain populations more than others. If so, explain which populations.

Student answers will vary based on their answer to Question #2 above. However, several conditions are more prevalent in certain populations.

(+1 for listing populations more affected or for answering 'No, it is not currently known.')

6. Go back to the “Health Reports: Complete List.” In your own words, describe a Preliminary Research Report.

A Preliminary Research Report contains information from one or a few scientific reports that have been peer-reviewed (reviewed by the scientific community), but there is not sufficient data to confirm the results of the study. There may be cases in which two studies about the same gene(s) or condition(s) are actually contradictory. Once enough data and studies have been conducted to confirm an association, Preliminary Research Reports may become Established Research Reports.

(+1 for including bolded ideas or indicating that information comes from a small number of studies.)

7. Find a condition that has a Preliminary Research Report. Write down the name of that condition and click the link to the report for that condition.

Student answers will vary.

8. Using the information under the “Example Data” tab, in your own words, summarize the description paragraph about the disease or condition and describe how much genes contribute to the condition.

Student answers will vary based on their answer to Question #6. However, many conditions have both genetic and environmental factors.

(+1 for accurate description of condition; +1 for description of relative contributions of genes and environment.)

9. Are Established Research Reports or Preliminary Research Reports more scientifically reliable? Explain.

Established Research Reports are more reliable than Preliminary Research Reports, because they draw on information from multiple, large, peer-reviewed scientific studies. Preliminary Research Reports are based on one, or only a few, scientific studies which often contain fewer research subjects.

(+1 for Established Research Reports, with explanation.)

10. Clinical validity is a term that bioethicists, doctors and genetic counselors use to describe how accurately a genetic test predicts whether a person will get a particular disease or symptom. Are all the tests offered on 23andMe equally clinically valid? Explain.

No, all of the tests offered on 23andMe are not equally clinically valid. For example, conditions for which there are Established Research Reports tend to be more clinically valid than conditions for which there are only Preliminary Research Reports. In addition, some students may note that some genetic associations only account for a small portion of the risk of a disease or condition (traits for which heritability is low). In this case, if a particular gene or allele only conveys a small portion of the risk, the genetic test for that condition may not be considered clinically valid. Students will explore this issue in depth in Lesson Six.

(+1 for reference to Established Research Reports; +1 for reference to different genetic contributions.)

1

Understanding Genetics and SNPs

[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 **7 points**.]

1. Click the "How it Works" button under the green bar at the top of the page.

a. How is this like the Gene Machine?

A genetic sample (saliva) is sent to 23andMe for analysis, and results are given to the client. (+1 point.)

b. How is it different from the Gene Machine?

It takes two to four weeks to analyze the sample; the results are not instant, as with the Gene Machine. Although not shown, the process requires a numbers of steps, not one machine. 23andMe looks at over 120 traits and topics; it is unclear what is (or is not) analyzed with the Gene Machine. (+0.5 for each difference noted between the Gene Machine and 23andMe, up to +1.5 points.)

2. From the "How it Works" section, scroll down to the cartoon picture on the left, under "Genetics 101." Click to begin where it says "Watch an animated guide to your genes, SNPs, phenotype, and more."

a. Approximately how many genes does each human cell contain?

20,000. (+0.5 point.)

b. Approximately how similar are human beings to chimpanzees?

Human beings and chimpanzees share 98.5% of their genes. (+0.5 point.)

c. Approximately how similar are human beings to one another?

Human beings share 99.5% of their genes with one another. (+0.5 point.)

3. From the column on the left, under "Genetics," click "What are SNPs?" and "What is phenotype?"

a. What is an SNP? Explain not only what the abbreviation stands for, but also what an SNP is.

A SNP is a single nucleotide polymorphism. A SNP is a single base pair change among individuals. (+1 for explaining what "SNP" stands for; +1 for the explanation of what an SNP is.)

b. Most genetic tests offered by 23andMe evaluate SNPs. Do all SNPs result in a change in phenotype? Explain.

Most SNPs do not result in a change in phenotype. These are "silent" changes that do not result in a different amino acid within the coding sequence of a protein. (+0.5 points for noting that all (or most) SNPs do not result in a change in phenotype; +0.5 points for explaining why.)

LESSON 1

KEY

2 Navigating the NCBI

Introduction

Students navigate parts of the **National Center for Biotechnology Information** (NCBI) website and work independently to explore **databases**, focusing on the *BRCA1* gene and the **bioinformatics** tool *Map Viewer*. Through an analogy that compares two collections of databases (iTunes® and the NCBI), students connect with their own prior knowledge to better understand database structure and function. In *Lesson Two*, students learn how **veterinarians** might use bioinformatics tools in their career.

Learning Objectives

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

- Databases like those available at the National Center for Biotechnology Information (NCBI) are used to organize and search vast amounts of biological information.
- Genetic tests are developed using the biological information available in databases like those at the NCBI.
- Bioinformatics tools are used by people in many careers, including veterinarians.

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

- Navigate databases at the National Center for Biotechnology Information (NCBI) to find biological information.
- Explain the need for searchable databases as applied to genetic testing.

Key Concepts

- The NCBI is a central repository for many types of biological data freely available to scientists and the general public including: nucleotide and protein sequences, structures, scientific publications and much more. The many databases at the NCBI can be searched simultaneously using the NCBI's search engine **Entrez**.
- Companies that offer direct-to-consumer genetic testing rely on biological information available through the NCBI.
- The NCBI resource *Map Viewer* allows users to view and search an organism's complete genome and display chromosome maps.
- Bioinformatics tools are used by and benefit people in many careers, including veterinarians.

Class Time

One class period (50 minutes).

Prior Knowledge Needed

- An introduction to taxonomy.
- An understanding of the relationship between genes and proteins.
- The ability to translate DNA nucleotide codons into amino acids.

Common Misconceptions

- Only humans have BRCA genes.
- BRCA genes are only located in breast tissue.

Materials

Materials	Quantity
Copies of Student Handout— <i>Careers in the Spotlight</i> (handed out in <i>Lesson One</i>)	1 per student
Class set of Student Handout— <i>Navigating the NCBI Instructions</i>	1 per student (class set)
Copies of Student Handout— <i>Navigating the NCBI Worksheet</i> [Note: This worksheet is for students' answers to lesson questions.]	1 per student
Teacher Answer Key— <i>Navigating the NCBI</i>	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.
<i>Lesson Two</i> PowerPoint Slides— <i>Navigating the NCBI</i> . 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 .
Computer lab with internet access for students.

Teacher Preparation


- Load the classroom computer with the *Lesson Two* PowerPoint slides.
- Make copies of the Student Handout—*Navigating the NCBI Instructions*, one per student. This handout is designed to be re-used as a class set.
- Make copies of the Student Handout—*Navigating the NCBI Worksheet*, one per student. This worksheet is used for students to write their answers to the lesson questions.

Procedure

WARM UP

1. As students enter the classroom, display the PowerPoint **Slide #1**. This slide highlights veterinarian Deborah Tegarden, DVM.

Navigating the NCBI: **Slide #1**



VETERINARIAN
DEBORAH TEGARDEN, DVM

Place of Employment:
Elliot Bay Animal Hospital
Seattle, Washington

"While we often think of genetic testing in humans, vets are seeing more and more tests being developed for animal patients. This is the most exciting time I can imagine in veterinary medicine, when things are getting more and more cutting edge and technology is developing at lightning speed."

2. Have students retrieve Student Handout—*Careers in the Spotlight* from Lesson One.
3. Students should think about, and write down, what kind of work a veterinarian might do (Veterinarian Question #1). This will be revisited at the end of the lesson, including how a veterinarian 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: What does the Gene Machine really do?

5. Explain to students the **aim of this lesson**. Some teachers may find it useful to write the aim on the board.

Lesson Aim:

- To understand the role of databases in organizing and searching for biological information.
6. Remind students that, in Lesson One, they watched the play *Meet the Gene Machine* and explored the 23andMe website to learn more about genetic testing.
 7. Share with students that in today's lesson, they will explore more about the inner workings of the "Gene Machine" by learning about the role bioinformatics plays in genetic testing. Another goal for today's lesson is to explore how databases, particularly those at the National Center for Bioinformatics Information (NCBI).
 8. Ask students, "What does the Gene Machine **do**?" It may be helpful to walk over to the prop used for the Gene Machine in the play from Lesson One, if it is available.
 9. Let students brainstorm answers. Students may say that it "analyzes DNA," or "tests for genetic diseases," or "gives a person a printout of his or her genes." For each case, ask students **how** this might happen (i.e., "**How** do you think it analyzes DNA?").
 10. Highlight for students that it is not enough to know just the patient's DNA sequences for a genetic test. A genetic test must **compare** the patient's DNA sequences to known sequences. These known sequences are called **reference sequences**.
 11. Tell students that the fictional Gene Machine from the play represents a number of steps necessary for genetic testing, from purifying and sequencing the DNA to comparing the patient's DNA with a known reference sequence.

PART II: The Need for Databases

12. Share with students the importance of databases in science: **Databases** allow scientists to store, manage, and retrieve information in an organized way.
13. Remind students of how much genetic information was returned for just one person in the play, *Meet the Gene Machine*. How would scientists handle the amount of data if everybody in the class got tested? Everybody in the school? Everybody in the school district? Where are all the reference sequences kept and how do scientists access them?

[**Note:** Teachers may also wish to discuss the Learning Objectives of the lesson, which are listed at the beginning of this lesson.]

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.

NCBI has a database dedicated to reference sequences, called the RefSeq database. The goal of RefSeq is to accurately represent all naturally occurring DNA, RNA, and protein molecules for major organisms.

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

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 organized in over 30 cross-referenced databases related to genetics and molecular biology. It's not just DNA – there are all kinds of data about how cells, organisms, and even ecosystems function.

Entrez: The search engine used to simultaneously search all of the databases at the NCBI. Items stored in different databases are cross-referenced and inter-linked, making it easier to find all the database records that are related to any one subject.

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

14. Tell students the following:

This information, and more, is stored in biological databases such as those at the **National Center for Biotechnology Information (NCBI)**, which is part of the National Library of Medicine, which in turn is part of the National Institutes of Health.

The NCBI houses biological information in over 30 databases related to genetics and molecular biology. All of these databases can be searched using one search engine (**Entrez**). Items stored in different databases are cross-referenced and inter-linked, making it easier to find all the database records that are related to any one subject. When students visit the NCBI in this lesson, they will primarily use the **Nucleotide database**.

Genetic tests like those available through 23andMe could not exist without the information available at the NCBI.

15. Ask students if they are familiar with using databases to search for and retrieve information. It is likely that many students have used iTunes®, a collection of databases related to music, podcasts, videos, and ringtones. iTunes® can be searched in multiple ways and can yield many results from one query, just as students will find at the NCBI.

16. Tell students the following:

Biology is undergoing a tremendous change because of the explosion of data that are being collected.

Because DNA is an information molecule, and we are gathering more and more genetic information, we need new strategies to understand the data.

NCBI can be thought of as a point for “one stop shopping” when searching for biotechnology information. The databases found at NCBI are crucial for scientists conducting biological research.

PART III: What is Bioinformatics?

17. Tell students that **bioinformatics is the application of computer science and information technology to biology and medicine**.

Teachers are encouraged to write this definition on the board, and ask students to write it in their notes. The definition can also be found at the top of students' *Careers in the Spotlight* handout.

18. Break this definition down to look at its parts. Bioinformatics is used to:

Create databases: Building cross-referenced, interlinked databases used to store information.

Search databases: Designing a way to search and retrieve information from the databases. When students go to the NCBI and search the databases, they are using the tools of bioinformatics.

Compare sequences: Comparing an individual's DNA to reference sequences that are uploaded and stored in the NCBI databases. These reference sequences have been reviewed by NCBI researchers to confirm that they contain no errors or mutations. They are the result of much collaboration between scientists. The programs used to compare these sequences are the work of bioinformatics.

Represent molecular structures: Identifying and representing the three-dimensional structure of biological molecules such as proteins. Computer programs that determine and represent these 3D structures are the work of bioinformatics.

19. Tell students that bioinformatics offers many different types of tools to use in different circumstances. Like any set of tools, bioinformatics is useful when applied.

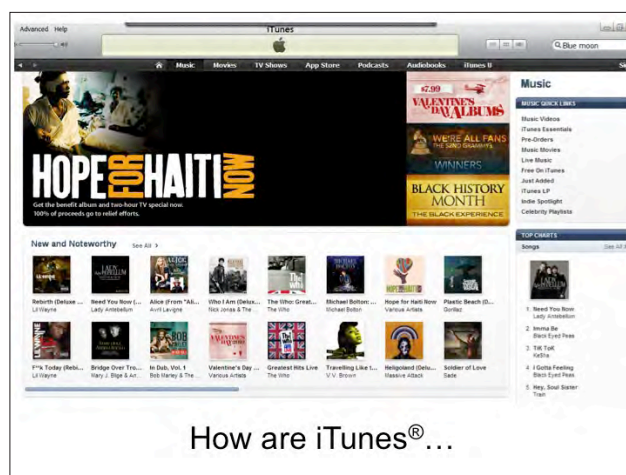
PART IV: Navigating the NCBI

20. Tell students that while they are at the NCBI website, they will focus on a gene they explored in *Lesson One: BRCA1*. Remind student that *BRCA1* plays a crucial role in DNA repair as a tumor suppressor that normally prevents cancer.
21. Acknowledge that students may feel overwhelmed by the amount of information at the NCBI. The purpose of this exercise is **not** for students to learn detailed, specific information from the site, but to gain an appreciation for the amazing depth and breadth of information available at the NCBI.
22. Distribute copies of Student Handout—*Navigating the NCBI Instructions* and copies of Student Handout—*Navigating the NCBI Worksheet* and allow students to start working on the activity independently at a computer.

PART V: A Database Analogy: iTunes® and the NCBI

23. After students have worked through Student Handout—*Navigating the NCBI Instructions*, tell them that they will now tie what they have learned about databases at the NCBI to what they already know about the iTunes® database. It may be helpful to ask students if they are familiar with the music database iTunes®, and ask them what they know about the service. For example, students might say that they use iTunes® to download songs, to find out which songs were published by which artist, or to learn which album a particular song was released on.
24. Show **Slide #2**, “How are iTunes®...”

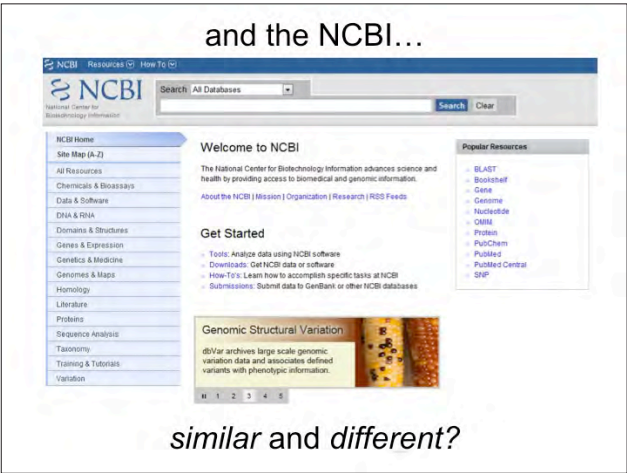
[**Note:** While working through Student Handout—*Navigating the NCBI Instructions*, students may feel overwhelmed with the information available in the databases. Working through it as a group or as a class may be helpful. It is important to realize that neither teachers nor students are going to understand everything at the NCBI. Students and teachers are encouraged to learn together. The NCBI contains a great deal of information and is updated regularly, making learning an on-going process.]



Navigating the NCBI: **Slide #2**

25. Show **Slide #3**, "and the NCBI...similar and different?"

Navigating the NCBI: **Slide #3**



[Note: You may wish to have students follow along with you by taking their own notes. They too can write "iTunes®" at the top of one column, and "NCBI" at the top of the second column. Rows on the top half of their paper can contain "Similarities," while rows on the bottom half of the page can be for noting "Differences." Under the columns, they can write down what these two databases have in common, and then how these two databases differ. This is also a useful way for students to take notes during the iTunes® versus NCBI brainstorming session.]

26. In pairs, have students brainstorm ways in which iTunes® and the NCBI are similar and ways they are different. Students could think about search functions, content, cost, and the type of results returned.
27. To review with students the results from their brainstorming session, **Slides #4–6** may be used. Alternatively, teachers may wish to draw tables on the board, and have students supply answers from their brainstorming. If using PowerPoint slides, project **Slide #4** "iTunes® and the NCBI" for all to see, and review with students the similarities between the two databases. If writing on the board, write "iTunes®" at the top of one column, and write "NCBI" at the top of the other column, listing the similarities, and then the differences, between the two databases. In addition to student answers, **Table 1** and **Table 2** on the following pages may be used to fill in the "similarities" and "differences;" write on the board only the bolded words found in the tables on the following pages, and discuss with students the ideas in italics.

Navigating the NCBI: **Slide #4**

iTunes® and the NCBI <i>are</i> similar because they both...	
iTunes®	NCBI
Contain Multiple Databases	
<i>iTunes® houses several organized collections of certain types of "data" (music, ringtones, videos, TV programs, and podcasts).</i>	<i>NCBI houses several organized collections of certain types of "data" (biological information).</i>
Use Search Functions	
<i>The iTunes® music database can be searched in a variety of ways, such as by song title, album title, artist, and more.</i>	<i>The NCBI nucleotide sequence database can be searched in a variety of ways, such as by topic, DNA sequence, accession number, author, and more.</i>

28. If using PowerPoint slides, next show **Slide #5**, which illustrates additional ways in which the NCBI and iTunes® are similar.

iTunes® and the NCBI are similar because they both...

iTunes®

NCBI

Yield Large Numbers of Results

Searching for a song title may yield a large number of closely-related results.

Searching for a DNA sequence may yield a large number of closely-related results.

Provide Additional Information

Each song search result includes information about the artist, album, release date, and more.

Each sequence search result includes information about the organism, location of sequence, date of database entry, and more.

Navigating the NCBI: **Slide #5**

iTunes®	NCBI
Contains multiple databases <i>iTunes® houses several organized collections of certain types of “data” (music, ringtones, videos, TV programs, and podcasts).</i>	Contains multiple databases <i>NCBI houses several organized collections of certain types of “data” (biological information).</i>
Uses search functions <i>The iTunes® music database can be searched in a variety of ways, such as by song title, album title, artist, and more.</i>	Uses search functions <i>The NCBI Nucleotide sequence database can be searched in a variety of ways, such as by topic, DNA sequence, accession number, author, and more.</i>
Yields large number of results <i>Searching for a song title may yield a large number of closely-related results.</i>	Yields large number of results <i>Searching for a DNA sequence may yield a large number of closely-related results.</i>
Provides additional information <i>Each song search result includes information about the artist, album, release date, and more.</i>	Provides additional information <i>Each sequence search result includes information about the organism, location of sequence, date of database entry, and more.</i>

Table 1: How iTunes® is Similar to the NCBI.

29. If using PowerPoint slides, next show **Slide #6**, and discuss with students ways in which iTunes® and the NCBI are different.

Navigating the NCBI: **Slide #6**

iTunes® and the NCBI are **not** similar because they both...

iTunes®	NCBI
Search Scale <i>The user cannot search for an exact sequence of notes that occur in more than one song in the music database.</i>	Search Scale <i>The user can search for an exact DNA sequence that occurs in many organisms in the DNA Nucleotide database.</i>
Content <i>iTunes® provides audio and audiovisual content.</i>	Content <i>NCBI provides biological content.</i>
Cost <i>iTunes® charges a fee for downloading most items.</i>	Cost <i>Use of the NCBI is free! It is paid for by the federal government through tax dollars.</i>

Table 2: How iTunes® is NOT Similar to the NCBI.

iTunes®	NCBI
Search scale—can't search by musical note sequences <i>The user cannot search for an exact sequence of musical notes that occur in more than one song in the music database.</i>	Search scale—can search by DNA sequences <i>The user can search for an exact DNA sequence that occurs in many organisms or genes in the DNA Nucleotide database.</i>
Content—audio and visual <i>iTunes® provides audio and audiovisual content.</i>	Content—biological <i>NCBI provides biological content.</i>
Cost—fee-based <i>iTunes® charges a fee for downloading most items.</i>	Cost—free <i>Use of the NCBI is free! It is paid for by the federal government through tax dollars.</i>

30. Show **Slide #7**. Draw students' attention to the first similarity between iTunes® and the NCBI (contains multiple databases). Tell students that the three databases they will be using the most during this unit are the **Nucleotide**, **Protein**, and **Structure** databases. Emphasize for students the types of information that are available in each database.

Navigating the NCBI: **Slide #7**

Two types of searchable databases...
not so different.

- **Nucleotide** database—this contains nitrogenous base sequences written in the form of A (adenine), T (thymine), C (cytosine), G (guanine), or U (uracil). Nucleotides are the building blocks of DNA and RNA. Three nucleotides code for one amino acid.
- **Protein** database—this contains the amino acid sequences written using the one-letter abbreviations for each of the 20 amino acids. For example, the amino acid *glycine* is abbreviated G and the amino acid *alanine* is abbreviated A. Amino acids are the building blocks of proteins.
- **Structure** database—this contains the three-dimensional representations of the protein structures. The amino acid sequence for the protein is also included in the structure database. Structures can be viewed with molecular viewing programs like **Cn3D**.

PART VI: Closure—Careers in the Spotlight

31. Check for understanding of the student handout. Make sure to reinforce the major concepts:

- The NCBI is the “wizard behind the curtain” that provides biological information to 23andMe and other direct-to-consumer genetic testing companies, as well as to research scientists and others studying genetic diseases. These companies have their own databases as well, which they add to with patient samples that they analyze.
- NCBI has a number of different databases that can be searched using the search engine Entrez.

32. Check for understanding about the BRCA genes. Ask, “Do all people have the *BRCA1* and *BRCA2* genes?”

“Yes, we do.” It is important for students to realize that all people—and in fact many other species—have both *BRCA1* and *BRCA2* genes—as students saw in Student Handout—Navigating the NCBI Instructions. The *BRCA1* and *BRCA2* proteins play crucial roles in DNA repair. However, some individuals have inherited *BRCA* alleles that contain mutations. Those mutations can encode defective proteins and are associated with increased risk for cancer.

33. Inform students that in *Lesson Three* they will read a case study that introduces students to a family with a history of breast cancer, and focuses on some of the ethical principles involved deciding whether to proceed with genetic testing.

34. Return to the picture of the veterinarian from the *Careers in the Spotlight* **Slide #8**.

Navigating the NCBI: **Slide #8**



VETERINARIAN

DEBORAH TEGARDEN, DVM

Place of Employment:
Elliot Bay Animal Hospital
Seattle, Washington

"While we often think of genetic testing in humans, vets are seeing more and more tests being developed for animal patients. This is the most exciting time I can imagine in veterinary medicine, when things are getting more and more cutting edge and technology is developing at lightning speed."

35. Show **Slide #9**, which provides job information for a veterinarian. Review this information with students.

Navigating the NCBI: **Slide #9**

CAREERS IN SPOTLIGHT: VETERINARIAN

What do they do?
Veterinarians diagnose and treat animals. Some veterinarians specialize in a particular area (such as oncologists who treat cancer), and some perform research to improve animal and human health. Veterinary technicians assist veterinarians in their work.

What kind of training is involved?
Veterinarians complete a Bachelor's degree and a DVM (Doctor of Veterinary Medicine) degree, which requires four years. Veterinary technician training is usually a two year (Associate's) program.

What is a typical salary for a Veterinarian?
Veterinarians: \$45,000/year (\$22/hour), up to \$140,000/year (\$67/hour). Veterinary Technicians: \$20,000/year (\$10/hour), up to \$45,000/year (\$22/hour).

36. Ask students, "How does a veterinarian's job relate to today's lesson?" Point out that:

- A number of genetic tests have been developed for animals.
- Bioinformatics tools can be used to conduct research on opportunities for animal breeding programs.
- As students saw today, much of the biological information at NCBI is gathered from animals, and veterinarians, as well as research scientists, play a role in this.

37. Ask students to answer Veterinarian 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 veterinarian does.

38. Ask students to also answer Veterinarian Question #3 on their *Careers in the Spotlight* handout, which has students explain how a veterinarian might use bioinformatics in his or her work.
39. 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 Student Handout—*Case Study: A BRCA Genetic Testing Dilemma* from *Lesson Three* be provided as homework to allow more time in class to discuss the case study.

- A. As homework, ask students to write about the activities they learned in *Lesson Two* 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:
- Today I learned that...
 - An important idea to think about is...
 - Something that I don't completely understand yet is...
 - Something that I'm really confident that I understand is...
- B. Have students return to the NCBI homepage and search for something of interest to them, such as a disease, an animal, or a gene they've heard about in class or in the news. Based on the Entrez search results, what can students conclude about how much this topic has been studied? Do any of the search results surprise them? Encourage them to explore some of the links to their search results, such as the Nucleotide sequences or scientific articles found in **PubMed** Central [which are all freely available], and describe what they've found.
- C. Distribute Student Handout—*Case Study: A BRCA Genetic Testing Dilemma* from *Lesson Three* as homework before the class discussion session. Encourage students to read through the case study and discuss the issues with family members and/or friends. Answers to the Homework Questions can serve as the entry ticket to participate in the class discussion the following day.

[**Note:** Suggested scoring for homework: +5 points if all 4 prompts are complete.]

[**Note:** Suggested scoring for homework: Up to +10 points.]

Glossary

Bioinformatics: Bioinformatics is the application of computer science and information technology to biology and medicine. Bioinformatics makes it possible to analyze large and complex biological data and can be used to search biological databases, compare sequences, and draw molecular structures. Bioinformatic techniques are used to design and carry out the computer-based portion of genetic tests.

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

Entrez: The search engine used to simultaneously search all of the databases at the NCBI. Items stored in different databases are cross-referenced and inter-linked, making it easier to find all the database records that are related to any one subject.

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

Credit

Tegarden, Deborah. Personal Interview. 6 November 2009.

2 Navigating the NCBI Instructions

Aim: To become familiar with the resources available at the National Center for Bioinformatics (NCBI) and the search engine *Entrez*.



Instructions: Write the answers to your questions on the Student Worksheet, in your lab notebook, or on a separate sheet of paper, as instructed by your teacher.

1. Go to the NCBI homepage <http://www.ncbi.nih.gov/>

The screenshot shows the NCBI homepage. On the left, a vertical menu lists various resources: NCBI Home, Site Map (A-Z), All Resources, Chemicals & Bioassays, Data & Software, DNA & RNA, Domains & Structures, Genes & Expression, Genetics & Medicine, Genomes & Maps, Homology, Literature, Proteins, Sequence Analysis, Taxonomy, Training & Tutorials, and Variation. On the right, a 'Popular Resources' section lists: BLAST, Bookshelf, Gene, Genome, Nucleotide, OMIM, Protein, PubChem, PubMed, PubMed Central, and SNP. The central area features a 'Welcome to NCBI' message, a 'Get Started' section with links to Tools, Downloads, How-To's, and Submissions, and a 'Genomic Structural Variation' banner. The search bar at the top is highlighted with a green box.

Figure 1: Familiarize yourself with the NCBI homepage. Credit: NCBI.



2. Take a few minutes to look around the site. The goal is to familiarize yourself with a few key components of the NCBI.
 - a. What is the name of one interesting resource or database shown in the blue box on the left? What do you think is its function or purpose?
 - b. What is one interesting resource listed in the Popular Resources menu on the right? What do you think is its function or purpose?
3. Find the search box in the center of the webpage (black box in above image). This search box uses the NCBI search engine *Entrez* to look for your search term (or "**query**") across **all** of the databases at the NCBI.

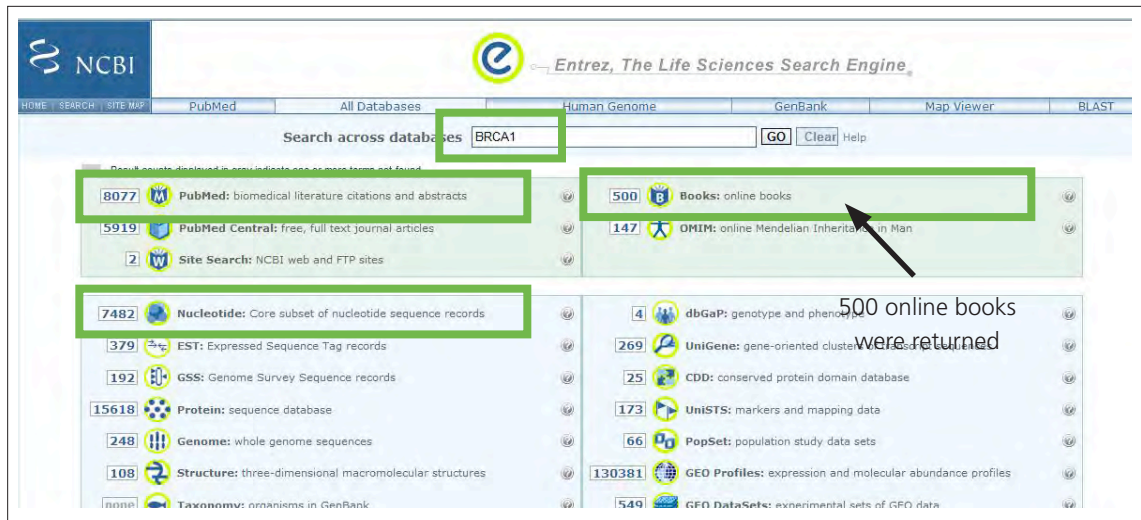


Figure 2: Search for “BRCA1.” Credit: NCBI.



4. Type “BRCA1” into the **Search** box. Make sure there is *no* space between BRCA and 1. Click **Search**.

BRCA1 is a tumor suppressor gene that normally prevents cancer. Mutations in this gene are associated with increased risk of hereditary breast cancer and ovarian cancer when normal function is lost.

The white box to the left of each database contains the number of “hits” returned from that database (see screen shot, above). This is like searching in iTunes® without specifying categories like ringtones, podcasts, movies, TV, or songs.

- a. Why are we searching for *BRCA1*?
 - b. The **Nucleotide** database has DNA sequences that have been loaded onto the NCBI database. How many times is ‘BRCA1’ cited in the Nucleotide database?
 - c. The **PubMed** database has the articles that have been published about a specific gene or disease. How many times is ‘BRCA1’ cited in the PubMed database?
 - d. Compare the numbers you got for Questions **a** and **c**. Do these relative numbers surprise you? What does this tell you about the *BRCA1* gene? Explain.
5. Go back to the NCBI homepage by clicking the **NCBI** logo in the upper left corner of the screen.
This search shows that there is a lot of information at the NCBI! It can be challenging to try to make sense of it all. Let’s start with something more familiar.

6. Click the “**All Resources**” link from the list of resources on the left side of the screen.
7. Find “**Map Viewer**.” Click on the “Tools” tab and either scroll through the alphabetical list, or use the “Find” feature (PC: “Control+F” Mac: “Command+F”) to Find “**Map Viewer**.” Click on the “**Map Viewer**” link.

The resulting page is called **Map Viewer** and it allows us to search the genomes of many different organisms, including humans.

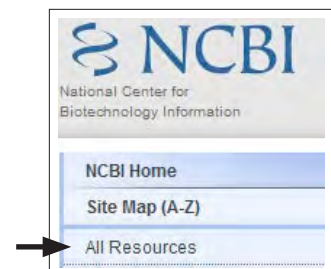


Figure 3: Click “All Resources” on the left side of the screen. Credit: NCBI.

8. Open the Search menu, select **Homo sapiens** from the pull-down menu, and click "Go."



9. Now we can see the **Homo sapiens (human) genome view**. A **genome** is all of the genetic information in an organism. Each figure you see in the "genome view" represents a pair of chromosomes. Most of the chromosomes are numbered, but a few are not. The abbreviations "X" and "Y" refer to the human sex chromosomes.

a. How many different types of chromosomes do you see?

b. What does "MT" represent?

[Note: you can click the "MT" link to find out.]

c. With the exception of MT, the chromosomes of the human genome are in pairs. X and Y are a pair. Using this information and the information from your answer to Question 9A, how many **pairs** of chromosomes are in the human genome?

Search
pull-down
menu

Select
*Homo
sapiens*

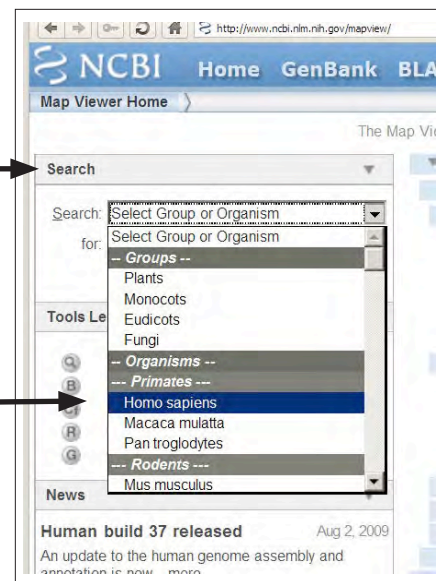


Figure 4: Select "Homo sapiens" from the list of groups or organisms. Credit: NCBI.



10. The Breast Cancer Susceptibility gene *BRCA1* is on chromosome 17 in humans. [Click on the link below chromosome 17.] Explore some of the links and views.

What do you see when you click on chromosome 17? Explore some of the links on the picture, and write down two things you found interesting, such as the description of other genes that are also found on chromosome 17.



11. To find the location of the *BRCA1* gene, type "BRCA1" in the "Search" box at the top left of the screen, and click "Find in This View." Scroll through the Map of Chromosome 17 and locate the *BRCA1* gene, which should be highlighted in pink. "BRCA1" will be found in the list of Symbols. You can also use the "Find" feature (PC: "Control+F" Mac: "Command+F"), which will highlight in yellow every mention of "BRCA1," including the *BRCA1* gene.

Draw a picture of chromosome 17 and show the *approximate* location of *BRCA1* on this chromosome.

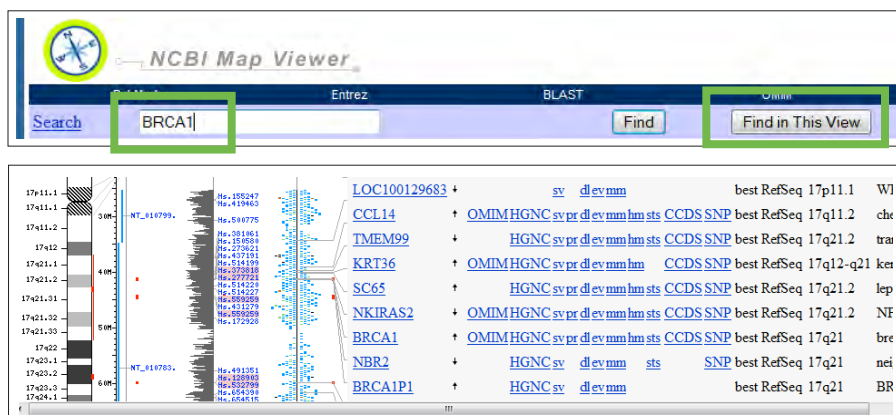


Figure 5: Find the location of the *BRCA1* gene by using the search function. Credit: NCBI.

12. Click on the **BRCA1** link. This will take you to **Entrez Gene**, which provides a summary of the information available at the NCBI for *BRCA1*. Scroll through the webpage and explore some of the information available. Scroll down the webpage to the section titled “Gene Ontology.” There is a table titled “Function.”



List three of the functions that the BRCA1 protein performs.

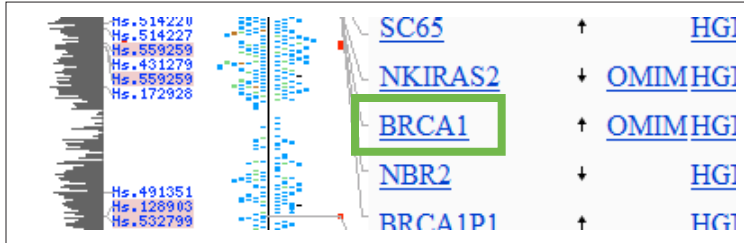


Figure 6: Click on the “BRCA1” link to launch **Entrez Gene**. Credit: NCBI.

Gene Ontology provided by [GOA](#)

Function	Evidence	
	Evidence Code	Pubs
DNA binding	IEA	

Figure 7: Scroll down to find the “Gene Ontology” section. Credit: NCBI.

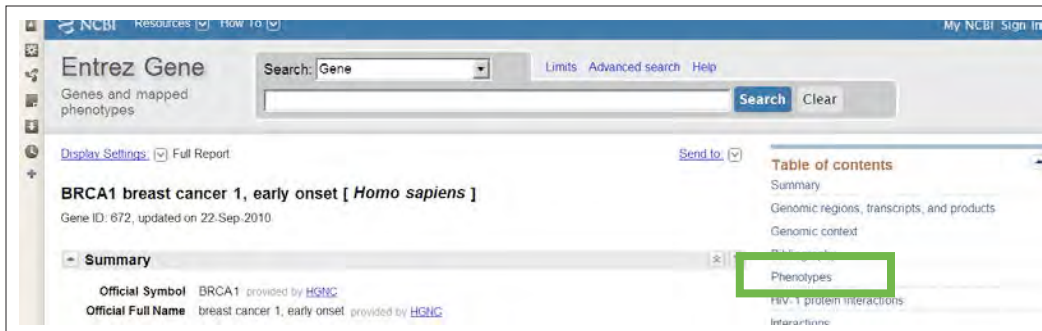


Figure 8: Select “Phenotypes” from the Table of Contents. Credit: NCBI.

13. To learn about all of the **phenotypes** associated with mutations of *BRCA1*, return to the top of the web page and from the “Table of Contents” on the right, select “Phenotypes.” This will bring you to the portion of the web page that contains the phenotype information for *BRCA1*.

- Based on what you’ve learned in class, what is a **phenotype**?
- What **phenotypes** are associated with mutations in the *BRCA1* gene? (You don’t need to click the links.)

14. Return to the **Table of Contents** at the top of the page and click “Reference Sequences.” This will take you to the portion of the webpage that contains the actual genetic sequence of the *BRCA1* gene.

15. **Reference sequences** are DNA or protein sequences that scientists, doctors and genetic counselors use to study genes like *BRCA1*. You can download these sequences in different formats. For this exercise, click “FASTA” (which is sometimes pronounced FAST-ay).



Figure 9: Click “FASTA” to obtain the FASTA sequence for *BRCA1*. Credit: NCBI.



Figure 10: Take a look at the FASTA sequence for *BRCA1*. Credit: NCBI.



16. This link takes you to the FASTA sequence for *BRCA1*. Scroll through the web page. This gene is very large!

- What four letters make up this long sequence?
- Based on what you’ve learned in class, what do these letters represent?

17. Return to the NCBI homepage by clicking on the NCBI icon on the top left of the web page.

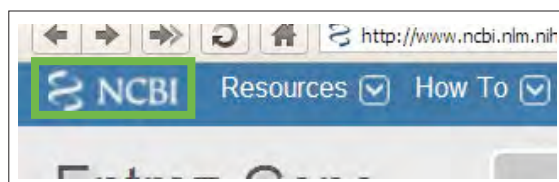


Figure 11: Return to the NCBI homepage by clicking on the logo. Credit: NCBI.

18. Type **BRCA1** in the **Search** box and select “**Nucleotide**” from the pull-down menu beside the **Search** box, to limit your search to the database containing all of the DNA and RNA (Nucleotide) sequences. Click the “Search” button.

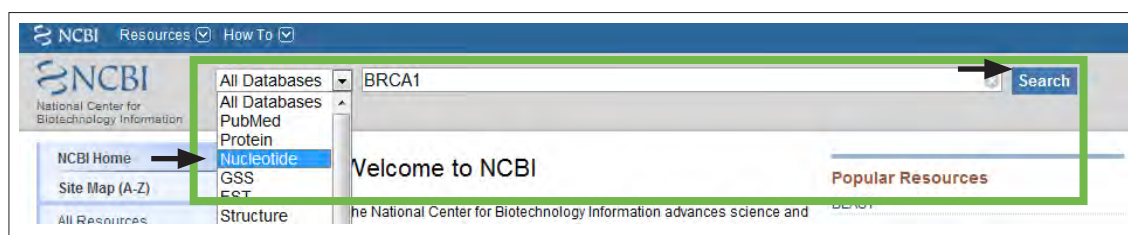


Figure 12: Type “BRCA1” into the Search box and choose “Nucleotide” from the pull-down menu. Credit: NCBI.

LESSON 2

CLASS SET

19. What other organisms have *BRCA1* genes? You can scroll through the list of organisms, but note that these are listed by the **scientific name** of the organism (Genus and species), not the common name. For example, *Homo sapiens* is the scientific name for humans. Also, the **Top Organisms** (or the organisms with the most “hits”) are listed on the right. **Helpful Hint:** Hold your cursor over the species name to see the common name appear. Alternatively, you can perform an internet search to find the common name(s) of your organisms.



List three organisms other than humans that have *BRCA1* genes. Include both the scientific and common names.

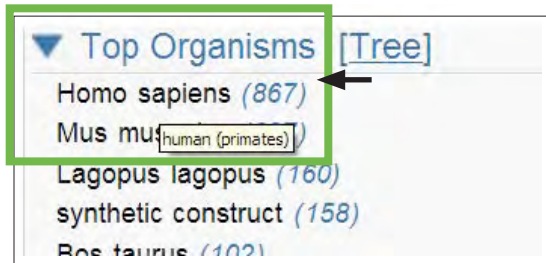


Figure 13: Scroll through the list of Top Organisms. Credit: NCBI.

20. Look back at your list of functions for the *BRCA1* gene (question #12).



Does it surprise you that so many organisms share the *BRCA1* gene? Explain.

21. What kind of information can you find at the National Center for Biotechnology Information?



Summarize what you have learned today by listing three types of information found at the NCBI.

Name _____ Date _____ Period _____

Navigating the NCBI Worksheet

Aim: To become familiar with the resources available at the National Center for Bioinformatics (NCBI) and the search engine **Entrez**.



Instructions: Use Student Handout—*Navigating the NCBI Instructions* to complete this worksheet.

- 2a. What is the name of one interesting resource or database shown in the blue box on the left? What do you think is its function or purpose?
- 2b. What is one interesting resource listed in the Popular Resources menu on the right? What do you think is its function or purpose?
- 4a. Why are we searching for *BRCA1*?
- 4b. The **Nucleotide** database has DNA sequences that have been loaded onto the NCBI database. How many times is 'BRCA1' cited in the **Nucleotide** database? _____
- 4c. The **PubMed** database has the articles that have been published about a specific gene or disease. How many times is 'BRCA1' cited in the **PubMed** database? _____
- 4d. Compare the numbers you got for Questions B and C. Do these relative numbers surprise you? What does this tell you about the *BRCA1* gene? Explain.
- 9a. How many different types of chromosomes do you see? _____
- 9b. What does "MT" represent? [**Note:** you can click the "MT" link to find out.]
- 9c. With the exception of MT, the chromosomes of the human genome are in pairs. X and Y are a pair. Using this information and the information from your answer to Question 9A, how many **pairs** of chromosomes are in the human genome? _____

LESSON 2

HANDOUT

10. What do you see when you click on chromosome 17? Explore some of the links on the picture, and write down two things you found interesting, such as the description of other genes that are also found on chromosome 17.

1:

2:

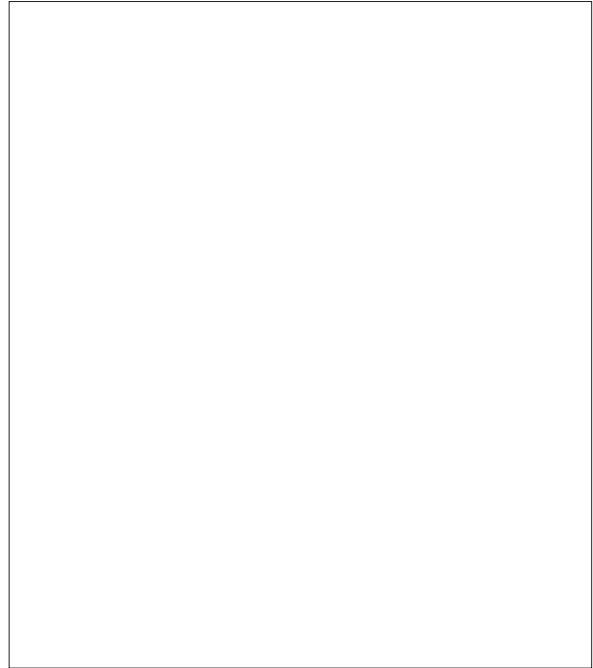
11. Draw a picture of Chromosome 17 in the box to the right and show the *approximate* location of *BRCA1* on this chromosome.

12. List three (3) functions that the protein produced by the *BRCA1* gene performs.

1: Function 1:

2: Function 2:

3: Function 3:



13a. Based on what you've learned in class, what is a **phenotype**?

13b. What **phenotypes** are associated with mutations in the *BRCA1* gene?
(You don't need to click the links.)

16a. What four letters make up this long sequence? _____

16b. Based on what you've learned in class, what do these letters represent?

19. List three organisms other than humans that have *BRCA1* genes.

1: Common Name: _____

Scientific name: _____

2: Common Name: _____

Scientific name: _____

3: Common Name: _____

Scientific name: _____

20. Does it surprise you that so many organisms share the *BRCA1* gene? Explain. (Hint: Look back at the functions of the BRCA1 protein (Question 12).)

21. Summarize what you have learned today by listing three types of information found at the NCBI.

1:

2:

3:

LESSON 2

HANDOUT

2

Navigating the NCBI 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 **30 points**.]

Aim: To become familiar with the resources available at the National Center for Bioinformatics (NCBI) and the search engine **Entrez**.

2a. What is the name of one interesting resource or database shown in the blue box on the left? What do you think is its function or purpose?

This will vary, as more resources are added to the NCBI, but students should list at least one Resource (such as "Nucleotide" and one function, such as "DNA sequences."
(+1 for listing a resource and +1 for attempting to name its function/purpose.)

2b. What is one interesting resource listed in the Popular Resources menu on the right? What do you think is its function or purpose?

This will vary, as more resources are added to the NCBI, but students should list at least one Resource, such as "Nucleotide," and one function, such as "DNA sequences."
(+1 for listing a resource and +1 for attempting to name its function/purpose.)

4a. Why are we searching for *BRCA1*?

To learn more about this gene and why mutations in this gene can lead to breast and ovarian cancer. (Students should draw on their experiences in *Lesson One*.)
(+1 for referring to understanding how it causes breast and ovarian cancer.)

4b. The **Nucleotide** database has DNA sequences that have been loaded onto the NCBI database. How many times is 'BRCA1' cited in the **Nucleotide** database?

New sequences are added every day. As of May 19, 2011, there were 7482
(+0.5 for number.)

4c. The **PubMed** database has the articles that have been published about a specific gene or disease. How many times is 'BRCA1' cited in the **PubMed** database?

New articles are added every day. As of May 19, 2011, there were 8077
(+0.5 for number.)

4d. Compare the numbers you got for Questions B and C. Do these relative numbers surprise you? What does this tell you about the *BRCA1* gene? Explain.

Students should note that these numbers are close, but not all published studies will contain new *BRCA1* sequences, and some studies will contain more than one sequence.

(+1 for response to relative numbers and +1 for explanation of response.)

9a. How many different types of chromosomes do you see? _____ (+0.5 for 25: 22 pairs, plus X, Y and MT.)

25

9b. What does "MT" represent?

MT is the mitochondrial genome/chromosome (+1 for mitochondrial).

[Note: you can click the "MT" link to find out.]

9c. With the exception of MT, the chromosomes of the human genome are in pairs. X and Y are a pair. Using this information and the information from your answer to Question 9A, how many **pairs** of chromosomes are in the human genome? _____ (+0.5 for 23.)

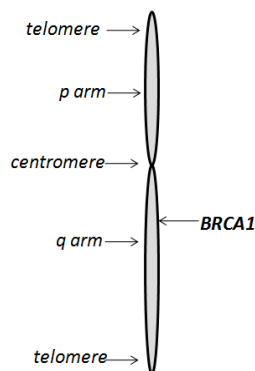
23

10. What do you see when you click on chromosome 17? Explore some of the links on the picture, and write down two things you found interesting.

Answers will vary. Students will see many genes listed, with links to other NCBI resources.

(+1 for each thing listed up to +2.)

11. Draw a picture of chromosome 17 in the box to the right and show the approximate location of *BRCA1* on this chromosome.



(+1 for drawing and +1 for *BRCA1* label in approximate location.)

[Note: Labeling of the centromere, telomeres, p and q arms is optional.]

12. List three (3) functions that the protein produced by the *BRCA1* gene performs.

1. DNA binding
 2. RNA binding
 3. Androgen receptor binding
 4. Protein binding
 5. Tubulin binding
 6. Transcription activator activity
- (+1 for each correct function listed, up to +3.)

13a. Based on what you've learned in class, what is a **phenotype**?

A phenotype is an observable characteristic or trait (+1.)

13b. What **phenotypes** are associated with mutations in the *BRCA1* gene? (You don't need to click the links.)

Breast cancer; ovarian cancer; breast-ovarian cancer; pancreatic cancer susceptibility; Papillary serous carcinoma of the peritoneum

(+0.5 for each phenotype listed, up to +2.)

16a. Based on what you've learned in class, what four letters make up this long sequence? _____

(+1 for including all 4 letters.)

A, T, C, G

16b. What do these letters represent? A = adenine; T= thymine; G = guanine; C= cytosine

These are the bases of the *BRCA1* gene. (+1 for either listing all 4 bases or for stating 'the bases.')

19. List three other organisms that have *BRCA1* genes. (+1 for each organism; – 0.5 for common name and 0.5 for scientific name; up to +3.)

Answers will vary, as almost all animals have *BRCA1* genes, and new sequences are being added every day, Possible answers include:

1. *Mus musculus* (mouse)
2. *Bos taurus* (cow)
3. *Macaca mulatta* (rhesus macaque/monkey)
4. *Sus scrofa* (pig or boar)
5. *Lagopus lagopus* (willow ptarmigan, a type of bird)

20. Does it surprise you that so many organisms share the *BRCA1* gene? Explain. (Hint: Look back at the functions of the BRCA1 protein (Question #12).)

All organisms need to bind DNA, RNA and other proteins.

(+1 for statement of surprise and +1 for reference to functions of BRCA1 protein.)

21. Summarize what you have learned today by listing three types of information found at the NCBI. (+1 for each type listed, up to +3.)

1. Scientific publications
2. Scientific books
3. Gene/nucleotide sequences
4. Protein sequences
5. Protein structures
6. Information about gene functions

3

Exploring Genetic Testing:
A Case Study

Introduction

In this lesson, students engage in a case study about a family with a history of breast cancer. Students consider ethical issues surrounding genetic testing as they decide whether family members should get tested for *BRCA1* or *BRCA2* mutations. Students then evaluate the case through the principles-based bioethics concepts of: **Respect for Persons**, **Maximize Benefits/Minimize Harms**, and **Justice**. Students apply the principles to help them reason through their decision as they participate in a Structured Academic Controversy. In *Lesson Three*, students learn how **genetic counselors** might use bioinformatics tools in their careers.

Learning Objectives

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

- Genetic testing involves screening a patient's DNA for the presence of mutations that may cause diseases such as cancer.
- Genetic testing can have implications for family members of the patient, as they share some of the same genetic material.
- Bioinformatics tools are used by people in many careers, including genetic counselors.
- In the case of *BRCA1* and breast cancer, no treatment is 100% effective, and the test is not 100% predictive about whether the patient will develop cancer.

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

- Identify ethical issues involved in genetic testing.
- Apply their understanding of bioethical principles to a case study.
- Explain why someone would or would not choose to have the genetic test for *BRCA1* mutations.
- Consider alternative perspectives and engage in discussion and decision making during a Structured Academic Controversy.

Key Concepts

- Genetic testing involves screening individuals for the presence or absence of mutations that can cause cancer (i.e., cancer-associated alleles).
- A mutation in a particular gene does not mean that the person carrying that allele will definitely get cancer.
- Knowledge of ethical principles can provide a structure for making complex decisions. The bioethical principles introduced are:
 - o **Respect for Persons:** Respecting the inherent worth of an individual and his or her **autonomy**.
 - o **Maximize Benefits/Minimize Harm: Beneficence/nonmaleficence.**
 - o **Justice:** Being fair.

Class Time

- One homework assignment before class.
- One class period (50 minutes) for the Structured Academic Controversy.

Prior Knowledge Needed

- Understanding of the Central Dogma of Molecular Biology.

Common Misconceptions

- Only women can get breast cancer.
Fact: 1 in 1,000 men will contract breast cancer in their lifetime.
- Not everyone has BRCA genes.
Fact: Everyone has BRCA genes. Some of the mutant or altered forms (alleles) can cause cancer.

- In the case of *BRCA1* and breast cancer, no treatment is 100% effective.
- Bioinformatics tools are used by people in many careers, including genetic counselors

Materials

Materials	Quantity
Copies of Student Handout— <i>Careers in the Spotlight</i> (handed out in <i>Lesson One</i>)	1 per student
Class set of Student Handout— <i>Case Study: A BRCA Genetic Testing Dilemma</i>	1 per student (class set)
Class set of Student Handout— <i>Focus on the Principles</i>	1 per student (class set)
Copies of Student Handout— <i>Structured Academic Controversy Worksheet</i>	1 per student
Class set of Student Handout— <i>Structured Academic Controversy FOR Position Statement</i>	1 per every 2 students (half of class) (class set)
Class set of Student Handout— <i>Structured Academic Controversy AGAINST Position Statement</i>	1 per every 2 students (half of class) (class set)
Teacher Answer Key— <i>Focus on the Principles</i>	1
Teacher Answer Key— <i>Structured Academic Controversy Worksheet</i>	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.
<i>Lesson Three</i> PowerPoint Slides— <i>Exploring Genetic Testing: A Case Study</i> . 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 .
Computer lab with internet access for students.

Teacher Preparation

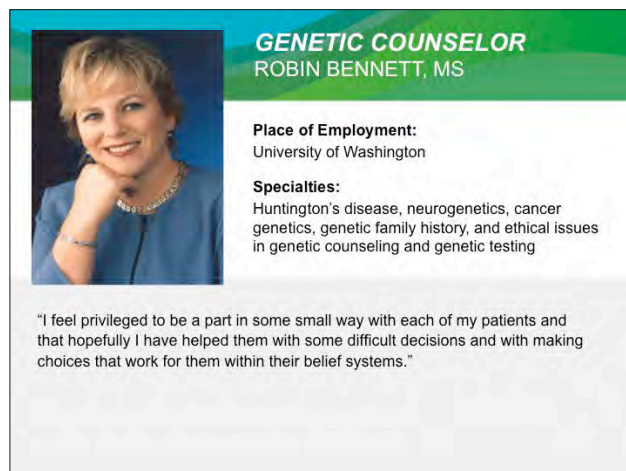
- Load the classroom computer with the *Lesson Three* PowerPoint slides.
- Make copies of the Student Handouts, one per student. The following handouts are designed to be used as class sets: Student Handout—*Case Study: A BRCA Genetic Testing Dilemma*; Student Handout—*Focus on the Principles*; Student Handout—*Structured Academic Controversy FOR Position Statements*; and Student Handout—*Structured Academic Controversy AGAINST Position Statement*.
- Student Handout—*Case Study: A BRCA Genetic Testing Dilemma* should be assigned as homework before the lesson.

[**Note:** Half of the students will receive copies of Student Handout—*Structured Academic Controversy FOR Position Statements* and half of the students will receive copies of Student Handout—*Structured Academic Controversy AGAINST Position Statements*.]

Procedure

WARM UP

1. As students enter the classroom, display PowerPoint **Slide #1**. This slide highlights genetic counselor Robin Bennett.



Exploring Genetic Testing: **Slide #1**

2. Have students retrieve Student Handout—*Careers in the Spotlight* from Lesson One.
3. Students should think about, and write down, what kind of work a genetic counselor might do (Genetic Counselor Question #1). This will be revisited at the end of the lesson, including how a genetic counselor 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: Applying Bioethical Principles to a Case Study

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

Lesson Aim:

- To understand the ethical issues involved in genetic testing.

Some teachers may find it useful to write the lesson 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 plan.

6. Ensure that everyone has read the case study. To participate in the discussion and the following Structured Academic Controversy, it is very important that every student has read the case study. If the case study was not assigned previously for homework, provide time in class for students to read the case study found on Student Handout—*Case Study: A BRCA Genetic Testing Dilemma*.
7. Pass out Student Handout—*Focus on the Principles*.
8. Show **Slide #2**. Tell students that they may already be familiar with the important concepts behind the bioethical principles found on Student Handout—*Focus on the Principles*, which include concepts such as **fairness**, **doing good**, and **respect**. Bioethicists use a number of different ethical

perspectives to help defend their position. In this unit, we will focus on the principles **Respect for Persons**, **Maximize Benefits and Minimize Harms**, and **Justice**.

Exploring Genetic Testing: **Slide #2**

BIOETHICAL PRINCIPLES

Respect for Persons

- Respecting the inherent worth of an individual and his or her autonomy

Maximize Benefits/Minimize Harm

- Beneficence/Nonmaleficence
- The most good for the most people

Justice

- Being fair; giving what is "owed" or "due"
- Distributing benefits/burdens equitably across a group of individuals

- Encourage students to engage in a Think-Pair-Share. First, using their answers to the case study questions found on Student Handout—*Case Study: A BRCA Genetic Testing Dilemma*, have students use a separate sheet of paper to respond to the prompts on Student Handout—*Focus on the Principles* as best they can individually.
- After about five minutes, have individual students come together in pairs to compare answers to Student Handout—*Focus on the Principles*. Students can revise their written responses as needed.
- After a few minutes, have two pairs of students meet as a group of four. Again, students can compare answers and revise their written responses.
- Bring the class together to share students' answers and thoughts on Student Handout—*Focus on the Principles*. During the class discussion, draw out how the bioethical principles relate to Deb Lawler and her decision to have, or not to have, genetic testing for mutations in the BRCA genes. Teacher Answer Key—*Focus on the Principles* can be used as a guide for this discussion.
- Point out that the principles can be used to support either a 'yes' or a 'no' answer to the ethical question. The **reasoning** behind the decision is more important than the decision itself for these purposes.

PART II: Structured Academic Controversy

- Show **Slide #3**. Tell students about the framework of a Structured Academic Controversy. The basic framework is:
 - A group of four subdivides into two pairs of students.
 - Two students are assigned (or choose) the FOR position; two argue the AGAINST position.
 - Each pair reads background material on their position and prepares their argument.
 - Pair FOR presents while Pair AGAINST listens.

[**Note:** Structured Academic Controversy is a text-based, small group deliberation model where students learn about both sides of an issue before examining their own personal views. Active listening is an important part of the process. The ethical question the students will be considering is: "Should Deb Lawler have BRCA genetic testing?"]

- Pair AGAINST asks clarifying questions **only**.
- Pair AGAINST paraphrases FOR.
- Pair AGAINST presents while Pair FOR listens.
- Pair FOR asks clarifying questions **only**.
- Pair FOR paraphrases AGAINST.
- Students drop their assigned roles and discuss possible solutions.
- Students take turns stating their own positions.
- Students clarify areas of agreement and disagreement.

STRUCTURED ACADEMIC CONTROVERSY

Should Deb Lawler have BRCA genetics testing?

- Read background position statements
- Pair "FOR" presents, Pair "AGAINST" listens
- Pair "AGAINST" asks CLARIFYING questions **only**
- Pair "AGAINST" paraphrases "FOR"
- Pair "AGAINST" presents, Pair "FOR" listens
- Pair "FOR" asks CLARIFYING questions **only**
- Pair "FOR" paraphrases "AGAINST"
- Students drop their assigned roles and discuss possible solutions.
- Students take turns stating their own positions.
- Students clarify areas of agreement and disagreement.

Exploring Genetic Testing: **Slide #3**

15. Remind students of the classroom norms. For example, students should speak one at a time, hear all sides equally, listen well enough to respond, and back up their opinions with clear reasons.
16. Show **Slide #4**, which begins to walk students through the steps of the Structured Academic Controversy. Students should already be in groups of four from completing *Part I* of the lesson. Have them further subdivide into two pairs.

STRUCTURED ACADEMIC CONTROVERSY

Should Deb Lawler have BRCA genetics testing?

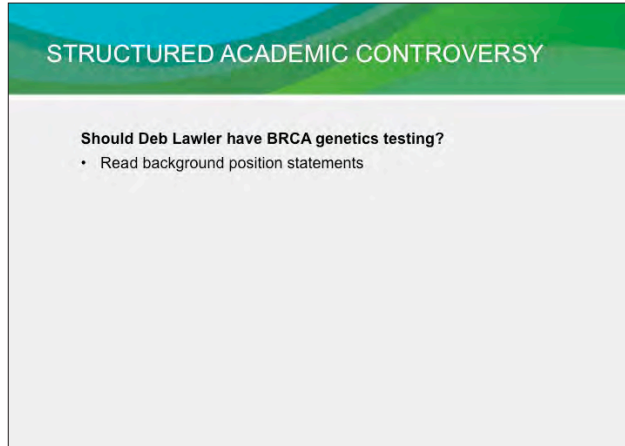
- Groups of four students subdivide into two groups of two
- Two students are "FOR" and two students are "AGAINST"

Exploring Genetic Testing: **Slide #4**

17. Pass out Student Handout—*Structured Academic Controversy Worksheet*. In their pairs, have students fill out the *Relevant facts and Stakeholders* sections using the case study (Student Handout—*Case Study: A BRCA Genetic Testing Dilemma*).

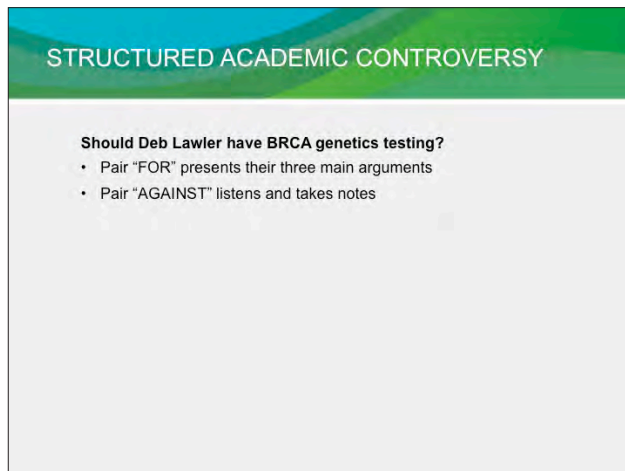
18. Pass out Student Handout—*Structured Academic Controversy FOR Position Statements* to half of the students (the FOR groups) and Student Handout—*Structured Academic Controversy AGAINST Position Statements* to half of the students (the AGAINST groups). Two students are assigned (or choose) the FOR position; two are assigned (or choose) the AGAINST position.
19. Show **Slide #5**. Each pair reads the background information supporting their position (about five minutes). Together, each pair plans a presentation of their position and arguments. Students should focus on their **three most important arguments**.

Exploring Genetic Testing: **Slide #5**

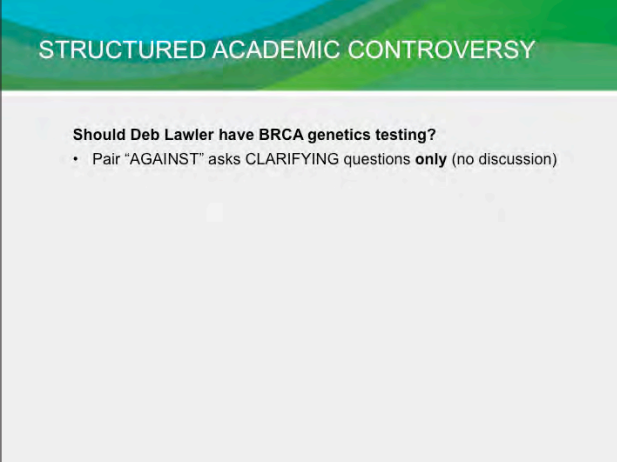


20. Show **Slide #6**. One side presents, the other side listens and takes notes. One side presents their three important arguments to the other side. The other side needs to listen carefully and take notes. The presenters should be satisfied that their position has been heard and understood.

Exploring Genetic Testing: **Slide #6**



21. Show **Slide #7**. To be sure that Pair AGAINST understands the arguments, they may ask clarifying questions as necessary. Emphasize that there is no discussion at this point.




STRUCTURED ACADEMIC CONTROVERSY

Should Deb Lawler have BRCA genetics testing?

- Pair "AGAINST" asks CLARIFYING questions **only** (no discussion)

Exploring Genetic Testing: **Slide #7**

22. Show **Slide #8**. Pair AGAINST paraphrases the three main arguments from Pair FOR (members of Pair AGAINST take turns restating the main points back). Pair FOR corrects any misunderstandings.



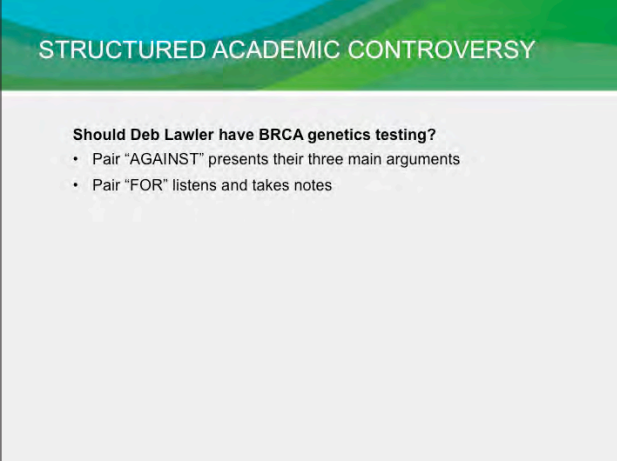
STRUCTURED ACADEMIC CONTROVERSY

Should Deb Lawler have BRCA genetics testing?

- Pair "AGAINST" paraphrases "FOR" (members of Pair "AGAINST" take turns restating the main points back)
- Pair "FOR" corrects any misunderstandings

Exploring Genetic Testing: **Slide #8**

23. Show **Slide #9**. **The pairs switch** and the process is repeated, with Pair AGAINST presenting their three main arguments, while Pair FOR listens and takes notes.



STRUCTURED ACADEMIC CONTROVERSY

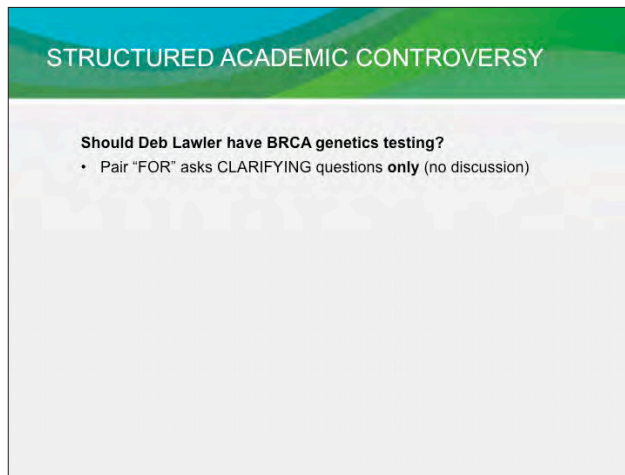
Should Deb Lawler have BRCA genetics testing?

- Pair "AGAINST" presents their three main arguments
- Pair "FOR" listens and takes notes

Exploring Genetic Testing: **Slide #9**

24. Show **Slide #10**. To be sure that Pair FOR understands the arguments, they may ask clarifying questions as necessary. Emphasize that there is no discussion at this point.

Exploring Genetic Testing: **Slide #10**



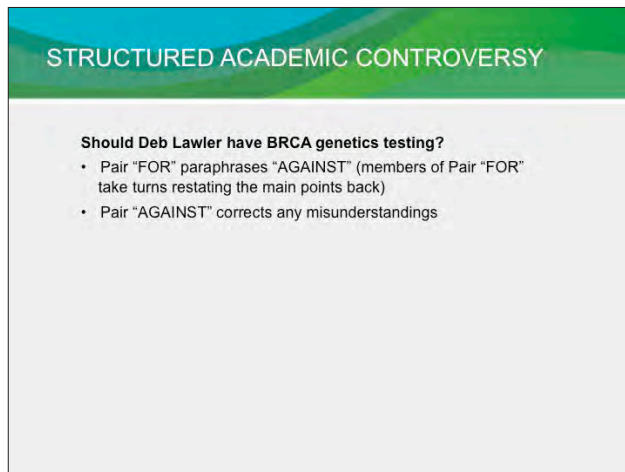
STRUCTURED ACADEMIC CONTROVERSY

Should Deb Lawler have BRCA genetics testing?

- Pair "FOR" asks CLARIFYING questions **only** (no discussion)

25. Show **Slide #11**. Pair FOR paraphrases the three main arguments from Pair AGAINST (members of Pair FOR take turns restating the main points back). Pair AGAINST corrects any misunderstandings.

Exploring Genetic Testing: **Slide #11**



STRUCTURED ACADEMIC CONTROVERSY

Should Deb Lawler have BRCA genetics testing?

- Pair "FOR" paraphrases "AGAINST" (members of Pair "FOR" take turns restating the main points back)
- Pair "AGAINST" corrects any misunderstandings

26. Show **Slide #12**. Students drop their roles. Students proceed as their own individual selves, using information from their own experiences as well as the background readings. **Prompt: See if you can clarify areas of agreement and disagreement. Feel free to change your mind.**

Exploring Genetic Testing: **Slide #12**


STRUCTURED ACADEMIC CONTROVERSY

Should Deb Lawler have BRCA genetics testing?

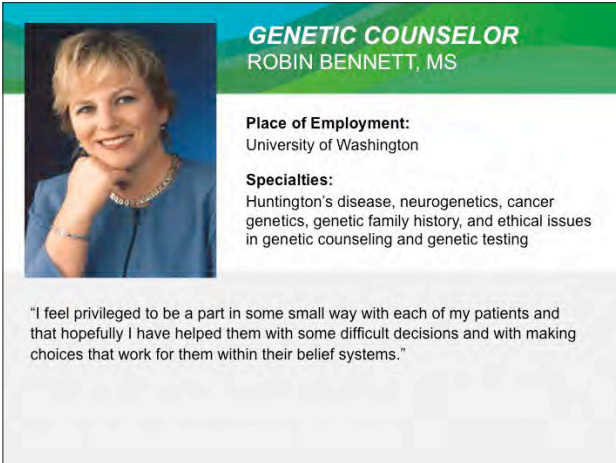
- Students drop their assigned roles and discuss possible solutions.
- Students take turns stating their own positions.
- Students clarify areas of agreement and disagreement.

27. While working through the Structured Academic Controversy, students should continue to fill out Student Handout—*Structured Academic Controversy Worksheet*.

Closure: Careers in the Spotlight

28. Convey this to students: “Today, we’ve taken a close look at a personal decision about whether to have genetic testing for mutations in the BRCA genes. Tomorrow we’ll learn more about the tools that make genetic testing possible, using sequences from the Nucleotide and Protein databases at the NCBI to compare with sequences from members of the Lawler family to find out if each person has a mutation in his or her *BRCA1* gene.”

29. Return to the picture of the genetic counselor from Student Handout—*Careers in the Spotlight*, **Slide #13**.

Exploring Genetic Testing: **Slide #13**


GENETIC COUNSELOR
ROBIN BENNETT, MS

Place of Employment:
University of Washington

Specialties:
Huntington's disease, neurogenetics, cancer genetics, genetic family history, and ethical issues in genetic counseling and genetic testing

"I feel privileged to be a part in some small way with each of my patients and that hopefully I have helped them with some difficult decisions and with making choices that work for them within their belief systems."

30. Show **Slide #14**, which provides job information for a genetic counselor. Review this information with students.

Exploring Genetic Testing: **Slide #14**

CAREERS IN SPOTLIGHT:
GENETIC COUNSELOR

What do they do?

- Review a patient's medical and family history.
- Advise patients and their families about the benefits and consequences of genetic testing, and about the nature of genetic disorders.
- Offer counseling consistent with the patient's belief system.

What kind of training is involved?
 Bachelor's degree and a two to three year Master's degree.

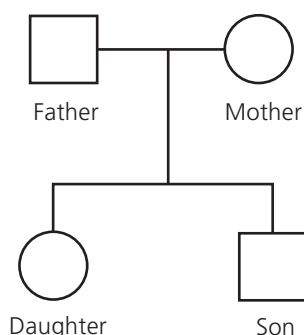
What is a typical salary for a Genetic Counselor?
 Start at \$60,000/year (\$29/hour), and may make up to \$120,000/year (\$58/hour).

31. Ask students, "How does a genetic counselor fit into today's stories?" Point out that:
- Genetic counselors provide support and guidance to help families identify risks, interpret genetic information, and analyze how conditions are inherited.
 - Recent advances in bioinformatics have revolutionized the field of genetic counseling by dramatically increasing the amount of information to which genetic counselors have access.
 - The tools of bioinformatics are used to compare genetic sequences (as students will see tomorrow) which is the basis for the genetic tests genetic counselors help interpret.
32. Ask students to answer Genetic Counselor 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 genetic counselor does.
33. Ask students to also answer Genetic Counselor Question #3 on their *Careers in the Spotlight* handout, which has students explain how a genetic counselor might use bioinformatics in his or her work.

Homework

The following are suggested homework activities to follow this lesson. It is highly recommended that Student Handout—*Lawler Family Pedigree* be provided as homework to allow more time for class discussion in *Lesson Four*.

- A. Students should complete Student Handout—*Lawler Family Pedigree* for homework (from *Lesson Four*). If students are not familiar with pedigrees, draw a simple pedigree chart for a nuclear family with two children, such as:



Explain the relationships detailed in the pedigree. For homework, students will be asked to fill in the Lawler family pedigree by coloring in the square or circle if that person has had breast cancer (**phenotype**). Tell students that they can't know a person's genetic component (**genotype**) until the individual has been tested. As such, it is impossible to know carrier status, and no circles or squares should be partially colored in.

If students are unfamiliar with how to use a Punnett Square, page three of Student Handout—*Lawler Family Pedigree* (from Lesson Four) should not be assigned.

- B. Teachers may also wish to include a reflective homework assignment. Ask students to write about the activities they did in Lesson Three 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:

- Today I learned that...
- An important idea to think about is...
- Something that I don't completely understand yet is...
- Something that I'm really confident that I understand is...

- C. Teachers may find it helpful to add a reflective paragraph for homework in lieu of the reflective questions listed above. Encourage students to share their specific thoughts about genetic testing, especially if students did not have sufficient time to explore and discuss their thoughts and feelings at the end of the Structured Academic Controversy. This assignment could take the form of a Supreme Court decision, with students writing the "Majority Decision" based on their own feelings and beliefs either "FOR" or "AGAINST" genetic testing, while acknowledging the merits of the opposite view in the "Minority Decision."

[**Note:** Suggested scoring for reflection: +5 points if all 4 prompts are complete.]

Extension

- Students can use Student Handout—*Focus on the Principles* to consider some additional ethical scenarios. The italicized ethical question can go in the center of the worksheet, and students can view each scenario through the lens of the bioethical principles. A generic copy of Student Handout—*Focus on the Principles* can be found in the *Appendix*.
 - a. Matt's grandfather died of Huntington's Disease at the age of 53. It is a genetic disease for which there is no cure. Matt is 15 and would like to be tested for the disease, but his mother doesn't want him to get tested. She thinks Matt should live his life without the burden of knowing, at least until he is older. ***Should he take the genetic test now?***
 - b. Through genetic testing, Gabriella has found out that she is a carrier for Duchenne muscular dystrophy, an X-linked disorder that can be passed on to boys. ***Should her insurance company pay for in vitro fertilization techniques to ensure that her next baby is a girl?***
 - c. For his last birthday, Anthony's wife bought him a full genetic screening package from a direct-to-consumer genetic testing business. The results showed that Anthony has familial hypercholesterolemia, a condition which puts him a much higher than average risk of having an early heart attack. Anthony is an airline pilot and fears that passenger safety might be at risk. ***What should he do?***
- Information about the Genetic Information Nondiscrimination Act of 2008 (GINA), a new federal law that prohibits discrimination in health coverage and employment based on genetic information, can be found in the *Appendix*. This reading may be assigned to students as an extension activity. Students may use this information to write a reflective piece about how GINA would impact their answers to Questions #3 and 4 on Student Handout—*Case Study: A BRCA Genetic Testing Dilemma*.
- *Pretty is What Changes: Impossible Choices, The Breast Cancer Gene, and How I Defied My Destiny*, by *The Gilmore Girls* TV writer Jessica Queller, is a compelling novel about one woman's struggle with *BRCA1* and a **prophylactic** double **mastectomy** at age 35. This book was recommended by a Bio-ITEST field test teacher, and may be assigned as an extension activity or recommended to students who wish to read more about this material.

Teacher Background

The rest of the story: This case study is based on a true story published by Amy Harmon in the *New York Times* on September 16, 2007 entitled “The DNA Age: Cancer Free at 33 but Weighing a Mastectomy.” In the original article, Deb Lindner decides in favor of getting the BRCA genetic test. Students will have to wait until the end of *Lesson Four* to find out the results of the test. The name Lindner has been changed to Lawler in this curriculum.

Assessment Suggestions

The extension to the lesson (described above) could also be used to assess students’ ability to apply the bioethical principles to a short scenario.

Glossary

Autonomy: Related to the bioethical principle of **respect for persons**, autonomy is independence or freedom to choose one’s own actions.

Beneficence/nonmaleficence: Bioethical principles in which decisions are based on doing the most good for the most people. Sometimes referred to as **maximize benefits/minimize harms**.

Chemoprevention: Regular doses of drugs that block estrogen and help prevent the development of breast cancer, but induce a form of menopause.

Justice: An ethical principle often referred to as “being fair.”

Mammogram: An X-ray of the breast, often used to detect breast cancer.

Mastectomy: Surgical removal of the breast.

Maximize benefits/minimize harms: Bioethical principles in which decisions are based on doing the most good or the least amount of harm for the most people. Sometimes referred to as **beneficence/nonmaleficence**.

Prophylactic: A preventative measure. For example, a prophylactic mastectomy is the removal of the breast(s) before cancer develops.

Respect for persons: Respecting the inherent worth of an individual and his or her autonomy.

Resources

The Genetic Information Nondiscrimination Act of 2008 (GINA) is a federal law that prohibits discrimination in health coverage and employment based on genetic information. A handout prepared by the Department of Health and Human Services (HHS) with information about GINA for researchers and health care professionals is provided in the *Appendix*. This handout is designed to provide a brief overview of what legal protections are now in place regarding genetic testing, genetic privacy, and genetic discrimination.

Background on ethical theories can be found in the *Appendix*.

For additional information and activities, NWABR’s *Ethics Primer and Bioethics 101* are helpful resources. These can be downloaded free of charge at: <http://nwabr.org/education/ethicslessons.html>.

Queller, Jessica. 2008. *Pretty Is What Changes: Impossible Choices, The Breast Cancer Gene, and How I Defied My Destiny*. New York, New York: Spiegel & Grau.

Credit

Bennett, Robin. Personal Interview. 18 March 2010.

Couch F.J., DeShano M.L., Blackwood M.A. et al. *BRCA1* mutations in women attending clinics that evaluate the risk of breast cancer. *New England Journal of Medicine*. 1997; 336: 1409–15.

Easton D.F., Steele L., Fields P. et al. Cancer risks in two large breast cancer families linked to *BRCA2* on chromosome 13q12–13. *American Journal of Human Genetics*. 1997; 61: 120–8.

Ford D., Easton D.F., Stratton M. et al. Genetic heterogeneity and penetrance analysis of the *BRCA1* and *BRCA2* genes in breast cancer families. *American Journal of Human Genetics*. 1998; 62: 676–89.

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

Struwing J.P., Hartge P., Wacholder S. et al. The risk of cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jews. *New England Journal of Medicine*. 1997; 336: 1401–8.

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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: Deb's mother had fought breast cancer when Deb was in high school, and Deb's maternal grandmother 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 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 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 to detect breast cancer), breast self-exams, and blood tests, and at least yearly physical exams with her doctor and other tests to detect potential ovarian cancer.

For every **1,000** women...

120 (12%) will get breast cancer.

6 of them inherited mutations in *BRCA1* or *BRCA2* (5% of all cases of breast cancer).

This may seem like a small number, but for a woman who inherits a cancer-causing *BRCA1* or *BRCA2* mutation, her **risk of developing breast cancer is up to 85%**.

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 was also undecided, though she thought that the results may benefit her two young children some day. Deb's Aunt Sue 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. Should Deborah take the test?

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



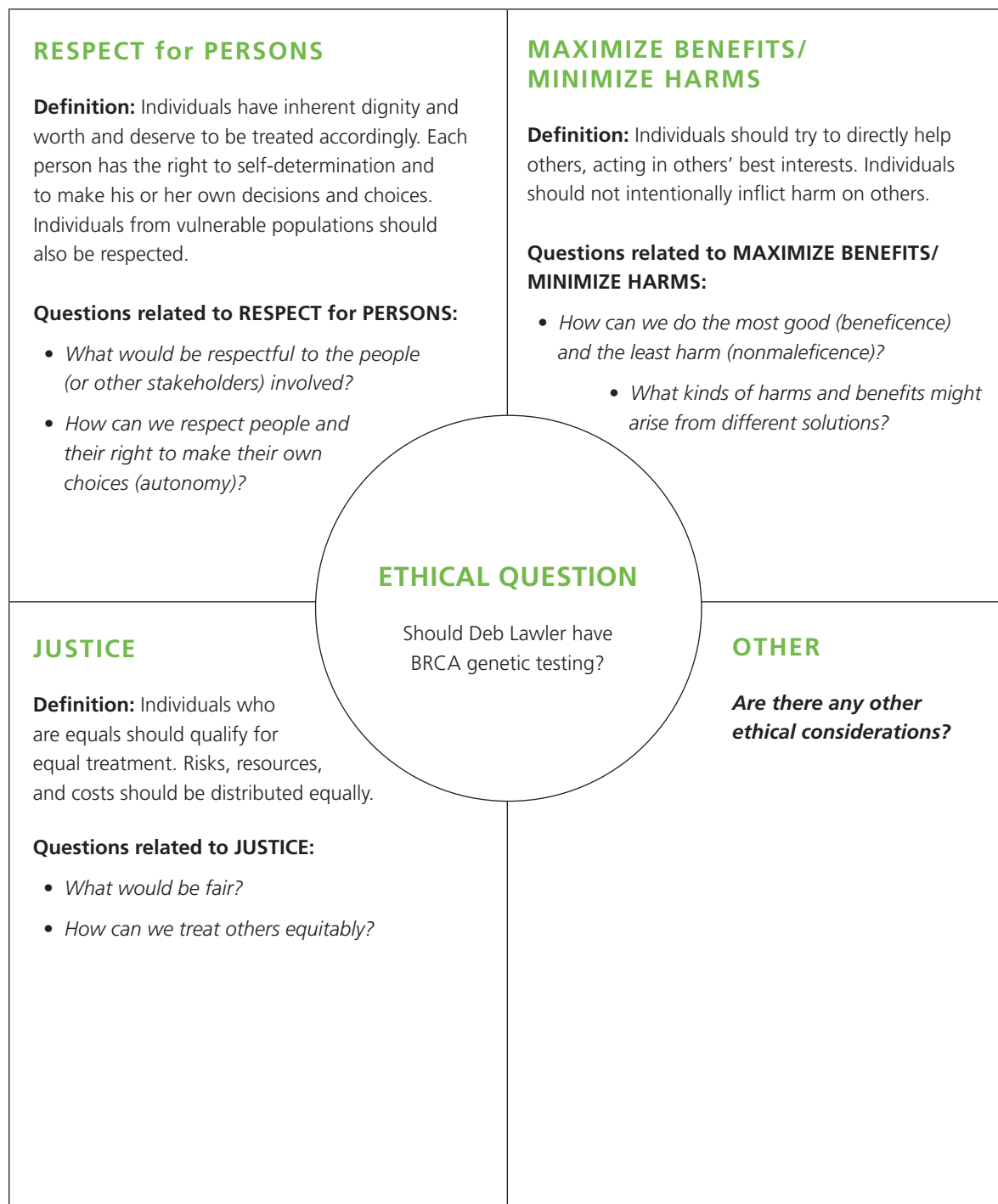
Homework Questions: Answer these questions in your lab notebook or on a separate sheet of paper.

1. One important principle of ethics is **Respect**. Part of Respect acknowledges a person's right to make choices, hold views, and to take actions based on personal values and beliefs. Describe one way that the principle of Respect applies to this case study.
2. Another principle of ethics is **Maximize Benefits/Minimize Harms**, which states that there is an obligation not to inflict harm, to provide benefits to persons, and to contribute to their welfare. Describe one way that the principle of Maximize Benefits/Minimize Harms applies to this case study.
3. Would you ever consider having a genetic test done? Why or why not?
4. Under what circumstances would you not want to have a genetic test done?

3 Focus on the Principles



Do these principles apply? In your lab notebook or on a separate sheet of paper, discuss how each of these bioethical principles applies to the ethical question, “Should Deb Lawler have BRCA genetic testing?” You may use the questions below each principle to guide your answers. Some principles may apply more than others for a particular situation.



LESSON 3

CLASS SET

Name _____ Date _____ Period _____

3

Structured Academic Controversy Worksheet



This Issue: *Should Deb Lawler have BRCA genetic testing?*

Team Members **FOR**

1. _____

2. _____

Team Members **AGAINST**

1. _____

2. _____

Relevant facts:

Individuals or groups who have a stake in the outcome ("stakeholders") and their concerns:
(Who is affected by Deb's decision? Why do they care?)

LESSON 3

HANDOUT

<p>Main arguments FOR:</p> <p>1.</p> <p>2.</p> <p>3.</p>	<p>Main arguments AGAINST:</p> <p>1.</p> <p>2.</p> <p>3.</p>
<p>List of possible solutions:</p> 	
<p>Areas of agreement:</p> 	<p>Areas of disagreement:</p>

3 Structured Academic Controversy FOR Position Statement

FOR Arguments: (Deb *should* proceed with BRCA genetic testing).

If Deb takes the test and the result is negative (meaning she does not have a mutation in her *BRCA1* or *BRCA2* genes that would increase her risk of breast and ovarian cancer), she will likely feel relieved and be less anxious about her future. She will also not have to worry about passing the mutation on to future children.

If Deb takes the test and the result is positive (meaning she does have a mutation in her *BRCA1* or *BRCA2* genes that would increase her risk of breast and ovarian cancer), she can begin to make some lifestyle and behavioral changes to reduce her risk, such as:

- Having physical exams more frequently to try to detect breast or ovarian cancer early.
- Increasing her number of medical screenings, such as mammograms (x-rays of the breast used to find cancer).
- Beginning **chemoprevention** medications, which are drugs that are taken regularly to help prevent cancer from developing.

She could also reduce her risk by having surgery to remove her breasts and/or ovaries.

Ethical Arguments Supporting the FOR Side Include:

Respect for Persons

Deb has the right and responsibility to make decisions and take action based on her values and beliefs. She appears to value the knowledge that would come from the test results. Deb is a competent adult, fully able to understand the results and take action on those results. Whether or not her mother supports her decision to take the test, Deb can make her own autonomous decision about her own health and care. It is her body and her decision.

Maximize Benefits and Minimize Harms

The benefits that come from knowing her BRCA status outweigh the harms that may result.

If the test is negative, the benefits (relief, less anxiety) far outweigh any harms that come from the knowledge.

If the test is positive, Deb will benefit by being able to be proactive about her health care. Being able to take action (see the bullets above) will outweigh the anxiety that may result from a positive test result.

3 Structured Academic Controversy AGAINST Position Statement

AGAINST Arguments: (Deb **should not** proceed with BRCA genetic testing).

- Not all people with a mutated *BRCA1* or *BRCA2* gene will develop breast or ovarian cancer as a result.
- Inherited mutations in *BRCA1* or *BRCA2* genes only account for about 5% of breast cancer.
- Not all family members may want to know the results of this test. A positive or negative result for Deb also informs family members who have a similar genetic makeup.
- There is a possibility that testing results may not remain entirely private.
- There is a possibility of discrimination from insurance companies based on results.
- While there are some behavioral changes Deb could make, there is really no “cure” and treatment options are limited.

Ethical Arguments Supporting the AGAINST Side Include:

Respect for Persons

While Deb has the right to make her own decisions and choices about her health, her test results also affect her family. A positive result for Deb identifies her mother as also having the mutation. This information has strong implications for both of Deb’s siblings and her maternal cousins. If a family member does not want to know his or her BRCA status, that individual’s right to make his or her own choices may be violated by Deb’s test results.

Maximize Benefits and Minimize Harms

The harms that come from knowing her BRCA status outweigh the benefits that may result.

Nothing in biology is 100%, and knowing that her chances of breast/ovarian cancer are increased does not mean that Deb will develop the disease. Acting on positive test results (meaning she does have a BRCA mutation that would increase her risk of breast and ovarian cancer), Deb may choose to have her breasts and/or ovaries removed. While there might be some unknowable benefit to this procedure, she would be intentionally inflicting harm on herself in the pursuit of health.

Many of the lifestyle changes that reduce the risk of breast cancer in all women (such as keeping a healthy diet, exercising regularly, and maintaining a healthy weight) have widespread benefits, regardless of BRCA status. Deb does not need to take the genetic test to benefit from embracing these health and lifestyle choices.

3

Focus on the Principles

Teacher Answer Key

[**Note:** Suggested point values are included after the sample answers 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 **10 or more points**.]

Do these principles apply? In your lab notebook or on a separate sheet of paper, discuss how each of these bioethical principles applies to the ethical question, “Should Deb Lawler have BRCA genetic testing?” You may use the questions below each principle to guide your answers. Some principles may apply more than others for a particular situation.

RESPECT for PERSONS

Definition: Individuals have inherent dignity and worth and deserve to be treated accordingly. Each person has the right to self-determination and to make his or her own decisions and choices. Individuals from vulnerable populations should also be respected.

Questions related to RESPECT for PERSONS:

- What would be respectful to the people (or other stakeholders) involved?
- How can we respect people and their right to make their own choices (autonomy)?

Deb has the right to make decisions and take action based on her values and beliefs; she appears to value the knowledge that would come from the test results. She is fully able to understand the results and take action. The test results, however, also affect her family. A positive result has strong implications for both of Deb’s siblings and her cousin. The family members’ right to know (and make his or her own choices) is affected by Deb’s choices. If Deb gets the genetic test, will her information remain private?

(+4 total, +2 points per reasonable application of principle to question.)

MAXIMIZE BENEFITS/MINIMIZE HARMS

Definition: Individuals should try to directly help others, acting in others’ best interests. Individuals should not intentionally inflict harm on others.

Questions related to MAXIMIZE BENEFITS/MINIMIZE HARMS:

- How can we do the most good (beneficence) and the least harm (nonmaleficence)?
- What kinds of harms and benefits might arise from different solutions?

If the test is negative, Deb (and her family members) will benefit by feeling relieved. If the test is positive, Deb can be proactive about her health care. However, a positive result does not mean that Deb will certainly develop breast or ovarian cancer. The knowledge that she could (but may not) develop cancer may cause her anxiety over time. A positive test may cause harm (such as psychological harm) to her family members.

(+4 total, +2 points per reasonable application of principle to question.)

JUSTICE

Definition: Individuals who are equals should qualify for equal treatment. Risks, resources, and costs should be distributed equally.

Questions related to JUSTICE:

- What would be fair?
- How can we treat others equitably?

Is it fair to Deb's siblings that they know more about their genetic predisposition to cancer because Deb gets tested, even if they choose not to know? Will Deb's insurance company pay for the testing? Should it? Should Deb's cousin have access to the test, even though she does not have health insurance? If the test is positive, can a future insurance company decline her coverage due to her preexisting risk of developing breast cancer? Does everybody have equal access to medical resources (clinics or hospitals that perform the test; genetic counselors and doctors who can help to interpret the risks)?

(+2 for at least one reasonable application of principle to ethical question.)

OTHER

Are there any other ethical considerations?

Students may come up with other answers or issues which do not neatly fit one category or the other.

(Additional points may be given to other considerations.)

3

Structured Academic Controversy 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**.]

This Issue: Should Deb Lawler have BRCA genetic testing?

Relevant facts:

- Deb is a doctor and understands the risks of mastectomy and cancer treatment.
 - Deb's mother, grandmother, and cousin all had breast cancer, suggesting a strong inherited (i.e., genetic) risk.
 - Deb's cousin Lynn has no health insurance.
 - *BRCA1* and *BRCA2* mutations are associated with a high risk of breast cancer.
 - Deb is still young enough that genetic testing could reveal risk before she gets sick (i.e., develops cancer), allowing her to make lifestyle choices (i.e., mastectomy or not, healthy diet, exercise).
 - There are a number of "controllable" factors that affect breast cancer risk, including getting enough exercise, eating a healthy diet, and maintaining a healthy weight.
 - Deb has health insurance, and can pay for the genetic test and cancer treatment.
 - The *BRCA1* and *BRCA2* genetic tests are not 100% predictive about whether or not Deb will get cancer, even if she inherited cancer-causing mutations.
 - Preventive treatment options like prophylactic or preventive mastectomies are not 100% effective.
- (+1 for each relevant fact, listed up to +4.)

Individuals or groups who have a stake in the outcome ("stakeholders") and their concerns: (Who is affected by Deb's decision? Why do they care?)

- Deb wants to know her cancer risk.
 - Deb's family shares some of her genetic information (brother, sister, mother, father, cousin, etc.), so if Deb tests positive for cancer-causing *BRCA1* or *BRCA2* mutations, her family is more likely to carry those mutations as well. Similarly, any children (i.e., Deb's nieces and nephews, or Deb's future children) could also be impacted, because they do (or will) share genetic material with Deb, too.
 - Deb's sister and brother are not sure if they want to know the test results, or if they want to be tested themselves.
- (+0.5 for each stakeholder and +0.5 for concerns, up to +3.)

Main arguments **FOR**:

1. A negative test result would relieve some worry for Deb about the increased risk of breast cancer, or passing mutations on to her children.
 2. A positive test result would allow or motivate Deb to make changes to her lifestyle that could impact her risk (i.e., healthy diet, exercising).
 3. If Deb tests positive, she could take medical action to minimize her risk (i.e., surgery, medication, increased screening).
 4. Deb has a right to know about her own health and health risks.
- (+1 for each main argument listed, up to +3.)

Please see Student Handout—*Structured Academic Controversy FOR Position Statement* for more information and additional possible answers.

Main arguments **AGAINST**:

1. A positive test does not mean that Deb will definitely get breast cancer.
 2. A negative test does not mean that Deb won't get breast cancer.
 3. Some of Deb's family members don't want to know if they carry cancer-causing mutations.
 4. No lifestyle changes are 100% effective at preventing cancer.
 5. No medical interventions are 100% effective at preventing cancer.
- (+1 for each main argument listed, up to +3.)

Please see Student Handout—*Structured Academic Controversy AGAINST Position Statement* for more information and additional possible answers.

List of possible solutions:

- Deb could get tested for *BRCA1* and *BRCA2* mutations and not tell her family the results.
- Deb could get tested and tell only those family members who wish to know her results.
- Deb could choose not to get tested but continue her medical testing and increased cancer surveillance with her doctor.
- There are other possible solutions students may arrive at.
- (+1 for each possible solution listed, up to +3.)

Areas of agreement:

- Deb and her family have a right to make their own decisions.
- (+1 for each area of agreement, up to +2.)

Areas of disagreement:

- Whether Deb or her family members want to know their genetic risk for breast cancer.
- (+1 for each area of disagreement, up to +2.)

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** (**B**asic **L**ocal **A**lignment **S**earch **T**ool) 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— <i>Careers in the Spotlight</i> (handed out in Lesson One)	1 per student
Copies of Student Handout— <i>Lawler Family Phenotype Pedigree: Who Has Breast Cancer?</i>	1 per student
Class set of Student Handout— <i>Instructions for Aligning Sequences with BLAST</i>	1 per student (class set)
Copies of Student Handout— <i>Aligning Sequences with BLAST Worksheet</i> [Note: This worksheet is for students' answers to lesson questions]	1 per student
Teacher Answer Key— <i>Lawler Family Phenotype Pedigree</i>	1
Teacher Answer Key— <i>Aligning Sequences with BLAST</i>	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— <i>Understanding Genetic Tests to Detect BRCA1 Mutations</i> . 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://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing .
Computer lab with internet access and a word processing program such as Microsoft Word® or Google Docs.

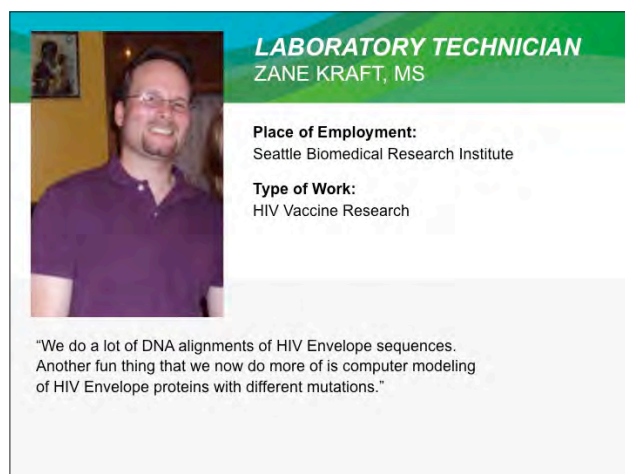
Teacher Preparation

- 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.

LESSON 4

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:

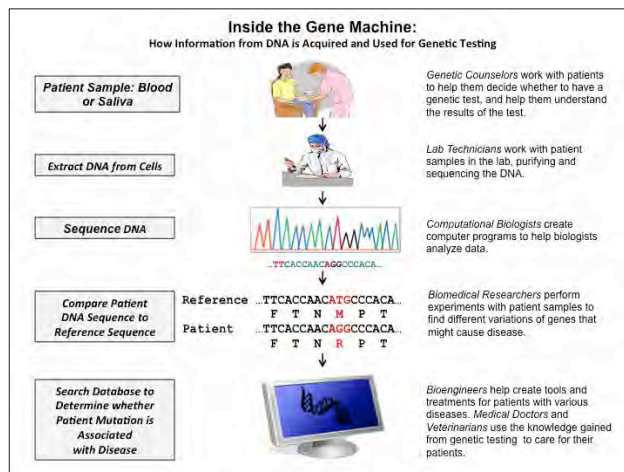
Person	Reason
Deb’s cousin Lynn	Doesn’t want to know. Did not give consent.
Lori’s children	Lori did not give consent for them.
Deb’s brother John	Doesn’t want to know. Did not give consent.
Deb’s Aunt Jackie	Doesn’t want to know. Did not give consent.
Deb’s grandparents	Deceased.

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.

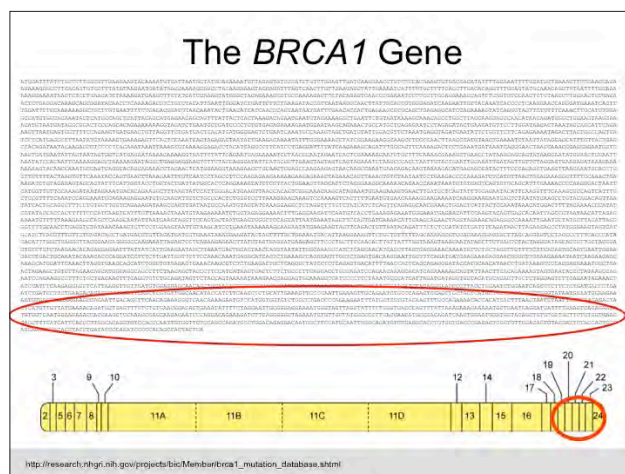
PART II: Inside the Gene Machine

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.
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.

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.

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.
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.

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


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**



LABORATORY TECHNICIAN

ZANE KRAFT, MS

Place of Employment:
Seattle Biomedical Research Institute

Type of Work:
HIV Vaccine Research

"We do a lot of DNA alignments of HIV Envelope sequences. Another fun thing that we now do more of is computer modeling of HIV Envelope proteins with different mutations."

CAREERS IN SPOTLIGHT:
LABORATORY TECHNICIAN

What do they do?
Laboratory technicians, sometimes called “lab techs” or research technicians, do much of the day-to-work in the lab, including purifying and sequencing DNA, caring for laboratory machinery, and analyzing samples.

What kind of training is involved?
Some laboratory technicians have a two year Associate’s degree or a four year Bachelor’s degree. Some, like Zane Kraft, also have a Master’s degree (additional two year training).

What is a typical salary for a Laboratory Technician?
Ranges with experience from \$30,000/year (\$14/hour) up to \$60,000/year (\$29/hour) or more.

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:

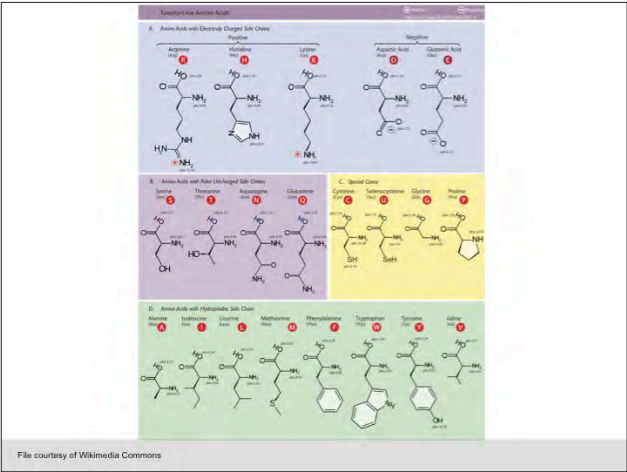
[Note: Suggested scoring for reflection:
+5 points if all four prompts are complete.]

- 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...

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:
- In its ionized form, arginine has a positive charge, while methionine is uncharged.
- The "size" of the R-groups differs. Arginine is larger.
- Methionine contains a sulfur.
- Arginine contains two amino groups.
- 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.
- 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.

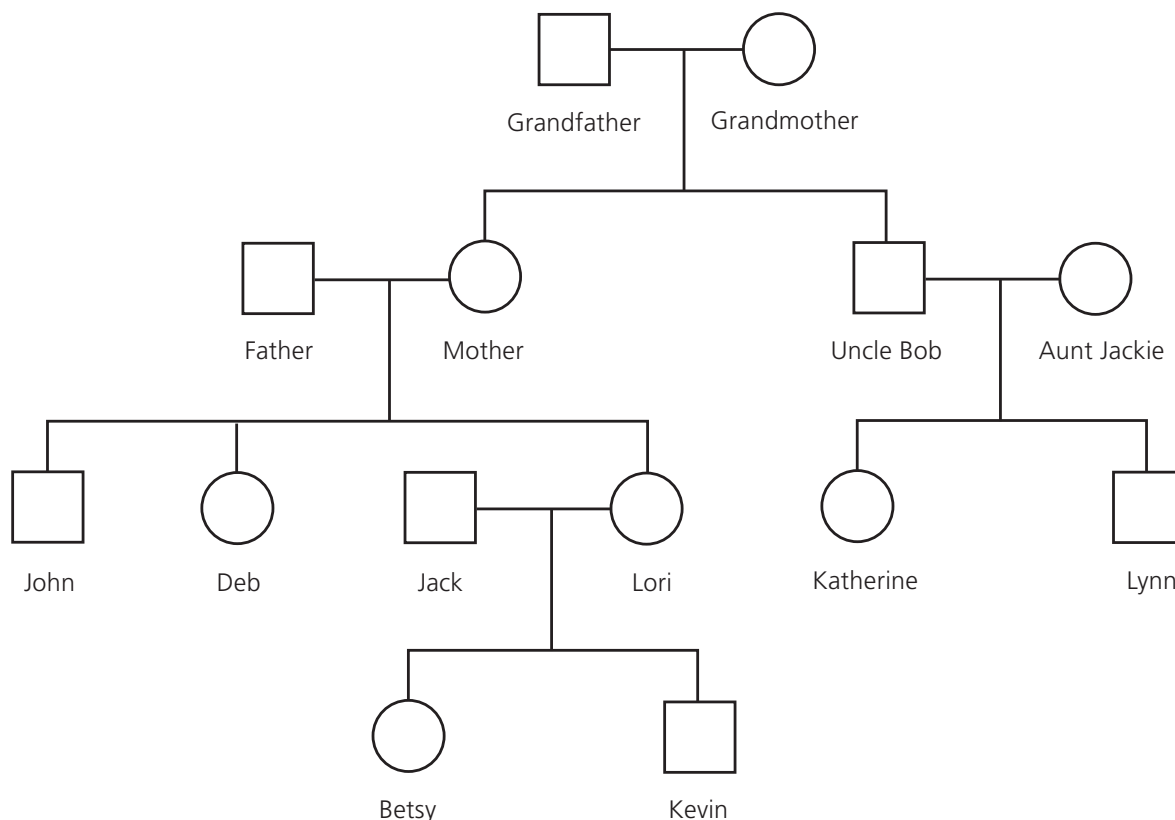
National Center for Biotechnology Information (NCBI). Basic Local Alignment Search Tool (BLAST). <http://blast.ncbi.nlm.nih.gov/Blast.cgi>.

Name _____ Date _____ Period _____

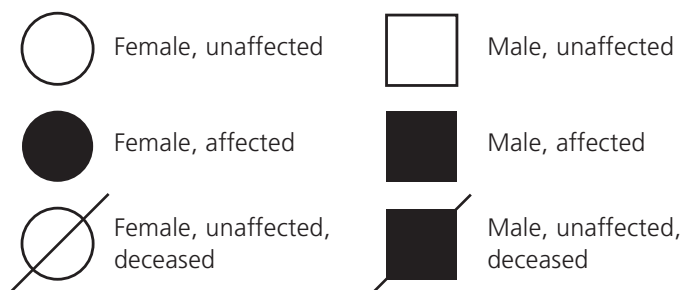
4 Lawler Family Phenotype Pedigree: Who has Breast Cancer?



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



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

LESSON 4

HANDOUT



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:

		Mother's Genotype	
		_____	_____
Father's Genotype	_____		

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:

		Mother's Genotype	
		_____	_____
Father's Genotype	_____		

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:

		Mother's Genotype	
		_____	_____
Father's Genotype	_____		

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?

LESSON 4

HANDOUT

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.

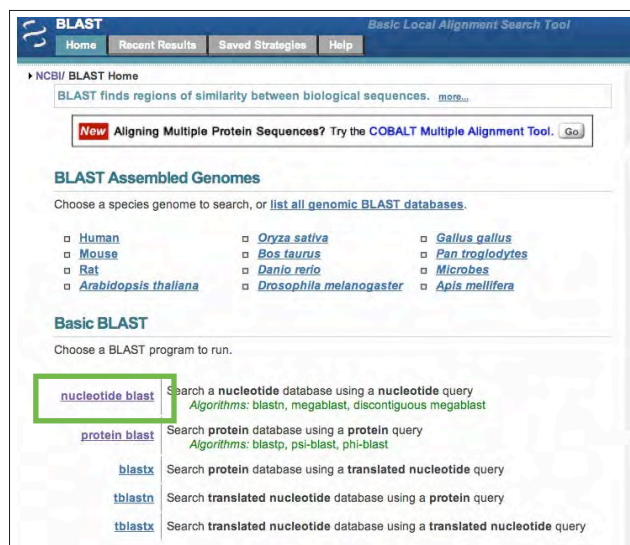


Figure 1: Select “Nucleotide BLAST.”
Credit: NCBI BLAST.

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

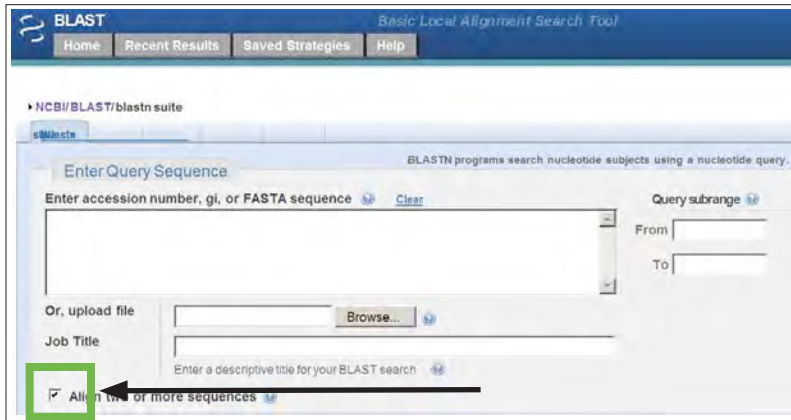


Figure 2: Align two or more sequences. Credit: NCBI BLAST.

- A second text box will appear.
- 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**).

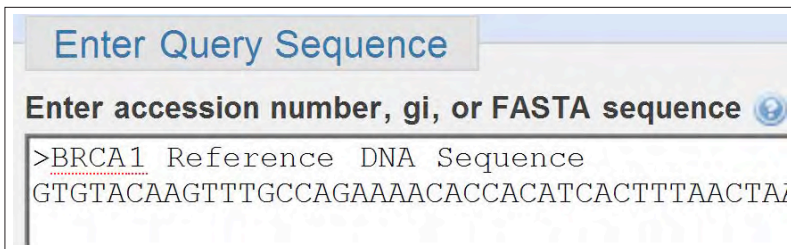


Figure 3: Copy the *BRCA1* reference sequence. Credit: NCBI BLAST.

- 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.**
- Click “BLAST.”
- 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.
- Click the “Formatting Options” link located near the top of the page (see **Figure 4**).

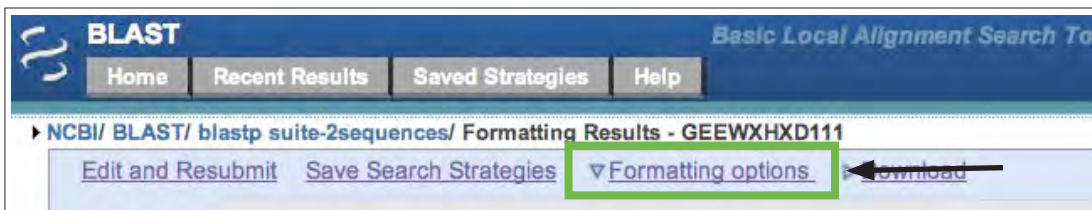


Figure 4: Click the “Formatting Options” link. Credit: NCBI BLAST.

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

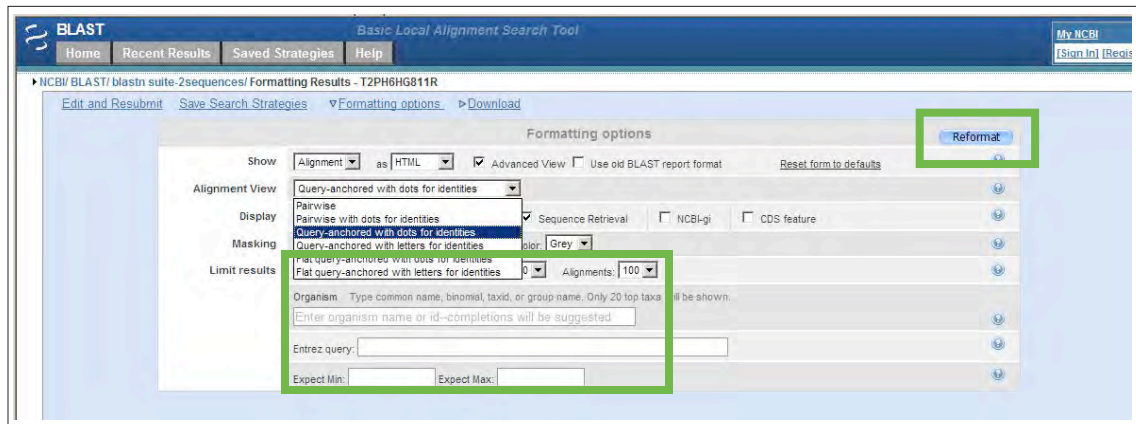


Figure 5: Click the "Reformat" button.
Credit: NCBI BLAST.

- 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**).

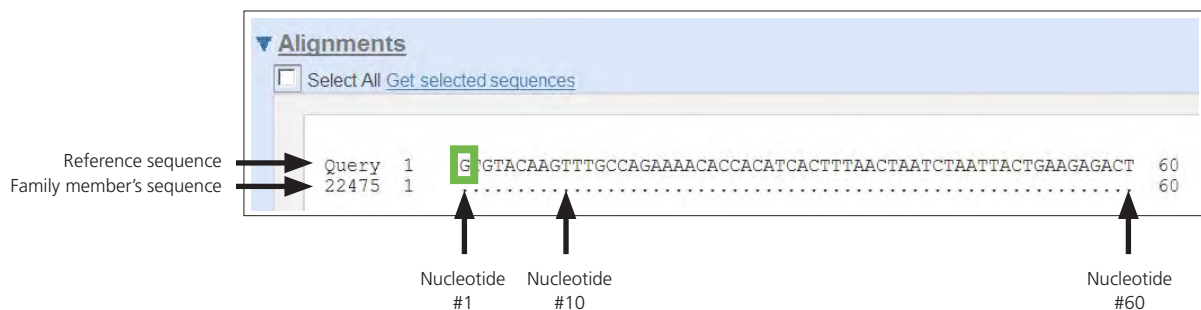


Figure 6: Comparing the query and subject sequences.
Credit: NCBI BLAST.



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



- 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**.

Alignments			
<input type="checkbox"/> Select All Get selected sequences Distance tree of results			
Reference sequence	Query	1	GTGTACAAGTTTGGCAGAAACACCATCACTTTAACTAATCTAATTACTGAAGAGACT 60
Family member 1	54818	1 60
Family member 2	54819	1 60
Family member 3	54820	1 60
Family member 4	54821	1 60
Family member 5	54822	1 60
Family member 6	54823	1 60

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:

- 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.**”
- Return to the BLAST results page.
- Scroll down until the sequence showing the *BRCA1* mutation is centered on the computer screen.
 - 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.
 - For Mac users:** Press the keys: **Command + Shift + 4** at the same time. The image will be saved on your desktop.
- Return to your Word® document.
 - For PC users:** Use the paste function to transfer the captured image (**Ctrl + V**).
 - For Mac users:** Open the **Insert** menu, choose **Picture** from file, and choose the image that you captured from the computer screen.
- 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**.

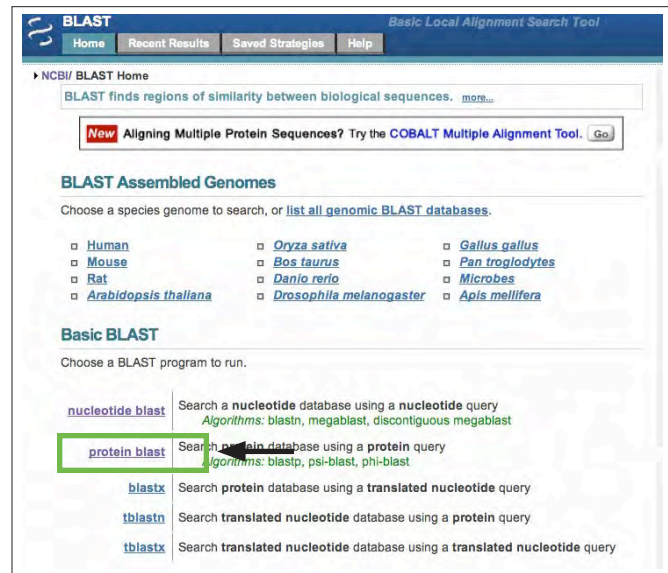


Figure 10: Select "Protein BLAST."
Credit: NCBI BLAST.

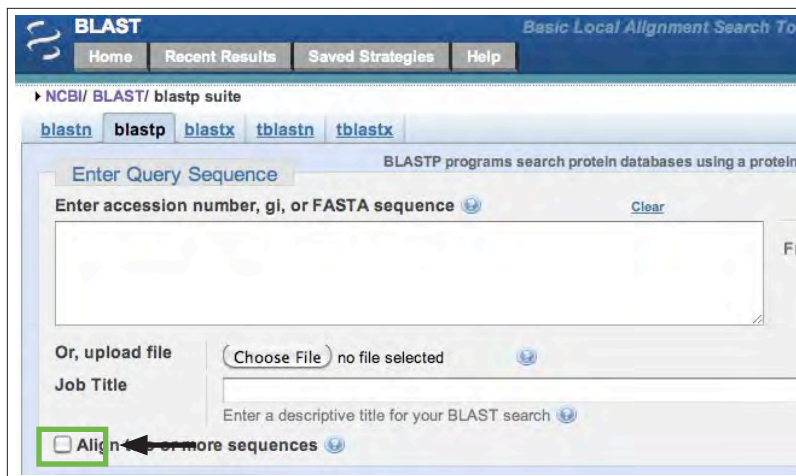


Figure 11: Select "Align two or more sequences."
Credit: NCBI BLAST.

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**.

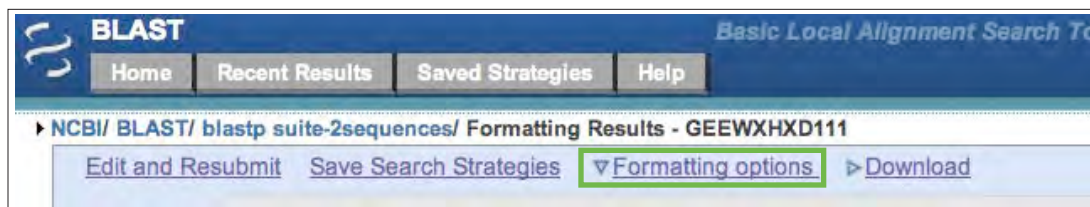


Figure 12: Click “Formatting options.”
Credit: NCBI BLAST.

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**.

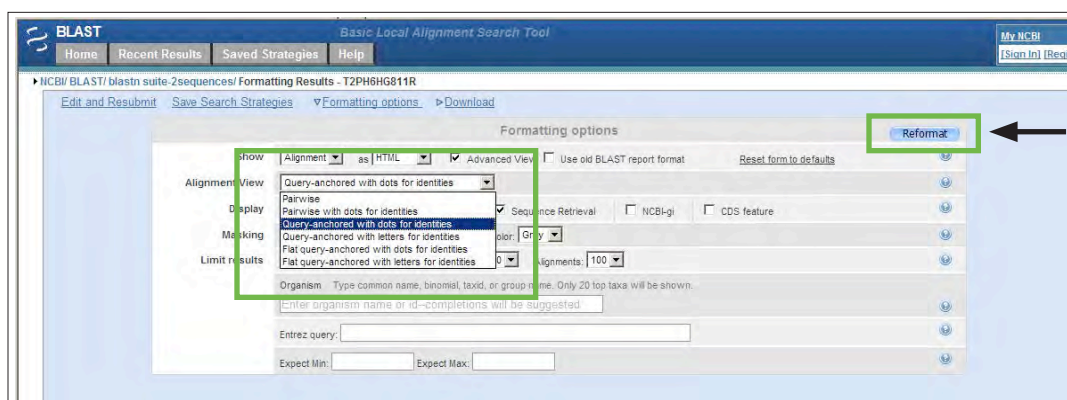


Figure 13: Locate “Alignment View” and the “Reformat” button.
Credit: NCBI BLAST.

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

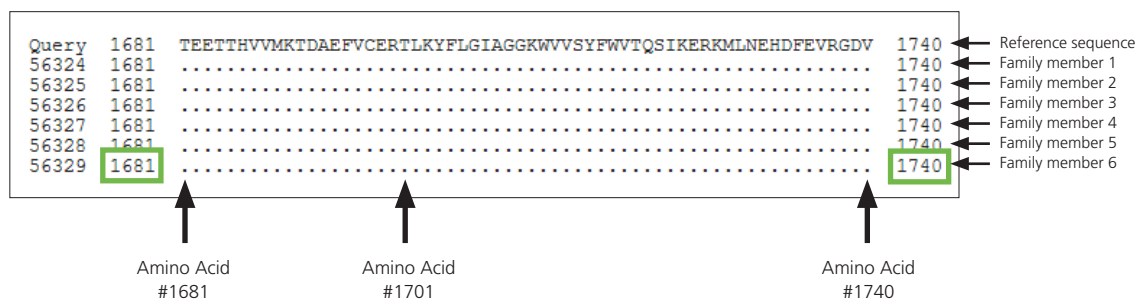


Figure 14: Compare the reference and subject sequences.
Credit: NCBI BLAST.

LESSON 4

CLASS SET



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:

- What is the amino acid in the reference sequence (as represented by a one-letter abbreviation)?
- What is the amino acid in the sequences containing the mutation?
- Is this the amino acid that you expected based on your DNA analysis in Part I, in the table “Lawler Family Sequence Analysis?”
- 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.

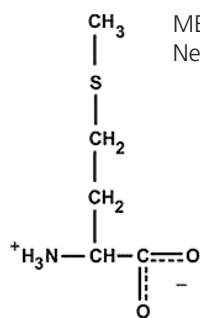


47. Now that you have some test results, return to Student Handout—*Lawler Family Pedigree*.

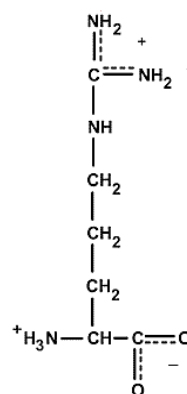
- Fill out as much additional information as you can for the pedigree.
- Which Punnett square most accurately represents the Lawler family? Why?

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 Local Alignment Search Tool

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).

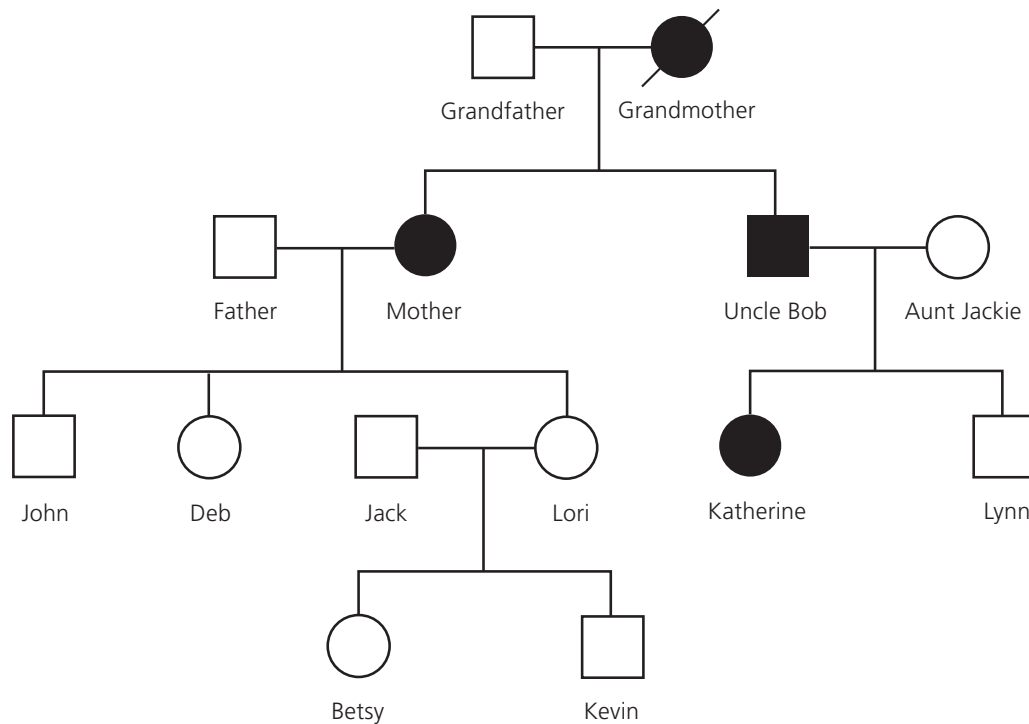
50. How might this mutation affect the protein?

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

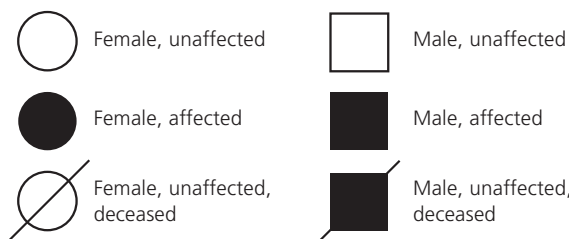
[**Note:** Suggested point values are included for each question/task, and are intended to provide general guidelines for the weight each question/task could be given. Using these suggested point values, the total value for this worksheet is **16 points** (Part 1: 5 points; Part 2: 7 points; Part 3: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



"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 B_N) 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:

		Mother's Genotype	
		B_N	B_1
Father's Genotype	B_N	B_N B_N	B_N B_1
	B_1	B_1 B_N	B_1 B_1

What is the percent chance children will inherit:

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

LESSON 4

KEY

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

		Mother's Genotype	
		B_N	B_1
Father's Genotype	B_N	B_N B_N	B_N B_1
	B_N	B_N B_N	B_N B_1

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:

		Mother's Genotype	
		B_N	B_N
Father's Genotype	B_N	B_N B_N	B_N B_N
	B_N	B_N B_N	B_N B_N

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* (B_1B_1), 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	<i>methionine</i>	<i>arginine</i>

(+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.)

5 Learning to Use Cn3D: A Bioinformatics Tool

Introduction

Up to this point, students have seen the BRCA1 protein represented in a linear, sequential form. In this lesson, students are introduced to the high importance of a protein's three-dimensional structure. Students first engage in a short activity in which they use a pipe cleaner to perform a simple function, as an analogy for the relationship between a protein's structure and function. Students then learn to navigate between linear protein sequences and three-dimensional structures by using the bioinformatics tool **Cn3D**. Students begin by viewing and manipulating DNA—a familiar molecule—using Cn3D. When students are familiar with the program, they visualize parts of the BRCA1 protein to show how a specific mutation in the *BRCA1* gene ultimately changes or destroys the protein's function. In *Lesson Five*, students learn how **3D animators** might use bioinformatics tools in their careers.

Learning Objectives

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

- Bioinformatics tools like Cn3D help scientists visualize proteins.
- A protein is a physical “thing” with a three-dimensional structure that determines its function.
- All proteins are comprised of amino acids linked together by **covalent bonds** and have the same general structure: a ‘beginning’ or **N-terminus**, which contains an amino group (NH_3^+), and an ‘end’ or **C-terminus**, which contains a carboxyl group (COO^-).
- Each amino acid has a chemical group that is unique to it, called an R-group (also known as a side-chain). The chemistry of the amino acid R-group is important for a protein's shape and function.
- Mutations can impact the three-dimensional structure of proteins, and thus impact the protein's function, as is the case with many genetic disorders.
- Bioinformatics tools are used and created by people in many careers, including 3D animators.

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

- Use Cn3D to view complex biological molecules in a three-dimensional format.
- Manipulate three-dimensional images in numerous ways to deepen understanding of molecular structure.

Key Concepts

- Genetic disorders are often caused by dysfunctional or absent proteins.
- Proteins are physical “things” with three-dimensional shapes. The shape of the protein is crucial to its function.

Class Time

One class period of 50 minutes.

Prior Knowledge Needed

- Basic DNA structure.
- An understanding of the Central Dogma.
- An understanding of how DNA, chromosomes, genes, and proteins relate to each other.
- The process of protein synthesis (transcription and translation).
- Basic amino acid structure.
- Basic protein structure (specifically the various forms of secondary protein structure such as alpha helix and beta sheet).

Common Misconceptions

- Biological molecules (such as DNA or proteins) actually look like their artistic representations.

- All proteins are comprised of amino acids linked together by covalent bonds and have the same general structure: a 'beginning' or N-terminus, which contains an amino group (NH_3^+), and an 'end' or C-terminus, which contains a carboxyl group (COO^-).
- Each amino acid has a different R-group (also known as a side-chain). Each R-group has different chemical properties. The chemistry of the amino acid R-group is important for a protein's shape and function.
- Mutations can cause changes to the three-dimensional shape of a protein. The change in shape can alter the function, resulting in genetic disorders, including cancer.
- Programs like Cn3D allow scientists (and students!) to view macromolecules in a number of different ways to enhance their understanding of the molecule's structure and function.
- Bioinformatics tools are used and created by people in many careers, including 3D animators.

Materials

Materials	Quantity
Copies of Student Handout— <i>Careers in the Spotlight</i> (handed out in Lesson One)	1 per student
Class set of Student Handout— <i>Instructions for Seeing DNA in 3D</i>	1 per student (class set)
Student Handout— <i>Seeing DNA in 3D Worksheet</i> [Note: This worksheet is for students' answers to lesson questions.]	1 per student
Pipe cleaners	1 per student
Teacher Answer Key— <i>Seeing DNA in 3D</i>	1
Teacher Resource— <i>Teacher Demonstration: Aligning 3D Structures with VAST</i>	1
Teacher Resource— <i>Installing Cn3D</i> [Note: If students will be asked to install Cn3D, make a class set of copies of this handout.]	1 -OR- 1 per student
Teacher Resource— <i>From Sequence to Structure</i> [Note: This is an optional lesson extension.]	1

Computer Equipment, Files, Software, and Media

Computer with internet access and projector to display PowerPoint slides and for the Teacher Demonstration described in Teacher Resource—*Teacher Demonstration: Aligning 3D Structures with VAST*.

Alternative: Print PowerPoint slides onto transparencies and display with overhead projector.

Lesson Five PowerPoint Slides—*Learning to Use Cn3D: A Bioinformatics Tool*. 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>.

VAST Protein Alignment for the Teacher Demonstration described on Teacher Resource—*Teacher Demonstration: Aligning 3D Structures with VAST*. Available for download from the Resources tab at:
<http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing>.

BRCA1 Animation. Available for viewing at: http://www.nwabr.org/education/animations/BRCA1_animation.html.

Computer lab with internet access, a word processing program such as Microsoft Word® or Google Docs, and the program Cn3D installed on every computer.

Access to the Microsoft Word® or Google Docs document that students created in Lesson Four.

Music files (mp3 format) for Teacher Resource—*From Sequence to Structure* are available for download from the Resources tab at: <http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing>.

Teacher Preparation

- Load the classroom computer with the *Lesson Five* PowerPoint slides.
- To maximize class time for the lesson activities, it will be useful to install the Cn3D program on all classroom computers. See Teacher Resource—*Installing Cn3D* for complete instructions on installing Cn3D. Alternatively, make a class set of Teacher Resource—*Installing Cn3D* and have students install the program.
- Download the VAST alignment file for the Teacher Demonstration on Teacher Resource—*Teacher Demonstration: Aligning 3D Structures with VAST*. The VAST alignment can be found under the Resources table at: <http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing>.
- Make copies of Student Handout—*Instructions for Seeing DNA in 3D*, one per student. This handout is designed to be reused as a class set.
- Make copies of Student Handout—*Seeing DNA in 3D Worksheet*, one per student. The worksheet is used for students to write their answers to the lesson questions.

[**Note:** You may need to contact your school administrator or IT support staff to be sure that Cn3D can be downloaded to your classroom computers. Cn3D is freely available through the National Center for Biotechnology Information, a division of the US government, US National Library of Medicine.]

Procedure

WARM UP

1. As students enter the classroom, display **Slide #1**. This slide highlights 3D animator Beth Anderson.

Learning to Use Cn3D: **Slide #1**

2. Have students retrieve Student Handout—*Careers in the Spotlight* from *Lesson One*.
3. Students should think about, and write down, what kind of work a 3D animator might do (3D Animator Question #1). This will be revisited at the end of the lesson, including how a 3D animator 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: Structure Meets Function: Pencil Transferase

- Explain to students the **aim of this lesson**. Some teachers may find it useful to write the aim on the board.

Lesson Aim:

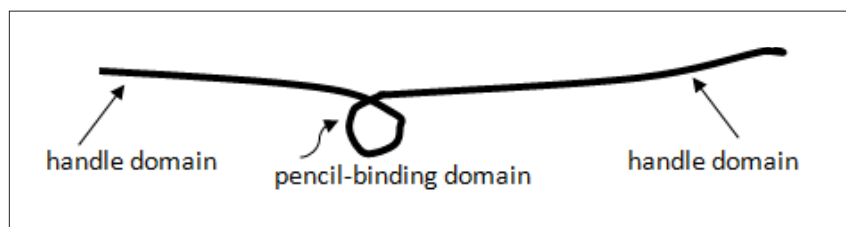
- To understand that protein structure can impact protein function, using the bioinformatics tool Cn3D to visualize molecules.

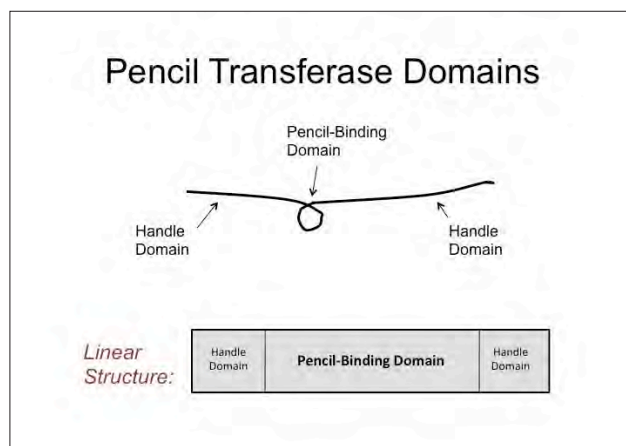
Teachers may also wish to discuss the *Learning Objectives* of the lesson, which are listed at the beginning of this lesson.

- Give each student a pipe cleaner. Tell students that their task is to **move a pencil from one desk to another desk** using the pipe cleaner.
- The rules are:
 - Students must use a pencil (not a pen or other implement with a cap).
 - Students may not touch the pencil with their hands when they are moving the pencil across desks.
 - Students may bend the pipe cleaner as needed.
 - After the pencil has been successfully moved, students should **retain the shape of the pipe cleaner** that successfully moved the pencil.
- Give students time to complete the task.
- When students have finished, have them hold up their pipe cleaners for everybody to see. The pipe cleaners will likely be very similar shapes, with a single or double loop in the middle, and a handle on each end.
- Ask students, “**What shape is your pipe cleaner? What shape is your neighbor’s pipe cleaner? Are there any shapes that are predominant in the class?**”
- Draw the shape(s) on the board.
- Finally, ask, “**Why do so many of the pipe cleaners have such similar shapes?**”
- Students will recognize that the **shape of the pipe cleaner allows it to perform its function**.
- Tell students that, like the pipe cleaner, proteins are folded into specific shapes to perform their functions.
- Show **Slide #2**, which shows the structure of “Pencil Transferase.” Drawn on the board, the “Pencil Transferase” protein may look like the image shown in **Figure 1**.

-ase is a common suffix for enzyme names.

Figure 1: “Pencil Transferase.”





16. Tell students that within “Pencil Transferase” there are areas with different structures that perform different functions. These units are called protein **domains**. Our “protein” has two handle domains and one pencil-binding domain.
17. Referring to **Slide #3**, show students the representation of the linear structure of “Pencil Transferase” on the board, as shown in **Figure 2**.

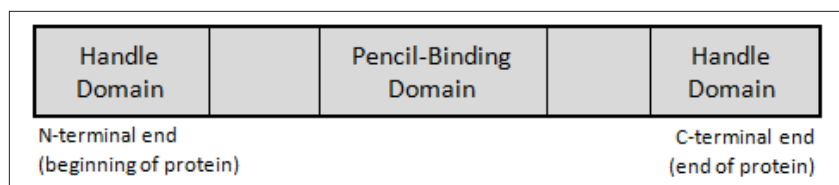


Figure 2: “Pencil Transferase” domains.

[**Note:** Need to move a piece of chalk instead of a pencil? Your “chalk-moving protein” will likely have similar domains. The amino acid sequence that folds itself into a loop to carry the pencil may be the same amino acid sequence that folds itself into a loop to carry the chalk in a different protein. The recurring domain units found in different proteins that move pencils, chalk, pens, or other similar shapes are called conserved domains.]

18. Point out that proteins are made up of amino acids that are held together by **covalent bonds**, and have a ‘beginning’ and an ‘end,’ labeled N-terminus and C-terminus respectively. The **N-terminus** or ‘beginning’ has an amino group (NH_3^+), while the **C-terminus** or ‘end’ has a carboxyl group (COO^-).
19. Show **Slide #3** or draw the representation of the linear structure of *BRCA1* on the board (see **Figure 3**). Tell students that the BRCA1 protein also has multiple domains, including a **DNA binding domain** and two **BRCT domains**. Their functions are:
 - **DNA binding domain:** To bind DNA! Remember, the function of the BRCA1 protein is to repair damaged DNA. This region binds to damaged DNA to repair it.
 - **BRCT domains:** BRCA1 needs to work with other proteins to repair DNA—it is one of many proteins that “cooperate” to do this. The BRCT domains facilitate protein-protein interactions involved in the DNA repair response. BRCT stands for Breast cancer C-Terminal domain (see **Figure 3**).

Covalent Bonds: A type of chemical bond that is characterized by the sharing of a pair of electrons between atoms.

N-terminus: The ‘beginning’ of a protein, containing an amino group (NH_3^+).

C-terminus: The ‘end’ of protein, containing a carboxyl group (COO^-).

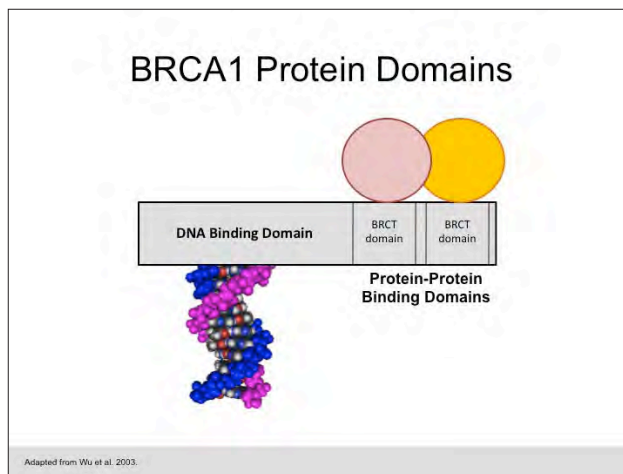
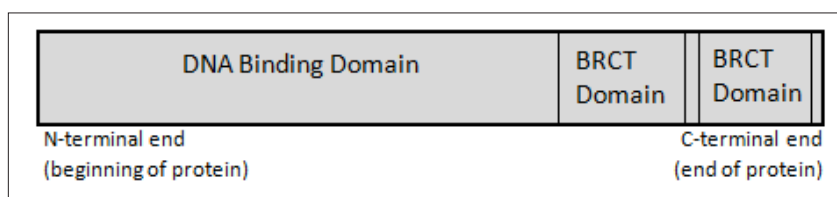


Figure 3: BRCA1 domains.



[**Note:** All amino acids have the same general structure: an alpha amino group (NH₃⁺), bound covalently to an alpha carbon, with the alpha carbon also bound to a carboxyl group (COO⁻) and a chemical group that is unique to each amino acid called an R-group. Amino acids are held together in proteins by bonds between the alpha amino group and the carbon from the carboxyl group. This is where the terms “N-terminus” (or “amino terminus”) and “C-terminus” (or “carboxyl terminus”) come from.]

BRCT Domain: The Breast Cancer C-Terminal domain, a protein domain in the BRCA1 protein located at the ‘end,’ or C-terminus, of the protein that is involved in protein-protein interactions.

Substitution Mutation: When one amino acid is substituted for another as a result of mutation.

20. Inform students that the BRCA1 protein is comprised of 1,863 amino acids. In *Lesson Four*, students learned that the mutation that affects Deb (and other family members) occurs at amino acid number 1775. At position number 1775, a methionine amino acid is replaced with an arginine amino acid. This is abbreviated as a M1775R mutation.

21. Ask students, “**Knowing the position of the mutation (at amino acid number 1775 out of a protein 1,863 amino acids long), which part of the protein is most likely affected by the mutation?**”

22. Tell students that the M1775R mutation occurs near the intersection of the two BRCT domains. As such, when the class looks at the structure at NCBI, the class will only be viewing the BRCT regions, not the whole protein. Proteins as large as BRCA1 can be difficult for biochemists to work with whole, so they often crystallize one piece (i.e., one or two domains) at a time.

23. Draw students’ attention back to their pipe cleaners and tell them that different types and locations of mutations can affect the protein in different ways. For example, a **substitution mutation** (in which one amino acid is substituted for another) **may** not have a harmful effect if it is a silent mutation (does not result in a change in amino acid sequence), or if it happens inbetween functional domains, like at point (A), as shown in **Figure 4**.

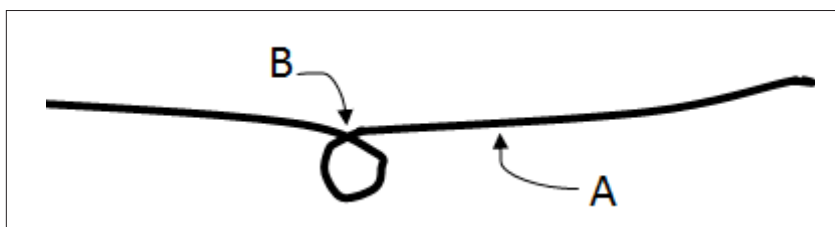
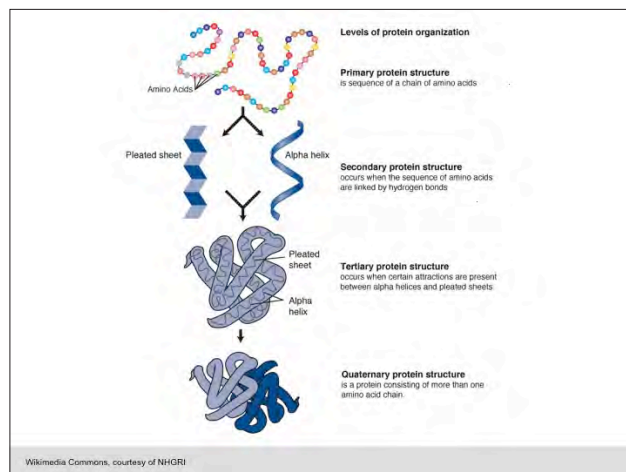


Figure 4: Substitution mutation on “Pencil Transferase.”

A substitution mutation at a crucial point in a functional domain, such as where the amino acids link together to make a loop (point B), may destroy the shape of the protein altogether, thus destroying its function. A **deletion** or **insertion** mutation could have similar consequences, depending on where in the protein the mutation occurs and how crucial the correct amino acid is at that point.

24. Show **Slide #4**. Briefly review levels of protein structure:

- **1° Primary:** The linear order of amino acids (the sequence of amino acids along the pipe cleaner model).
- **2° Secondary:** The alpha-helices and beta-sheets (similar to the 'loops' in the pipe cleaner model).
- **3° Tertiary:** The whole conformation or shape of the protein (including the handle domains in the pipe cleaner model) – the way the whole model folds.
- **4° Quaternary:** If the protein has more than one subunit or chain. (This can be demonstrated by putting several pipe cleaner models together.)



[Note: What if a point mutation in a sequence of DNA results in a premature stop codon? This causes protein translation to stop early, leading to a shorter protein.]

Teachers can demonstrate a **truncation** by simply cutting off part of the pipe cleaner and asking, "How well do you think the protein would work now?"

Unfortunately, insertions and deletions may not occur in multiples of three bases, resulting in shifting of the reading frame during translation (i.e. **frameshift mutations**). This often introduces stop codons and premature truncation of the protein.]

Learning to Use Cn3D: **Slide #4**

Part II: Seeing DNA in 3D

25. Tell students that, so far, they have been looking at the BRCA1 protein as a linear sequence of amino acids. Today they will be exploring the 3D shape of the BRCA1 protein to find out why the substitution of a single amino acid in the 1775th position has such serious consequences for the Lawler family.

26. The **BRCA1 Animation** illustrates the normal function of BRCA1, as well as the consequences of inherited mutations. Show the **BRCA1 Animation** to students. Alternatively, students can view the animation individually by visiting the Bio-ITEST website at: <http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing>.

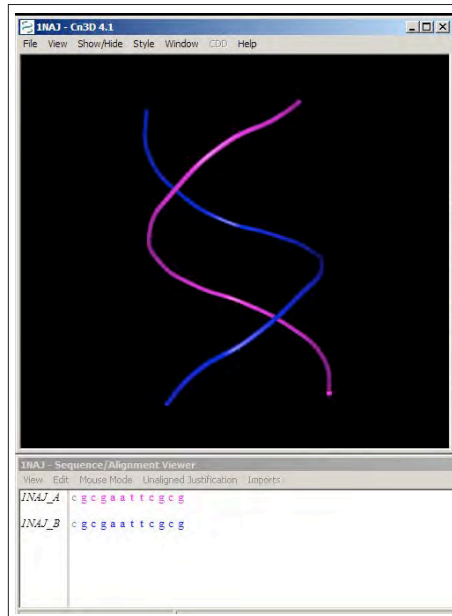
27. Tell students that they will learn how to view molecules in 3D through the use of the bioinformatics viewing tool **Cn3D**. They will learn about the program by viewing and manipulating a short DNA molecule.

Frameshift mutation: Also called a reading frame shift, a frameshift mutation is a genetic mutation caused by an insertion or deletion of a number of nucleotides not evenly divisible by three. Because codons are read as triplets, these insertions or deletions result in a change in the reading frame during protein translation. These reading frame shifts often result in premature stop codons and truncated proteins.

Cn3D (pronounced "see in 3D"): A molecular viewing software program from the National Center for Biotechnology Information. Cn3D simultaneously displays three-dimensional molecular structures along with the sequences for biological molecules such as proteins, RNA, and DNA.

28. When viewing the DNA molecule in Cn3D, students will see the anti-parallel strands, illustrated most clearly in Question #12 on Student Handout—*Seeing DNA in 3D*. When viewing the DNA structure, students may be confused by the sequence of the DNA shown in the **Sequence/Alignment Viewer** below the **Structure Window**. DNA sequences are written 5' to 3'. Therefore, both the 1NAJ_A and 1NAJ_B sequences are shown in the **Sequence/Alignment Viewer** as: cgcgattcgcg. (See **Figure 5**.)

Figure 5: Sequence/Alignment Viewer.
Credit: Wu et al. 2003.



However, the two strands of DNA are complementary and anti-parallel in the actual DNA structure:

1NAJ_A: 5' – cgcgattcgcg – 3'

1NAJ_B: 3' – gcgcttaagcgc – 5'

29. After students become familiar with Cn3D, students will view the “normal” (non-mutated) version of the BRCT region of the BRCA1 protein.
30. Tell students that neither students nor scientists can look at the entire BRCA1 protein, since its structure has not yet been “solved.” They can, however, look at the BRCT domains in that protein.
31. Remind students that BRCT stands for “**breast cancer carboxy-terminal**.” Although the BRCT domain is named for the *BRCA1* gene, it is present in many proteins that function in repairing DNA, in addition to BRCA1. Each BRCT domain is about 90-100 amino acids long and has a characteristic shape.
32. Pass out Student Handout—*Instructions for Seeing DNA in 3D* and Student Handout—*Seeing DNA in 3D Worksheet*. Allow time for students to work independently on the activity.


PART III: Teacher Demonstration—Aligning 3D Structures with VAST

33. **VAST** (Vector Alignment Search Tool) uses the locations of alpha helices and beta sheets to identify three-dimensional protein structures that might be related. Using the instructions in Teacher Resource—*Teacher Demonstration: Aligning 3D Structure with VAST*, teachers show two other protein structures associated with the BRCA1 mutations. Teachers demonstrate the structural changes between the non-mutated and mutated versions of the BRCA1 protein by aligning (overlying) the two protein structures using VAST.

VAST: VAST (Vector Alignment Search Tool) uses the locations of alpha helices and beta sheets to identify three dimensional protein structures that might be related. The two structures are overlaid on top of one another to compare structure similarities and differences.

Closure: Careers in the Spotlight

34. Today, students saw how a **single amino acid** substitution in a protein causes a very subtle shift in the protein's shape and impacts its binding to another molecule. This small change, however, has significant implications for many families, including the Lawlers. There are many mutations to BRCA1 that can cause cancer aside from the one viewed in class today. Tomorrow, students will take a closer look at the BRCA test and other genetic tests to determine how useful they may or may not be.
35. Return to the picture of the 3D animator from the *Careers in the Spotlight*, **Slide #5**.



3D ANIMATOR
BETH ANDERSON

Place of Employment:
Arkitek Studios

Type of Work:
Develops 3D animations that help scientists and students visualize biological processes.

"Bioinformatics interfaces in many ways with our work, from data that clients give us to visualize representationally, to creating actual visual data."

Learning to Use Cn3D: **Slide #5**

36. Show **Slide #6**, which provides job information for a 3D animator. Review this information with students.



CAREERS IN SPOTLIGHT:
3D ANIMATOR

What do they do?
3D animators are visual communicators and designers. The animations and illustrations they create help communicate scientific content and ideas to scientists, students, and educators.

What kind of training is involved?
Associate of Arts degree. Some also have training in biology, illustration, graphic design, and computer animation.

What is a typical salary for a 3D Animator?
Ranges with experience from very little when people are first starting out to \$100,000/year (\$48/hour) or more.

Learning to Use Cn3D: **Slide #6**

37. Ask students, "**What more do we know about 3D Animators after today's lesson?**" Point out that 3D animators in the biological sciences use computer programs like Cn3D and the information in protein crystal structures like the ones seen here for BRCA1 to create animations that help us visualize biological processes. Beth Anderson and her colleagues developed the BRCA1 animation seen in this lesson.
38. Ask students to answer 3D Animator 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 3D animator does.

39. Ask students to also answer 3D Animator Question #3 on their *Careers in the Spotlight* handout, which has students explain how a 3D animator might use bioinformatics in his or her work.
40. 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:

- A. Students should continue to prepare for the Socratic Seminar in Lesson Six using Student Handout—*Categorizing Genetic Tests* and/or Student Handout—*Weighing the Risks and Benefits of Direct-to-Consumer Genetic Testing*. Either Student Handout can also be passed out as homework to accompany the reading, which can be used as entry tickets for that class session. These are the reading and support materials for the Socratic Seminar students will participate in during *Lesson Six*. Students may need two days to prepare fully.
- B. If students answered the Extension Questions #48-50 on Student Handout – *Aligning Sequences with BLAST Worksheet* in Lesson Four, they can expand upon their answers to these questions, describing what they have learned about how this single amino acid change can impact the structure of the BRCT domain of the BRCA1 protein. Students can also answer the questions posed on their Word® document created in Lesson Four and augmented in Lesson Five as homework (see Student Handout—*Instructions for Seeing DNA in 3D* Part 2, Questions #18 and 19).
- C. As a reflective exercise, ask students to write about the activities they did 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 4 prompts are complete.]

X-ray crystallography: A method of determining the arrangement of atoms within a crystal, such as a crystal of a particular protein, in which a beam of X-rays strikes a crystal and deflects or diffracts into many specific directions. From the angles and intensities of these diffracted beams, a crystallographer can produce a three-dimensional picture of the density of electrons within the crystal, and thus calculate and estimate the three-dimensional shape of the molecule used to generate the crystals. This is often referred to as determining or “solving” the protein’s structure.

Extension

- For a musical analogy with MP3 clips to describe protein structure and conserved domains, a draft extension activity for *Lesson Five* can be found at the end of this lesson plan on Teacher Resource—*From Sequence to Structure*.

Adaptation

- Teachers can work through all or part of Student Handout—*Instructions for Seeing DNA in 3D* as a teacher-led demonstration, as desired.

Teacher Background

Protein Structures: Scientists know what DNA, a protein, or other macromolecules look like by using a number of different tools, including **X-ray crystallography** and **nuclear magnetic resonance (NMR)**, to determine molecular structures. In the case of x-ray crystallography, proteins are crystallized before being bombarded with x-rays. By measuring how the crystals deflect or diffract the x-rays, scientists can determine or “solve” the protein’s structure. With NMR, molecules are subjected to a strong magnetic field that causes the natural magnets in atomic nuclei to spin in the same direction, just like iron filings line up in the presence of a magnet. Radio waves are used to disrupt this state and scientists measure how each atom responds. The characteristics of the response are used to solve the molecular structure by determining which other atoms are close by. These structures are then added to the **Protein Data Bank (PDB)**, and later, to the structure database at the NCBI for others to view using special molecular-viewing programs like Cn3D. Molecular-viewing programs like Cn3D allow users to view and manipulate three-dimensional structures on a computer screen.

NMR spectroscopy: Nuclear Magnetic Resonance spectroscopy, usually abbreviated as “NMR,” is a technique used to determine the three-dimensional structure of molecules, including proteins. Molecules are subjected to a strong magnetic field that causes the natural magnets in atomic nuclei to spin in the same direction, just like iron filings line up in the presence of a magnet. Radio waves are used to disrupt this state, and scientists measure how each atom responds. The characteristics of the response are used to determine or “solve” the molecular structure by determining which other atoms are close by.

Protein Data Bank (PDB): A repository or collection of three-dimensional structures of large biological molecules, including proteins and nucleic acids, submitted by scientists from around the world. This data is typically obtained by X-ray crystallography or NMR spectroscopy.

Assessment Suggestions

Students can be assessed on their answers to the questions posed on their Word® document created in *Lesson Four* and augmented in *Lesson Five* (see Student Handout —*Seeing DNA in 3D*).

Glossary

Accession number: A unique identifier or code assigned to every entry in the National Center for Biotechnology Information (NCBI) databases. This unique code can be used to search the databases to find your gene, protein, or structure of interest.

Alpha helix (Plural: “alpha helices”): A common structure of proteins, characterized by a single, spiral chain of amino acids stabilized by hydrogen bonds.

Beta sheet: A structure that occurs in many proteins and consists of two or more parallel adjacent polypeptide chains arranged so that hydrogen bonds can form between the chains.

BRCT domain: The Breast Cancer C-Terminal domain, a protein domain in the BRCA1 protein located at the ‘end,’ or C-terminus, of the protein that is involved in protein-protein interactions.

Cn3D (pronounced “see in 3D”): A molecular viewing software program from the National Center for Biotechnology Information. Cn3D simultaneously displays three-dimensional molecular structures along with the sequences for biological molecules such as proteins, RNA, and DNA.

Covalent bonds: A type of chemical bond that is characterized by the sharing of a pair of electrons between atoms.

C-terminus: The ‘end’ of protein, containing a carboxyl group (COO⁻).

Domain: Specific area of a protein that performs a particular function.

DNA binding domain: Specific area of a protein (domain) that binds to DNA. The DNA binding domain in the BRCA1 protein is necessary for the BRCA1 protein to repair damaged DNA.

Frameshift mutation: Also called a reading frame shift, a frameshift mutation is a genetic mutation caused by an insertion or deletion of a number of nucleotides not evenly divisible by three. Because codons are read as triplets, these insertions or deletions result in a change in the reading frame during protein translation. These reading frame shifts often result in premature stop codons and truncated proteins.

Hydrophilic: A substance that is attracted to water. From the Greek “hydro” which means water, and “philos” meaning love. A synonym for polar. The opposite of hydrophobic or non-polar.

Hydrophobic: A substance which repels water. From the Greek “hydro” which means water, and “phobos” which means fear. A synonym for non-polar. The opposite of hydrophilic or polar.

NMR spectroscopy: Nuclear Magnetic Resonance spectroscopy, usually abbreviated as “NMR,” is a technique used to determine the three-dimensional structure of molecules, including proteins. Molecules are subjected to a strong magnetic field that causes the natural magnets in atomic nuclei to spin in the same direction, just like iron filings line up in the presence of a magnet. Radio waves are used to disrupt this state, and scientists measure how each atom responds. The characteristics of the response are used to determine or “solve” the molecular structure by determining which other atoms are close by.

Non-polar: A substance that repels water. A synonym for hydrophobic.

N-terminus: The ‘beginning’ of a protein, containing an amino group (NH_3^+).

Polar: A substance that is attracted to water. A synonym for hydrophilic. The opposite of hydrophobic or non-polar.

Protein Data Bank (PDB): A repository or collection of three-dimensional structures of large biological molecules, including proteins and nucleic acids, submitted by scientists from around the world. This data is typically obtained by X-ray crystallography or NMR spectroscopy.

Stop codon: A codon (series of three nucleotides in a row) that terminates, or stops, protein translation.

Substitution mutation: When one amino acid is substituted for another as a result of mutation.

Truncation: To shorten, as if by cutting off. During translation, a growing protein chain is truncated if it encounters a premature stop codon.

VAST: VAST (**V**ector **A**lignment **S**earch **T**ool) uses the locations of alpha helices and beta sheets to identify three dimensional protein structures that might be related. The two structures are overlaid on top of one another to compare structure similarities and differences.

X-ray crystallography: A method of determining the arrangement of atoms within a crystal, such as a crystal of a particular protein, in which a beam of X-rays strikes a crystal and deflects or diffracts into many specific directions. From the angles and intensities of these diffracted beams, a crystallographer can produce a three-dimensional picture of the density of electrons within the crystal, and thus calculate and estimate the three-dimensional shape of the molecule used to generate the crystals. This is often referred to as determining or “solving” the protein’s structure.

Credit

BRCA1 domain illustration from: <http://www.biochemsoctrans.org/bst/037/0597/bst0370597a01.gif>.

BRCA1 Animation developed by Beth Anderson, Arkitek Studios, and Jill DelSordi.

Anderson, Beth. Personal Interview. 2 July 2010.

Photo of Beth Anderson provided by Doug Huff.

Wu, Z., Delaglio, F., Tjandra, N., Zhurkin V.B., & Bax, A. (2003). Overall structure and sugar dynamics of a dna dodecamer from homo- and heteronuclear dipolar couplings and (31)p chemical shift anisotropy. *J. Biomol. Nmr.*, 26, 297.

Varma, A.K., Brown, R.S., Birrane, G., & Ladias, J.A.A. (2005). Structural basis for cell cycle checkpoint control by the brca1-ctip complex. *Biochemistry*, 44, 10941.

The authors wish to thank Wikimedia Commons for the definitions of some of the vocabulary terms found in the Glossary and throughout this lesson.

5

Instructions for Seeing DNA in 3D

Aim: To understand that protein structure can impact protein function, using the bioinformatics tool Cn3D to visualize molecules.

Instructions: Write the answers to your questions on Student Handout—*Seeing DNA in 3D Worksheet*, in your lab notebook, or on a separate sheet of paper, as instructed by your teacher.

PART I: Viewing DNA Structure

1. Go to the NCBI Structure database: (<http://www.ncbi.nih.gov/structure>).
2. The center top of the page has an open area for search terms. Type **1NAJ** into the search area and click the **Go** button.

We will begin our investigation of 3D structure with a look at a molecule you are already familiar with: DNA. "1NAJ" is the **accession number** for a file that contains structure information for a small piece of double-stranded DNA. An accession number is like a catalog number or bar code; it bears no resemblance to the product itself, but allows you to access information in the vast databases at the NCBI.

3. The 1NAJ record will appear as the only result from this search. **Click** the DNA image that looks like two colored strings winding loosely around each other with a black background.

You are on the **Structure Summary** page, which includes a brief description of the source of the structure.

4. Click the "**View structure**" button to download the structure file, as shown in **Figure 1**. Save this file to your desktop, or to a folder, as instructed by your teacher. Then double-click on the file to open it.

[**Note:** The file may appear in different places depending on the type of web browser and how the computer has been set up. You may need to look for your file in your Downloads folder. This file may have a name like "mmdb.cgi."]

5. If your file opens with the Cn3D program, skip to Step #7. Otherwise, find the Cn3D program icon on your desktop or in your program menu, and open the program. Select "**Open**" from the file menu and select your file from above.

[**Note:** The Structure Window is the black square box that shows the DNA structure. Your screen may also show the Sequence/Alignment viewer, below the Structure Window.]

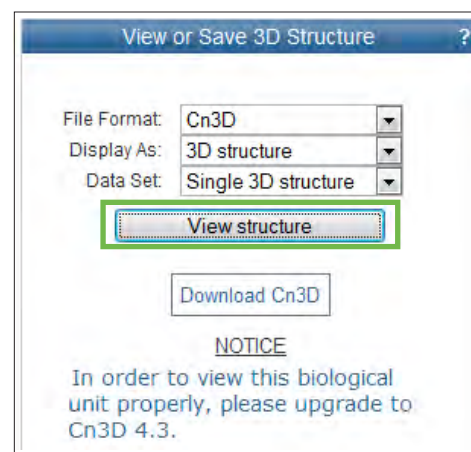


Figure 1: Click the "View structure" button to download the file.

LESSON 5

CLASS SET

- View the DNA in the Structure Window. Drag the structure with your mouse to turn it around. Hold the shift button down, and continue to hold it down, while dragging, to move your structure without turning it. Experiment with controlling the movement and viewing the molecule from multiple angles.

You can zoom in and out by using the **z** or **x** keys. Alternatively, you can change the size by holding down the left mouse button and dragging the mouse away from the screen.

Hold down the left mouse button and drag the mouse towards or away from the screen.



- Open the **View** menu and choose "**Animation.**" Choose the "Spin" option.

What happens when you click s and n?

Position the DNA molecule the way you want it before proceeding.



- Open the **Style** menu and select "**Rendering Shortcuts.**" There are a variety of rendering options available to view the DNA molecule. Explore each of the following:

Worms Tubes Wire Ball and Stick Space-fill

Imagine you are teaching a class about DNA. What rendering option(s) would you use to teach students about DNA structure? Explain the reasons for your decision.



- Open the **Style** menu and select "**Coloring Shortcuts.**" There are many options for changing the colors of molecules. Select each of the options below, and answer each of the following questions.

- Object:** How did this change the coloring of the DNA molecule?
- Rainbow:** 'Rainbow' uses the color red at the start (5' end) and continues through the rainbow. Why are there two red regions?
- Explore a few combinations using different rendering and coloring options. Which coloring option do you find most useful? Why?

- Go to the **Sequence/Alignment Viewer** window on your screen.

[**Note:** The Sequence/Alignment Viewer is the window below the Structure Window that shows the sequence being viewed, as shown in **Figure 2**. If there is no sequence visible, open the **Window** menu and choose "**Show Sequence Viewer.**"]

Click on the letter of a base in the Sequence/Alignment Viewer and see what happens.

Note that there are two sequences: *1NAJ_A* and *1NAJ_B*. Each sequence corresponds to a different strand of the DNA molecule.

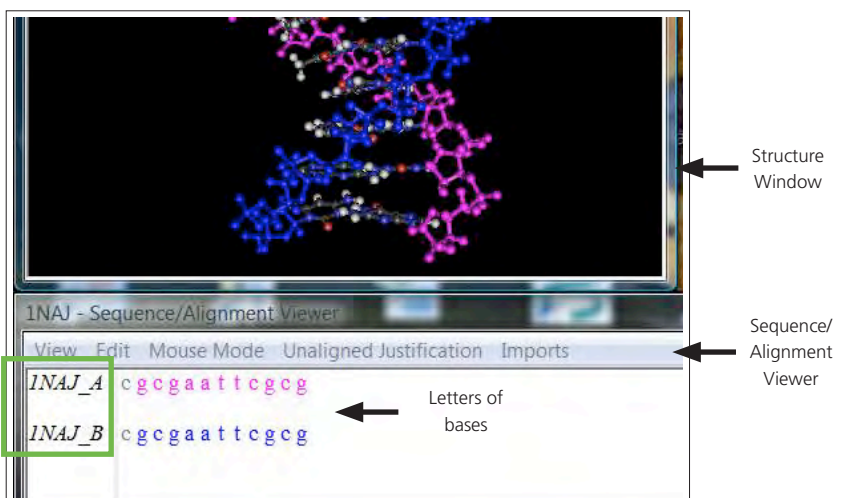


Figure 2: The Sequence/Alignment Viewer shows the sequences being viewed.
Credit: Wu et al. 2003.



11. In the sequence *1NAJ_A*, click the first “g.” Next, click the first “g” of the sequence *1NAJ_B*.

Where are these two guanines located on the DNA molecule? Explain why.

12. Choose a single base you would like to view, and click on it in the Sequence/Alignment viewer.



13. Change the **Rendering style** to “**Wire**” and the **Coloring style** to “**Object**.”

a. What does that do? Explain.

b. Move the DNA so that the ring structure(s) of the selected nucleotide’s base is clearly seen.

What do you see?



14. Open the **Select** menu and choose “**Show Selected Residues**.” Answer the following questions:

a. What do you see?

Zoom in to view the base more easily (refer to Step #7 if you need help).

b. **Challenge question:** Is the base you selected a purine or a pyrimidine, and how do you know?

From the Style menu choose “**Show Everything**” to see the complete strand.

PART II: Viewing the BRCA1 Protein

Now that you are familiar with the Cn3D program, we will view part of the BRCA1 protein and part of a second protein that it interacts with.

15. Go to the NCBI **Structure database**: (<http://www.ncbi.nih.gov/structure>) or click the “**Structure Home**” button from the DNA structure summary page.

16. At the top of the page, find the search option. Type “**1Y98**” into the blank and click the “**Go**” button.

17. The **1Y98** record will appear as the only result from this search. Click on the **link or title** of the structure above the black box with the picture of the protein in it. You are on the **Structure Summary** page, which includes a brief description of the source of the structure.

18. Click the “**View structure**” button to download and open the structure file.

19. Take a moment to explore different **Style** options.

20. Open the **Style** menu and choose “**Coloring Shortcuts → Secondary Structure**” to highlight each type of secondary structure in a different color.

21. Look at the **Sequence/Alignment Viewer** below the structure. When viewing the DNA structure, the Alignment/Sequence Viewer showed the letters of the bases. When viewing a protein structure, the viewer shows the one-letter abbreviation for the amino acids.

Note that colors of the amino acid sequence correspond to the colors of the structure.

22. The structure contains two BRCT domains. To see each domain individually, open the **Style** menu and choose “**Coloring Shortcuts → Domains**.”

This will show the two BRCT domains in different colors. **BRCT** stands for **B**reast cancer **C**-Terminal domain, and is involved in protein-protein interactions. The alpha helices are depicted as cylinders (like crayons) and the beta sheets are depicted as flat arrows. Both the helix and strand objects point in an amino to carboxyl direction (i.e., they point toward the end of the protein).

LESSON 5

CLASS SET

Note that there is a third domain – this is part of another, different protein to which BRCA1 normally binds to do its job of repairing DNA. This protein is called CtIP.

23. Find the portion of the second protein, CtIP, that BRCA1 interacts with during DNA damage repair.

It looks like a little brown squiggle off to the side. In the Sequence Window, the sequence of this portion of the second protein is labeled 1Y98B. If you can't find CtIP, highlight the 1Y98B sequence and the CtIP will light up yellow in the Structure Viewer. To remove the yellow highlights, click somewhere in the Sequence Window to activate the sequence menu. Then, open the **View** menu and choose "**Clear Highlights**."



24. Now that you know what the portion of the CtIP protein looks like, go back to the **Style** menu and select "**Coloring Shortcuts → Secondary Structure**." Move your cursor along the 1Y98A amino acid sequence in the Sequence/Alignment Viewer, as seen in **Figure 3**.

Note that the colors of the amino acids correspond to the colors in the protein structure (beta sheets, alpha helices, and random coils). The box on the lower left of the screen shows the location of the amino acid your cursor is over.

- What color are the beta sheets?
- What color are the alpha helices?

25. Move your cursor along the amino acid sequence until you get to amino acid **1Y98A_loc130 (PDB 1775)**.

Amino acid 1775 (PDB 1775) is the location of the M1775R mutation in BRCA1 that affects the Lawler family.

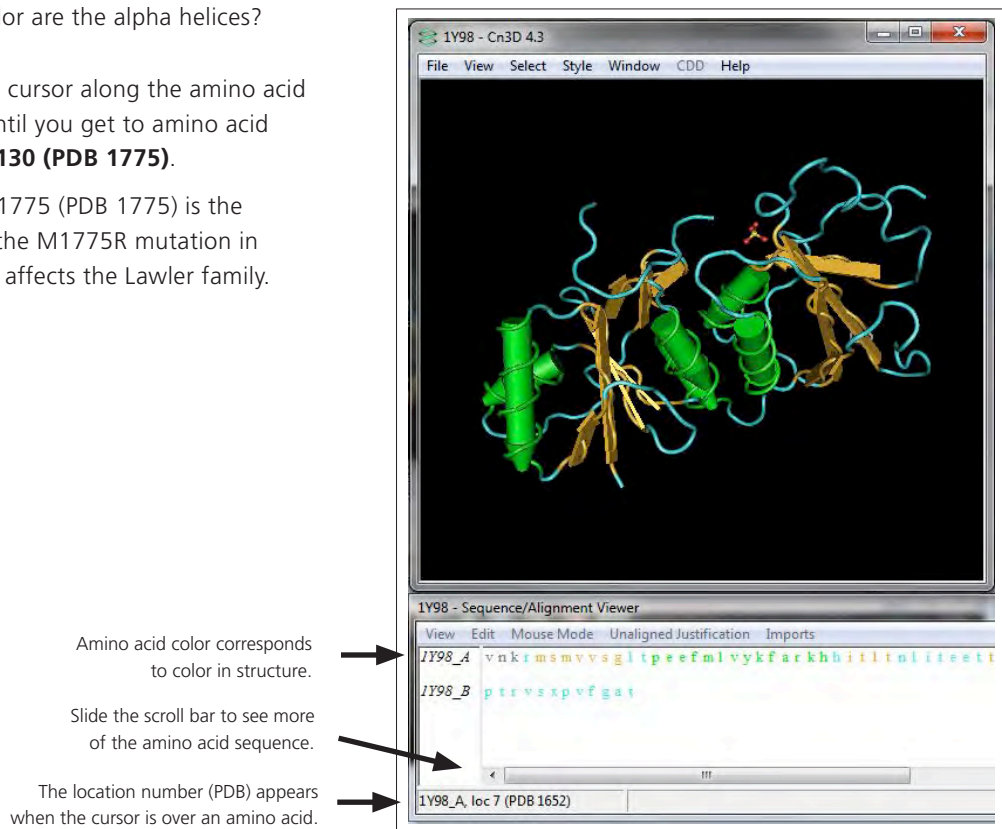


Figure 3: When you open the BRCA1 structure, you will see the BRCA1 protein that does not contain any mutations.
Credit: Varma et al. 2005.

26. Click on amino acid number “**1775**” (M, the amino acid methionine) in the Sequence Viewer to highlight it in the structure.

It should light up yellow in the structure. You are looking at where the mutation would be located, but this structure you are looking at does not have the mutation. (Recall that the mutation converts a M to a R.)

27. Open the **Select menu → Select by Distance**. Open the **Select menu → Select by Distance → Residues only**. Leave the distance cutoff at five Angstroms to select all molecules within a radius of five Angstroms. Make sure the options “Select protein residues” and “Select other molecules only” are checked. Click “OK.”

[**Note:** An Angstrom, Å, is 1×10^{-10} meters.]

28. Note that part of the CtIP protein now also lights up yellow, as do other BRCA1 amino acids (residues). It is very close to the site on BRCA1 where the mutation would be. You can imagine how a change in BRCA1 might impact its ability to interact with this second protein, CtIP, which is also required for DNA repair.

29. Record your results from Cn3D by capturing the image using the following instructions:

- Open up the Word® document you created in *Lesson Four*. It should be labeled with your LASTNAME_BRCA1_NCBI.
- Return to the 1Y98A Cn3D page with the protein structure.
- FOR PC USERS:** Hit the “Prnt Scrn” button on your computer. This is often in the top right of the keyboard, to the right of the F12 button.

FOR MAC USERS: Press the keys: “**Command + Shift + 4**” at the same time.

- Return to your Word® document.

- FOR PC USERS:** Use the paste function to transfer the screen shot.

FOR MAC USERS: Open the **Insert** menu, choose “**Picture from file,**” and choose the image that you captured from the computer screen.

[**Note:** The image may have saved to your desktop.]

- Type these instructions and questions at the bottom of your Word® document:

- Circle the location of the mutation in the picture of the BLAST alignment.
- Circle the location of the mutation in the Cn3D picture of the protein structure.
- Explain what these pictures represent.
- Explain how the pictures are connected to each other.

- Follow the instructions you typed above and answer the questions on your Word® document. You may choose to print out the document later and circle the points of mutation, or you can make the circles directly on your Word® document by doing the following:

Use your drawing tools in Word® to draw red circles around the mutation in the BLAST sequence window and the Cn3D view. (**Insert Shapes → Oval → Shape Fill: no fill, Shape outline: red line**). You can also use the “Crop” feature in Word® to crop your image.

- Save and close 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.



30. **Challenge:** To get a better sense of the interaction between BRCA1 and CtIP, Cn3D can show the amino acids that are interacting with each other in ball and stick form. Be sure that the amino acids in the previous step are highlighted before you begin.

- Open the **Style** menu and choose "**Annotate →**." Click on the box that says "**New**" to the right of "**Selection: (new)**." Another small Edit Annotation window will open.
- Click on "**Edit Style**" in the Edit Annotation window. There is no need to enter a description. A big Style Options window will open and will default to the "**Settings**" tab.
- Find the group **Protein Sidechains**, and click on the box next to it to check it.
- Immediately to the right, pull down the "**Rendering Options for Protein Sidechains**" and change them to "**Ball and Stick**."
- Click "**Apply**" and "**Done**" (and "**OK**" and "**Done**" on the remaining windows) to close all the windows and return to the structure.
- You may want to capture this image for your Word® document as well.



- Challenge Question:** What can you see now that you could not see before you annotated this structure? Does this help you understand the consequences of the Lawlers' M1775R mutation?

31. **Optional:** If time permits, you may wish to experiment with protein structures 1JNX and 1N5O. Make sure that you enter *capital O and not a zero* for "**1N5O**."

1JNX: This is the accession number for the BRCA domains of the **non-mutated** version of the BRCA1 protein.

1N5O: This is the accession number for the BRCA domains of the M1775R **mutation** of the BRCA1 protein.

Name _____ Date _____ Period _____

5

Seeing DNA in 3D Worksheet

Aim: To understand that protein structure can impact protein function, using the bioinformatics tool Cn3D to visualize molecules.



Instructions: Use Student Handout—*Instructions for Seeing DNA in 3D* to complete this worksheet.

PART I: Viewing DNA Structure

7. What happens when you click s and n?

s:

n:

8. There are many ways to view the DNA. Imagine you are teaching a class about DNA. What rendering option(s) would you use to teach students about DNA structure?

(circle one): **Worms** **Tubes** **Wire** **Ball and Stick** **Space-fill**

Explain the reasons for your decision:

9a. Look at **Coloring Shortcuts**, and click "**Object**." How did this change the coloring of the DNA molecules?

9b. Now look at **Rainbow**: "Rainbow" uses the color red at the start (5' end) and continues through the rainbow. Why are there two red regions of the DNA?

9c. Which coloring option do you find most useful? Why?

LESSON 5

HANDOUT

11. When you click on the two guanines that are in the same place for both DNA strands, where are they located on the DNA molecule? Explain why.

13a. After changing the **Rendering style** to “Wire” and the **Coloring style** to “Object,” what does that do? Explain.

13b. Move the DNA so that the ring structure(s) of the selected nucleotide’s base is clearly seen. What do you see?

14a. After selecting “**Show Selected Residues**,” what do you see?

14b. **Challenge question:** Is the base you selected a purine or a pyrimidine, and how do you know?

PART II: Viewing the BRCA1 Protein

24a. What color are the beta sheets?

24b. What color are the alpha helices?

30. **Challenge Question:** What can you see now that you could not see before you annotated this structure? Does this help you understand the consequences of the Lawlers’ M1775R mutation?

5

Seeing DNA in 3D

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 4 points for the optional Challenge questions**, (Questions #14B and #31).]

PART I: Viewing DNA Structure

7. What happens when you click s and n?

s: The image stops spinning.
n: The image begins to spin again.
(+1 for each correct response.)

8. There are many ways to view the DNA. Imagine you are teaching a class about DNA. What rendering option(s) would you use to teach students about DNA structure?

(circle one): **Worms** **Tubes** **Wire** **Ball and Stick** **Space-fill**

Explain the reasons for your decision:

Student answers will vary. Some students will prefer the simplified representation of the sticks or the worms, while others will prefer to see the structure details of the wires or ball and stick (which are both good for visualizing the rings of the DNA bases), or the space-filled, which makes it possible to view the full size of the atoms, including a representation of the electron clouds.
(+1 for selecting option; +1 for providing reasonable explanation.)

9a. Look at **Coloring Shortcuts**, and click "**Object**." How did this change the coloring of the DNA molecules?

The entire DNA molecule (the 'object') turns pink. (+1.)

9b. Now look at **Rainbow**: "Rainbow" uses the color red at the start (5' end) and continues through the rainbow. Why are there two red regions of the DNA?

Each of the two regions is a different strand of the DNA molecule.
(+1 for 'different strands' or 'two strands' or 'DNA is double stranded'.)

9c. Which coloring option do you find most useful? Why?

Student answers will vary. Some students will prefer the simplicity of the Object coloring, while others will find the Rainbow coloring useful to identify the 5' and 3' ends of each DNA strand.
(+1 for choosing option; +1 for providing reasonable explanation.)

11. When you click on the two guanines that are in the same place for both DNA strands, where are they located on the DNA molecule? Explain why.

The two guanines are located on opposite strands of DNA, so in the three-dimensional DNA structure, one guanine is located on the top (near the 5' end of one strand), and one is located on the bottom (near the 5' end of the second strand).

(+1 for providing location; +1 for explaining that they are on opposite strands.)

13a. After changing the **Rendering style** to “**Wire**” and the **Coloring style** to “**Object**,” what does that do? Explain?

Most of the DNA molecule is shown in pink, while the DNA base the student selected is highlighted in yellow, making this base easier to identify in the DNA structure.

(+1 for color change; +1 for explaining that the base is easier to identify.)

13b. Move the DNA so that the ring structure(s) of the selected nucleotide's base is clearly seen. What do you see?

You can visualize the structure of the base: the backbone, the ring, and the phosphodiester bond.

(+1 for structure or shape of the base.)

14a. After selecting “**Show Selected Residues**,” what do you see?

Most of the DNA structure has been removed, and only the selected base is visible. (+1.)

14b. **Challenge question:** Is the base you selected a purine or a pyrimidine, and how do you know?

The selected base is either a purine (if it has two rings) or a pyrimidine (if it has one ring).

(+1 bonus point for correct response; +1 bonus point for # of rings.)

PART II: Viewing the BRCA1 Protein

24a. What color are the beta sheets?

The beta sheets are gold/yellow (flat arrows). (+1.)

24b. What color are the alpha helices?

The alpha helices are green (round or tube-like arrows). (+1.)

29. You will need to open the document that you saved your BLAST Alignment to (the DNA and Protein sequence). Follow the instructions on Student Handout—*Instructions for Seeing DNA in 3D*.

Students should include a screen capture image of the BRCA1 protein structure. On this screen capture image, they should circle the location of the M1775R mutation on the BLAST alignment, as well as the location of the mutation on the Cn3D picture of the protein structure (circled in red in the sample image). They should explain that the BLAST picture represents an alignment of the Lawler family protein sequences compared to a reference sequence, which shows the location of the mutation (a methionine to arginine mutation at position 1775). The Cn3D image is a picture of part of the structure of the BRCA1 protein, which also has a circle around the location of the M1775R mutation found in the Lawler family.



Credit: Varma et al. 2005.

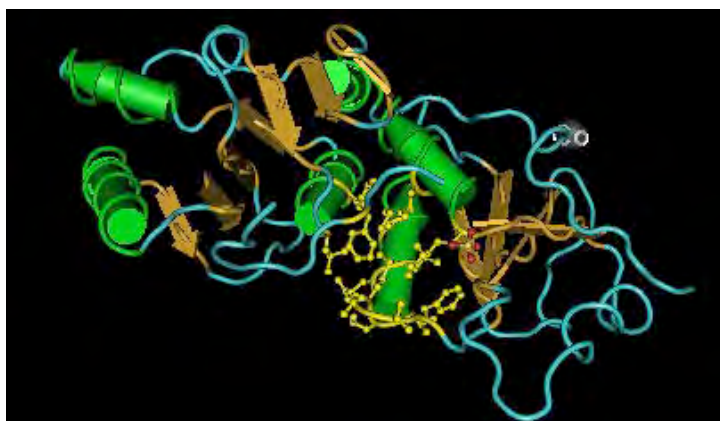
[Note: Some protein structures contain additional molecules, such as SO_4 or metal ions, which assist in protein crystallization but are not part of the protein structure.]

(+1 for screen capture; +1 for circling mutation; +1 for explaining the BLAST picture is the protein sequence with mutation; +1 for explaining the Cn3D picture represents the protein structure with mutation. 4 points total.)

31. **Challenge question:** What can you see now that you could not see before you annotated this structure? Does this help you understand the consequences of the Lawlers' M1775R mutation?

Students can now see the methionine impacted by the Lawler family mutation, as well as which amino acids methionine 1775 interacts with, including the CtIP protein. If methionine is mutated to an arginine at position 1775, interactions with multiple amino acids in the BRCA1 protein will be impacted, as seen in the screen capture image to the right.

(+1 bonus for methionine; +1 bonus for explaining multiple interactions.)



Credit: Varma et al. 2005.

5 Teachers Demonstration:

Aligning 3D Structures with VAST

VAST is a **V**ector **A**lignment **S**earch **T**ool. It uses the locations of alpha helices and beta sheets to identify three dimensional protein structures that might be related.

In this demonstration, teachers show the structural changes between the non-mutated and mutated versions of the BRCA1 protein by aligning two structures. The accession numbers for the two proteins are:

1JNX—This shows the BRCA domains of the **non-mutated** version of the BRCA1 protein.

1N50—This shows the BRCA domains of the M1775R **mutation** of the BRCA1 protein.

1. Go to the NCBI Structure database: (<http://www.ncbi.nih.gov/structure>).
2. The center top of the page has a search box next to the drop down menu that says **Structure**. Type "**1JNX**" into the blank box and click the **Search** button.
3. The 1JNX record will appear as the only result from this search. **Click the image** to get to the **Structure Summary** page.
4. Near the top right corner of the screen, click the VAST link to search for related structures identified by VAST, as shown in **Figure 1**.

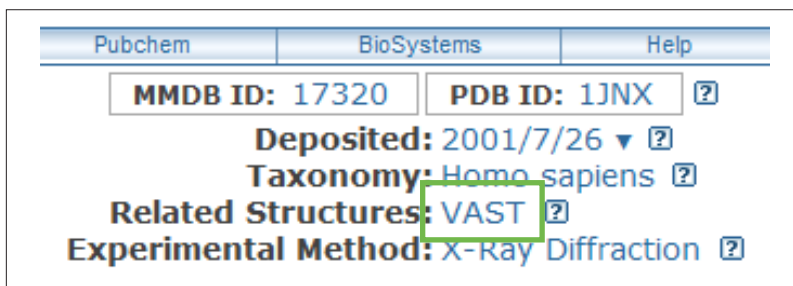


Figure 1: Click the VAST link near the top right corner of the screen. Credit: NCBI Structure, Molecular Modeling Database (MMDB).

5. When the VAST window opens, click on the "**Entire Chain**" link under **Domain Type** to find structures related to the full-length BRCT protein structure, as shown in **Figure 2**.

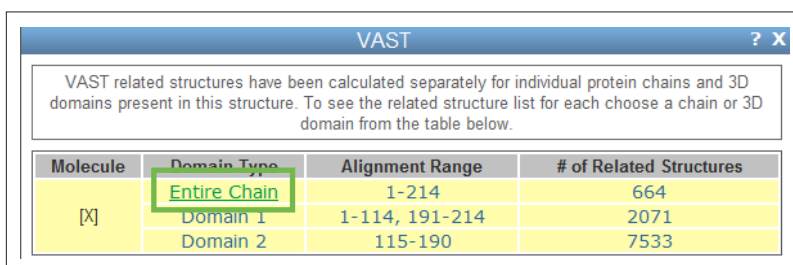


Figure 2: Select the "Entire Chain" option to view the entire BRCT domain. Credit: NCBI Structure, Molecular Modeling Database (MMDB).

6. To focus your search options on the 1N5O structure, which we know to be related to 1JNX, click on the + next to the phrase **Advanced related structure search** at the bottom of the yellow box in the top portion of the page (see **Figure 3**).

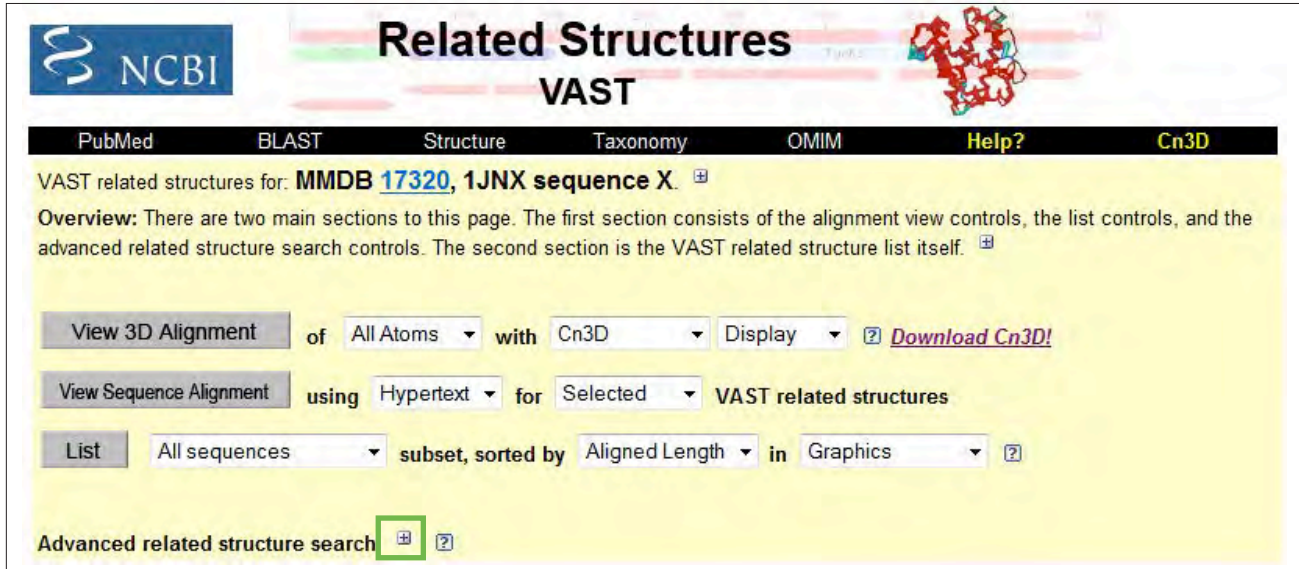


Figure 3: To expand your search options, click on the “+.” Credit: NCBI Structure, Molecular Modeling Database (MMDB).

7. Enter “1N5O” in the box labeled **related structures with MMDB or PDB ids**: Make sure the last character is a *capital O* and not a zero. Click the **Find** button (see **Figure 4**).

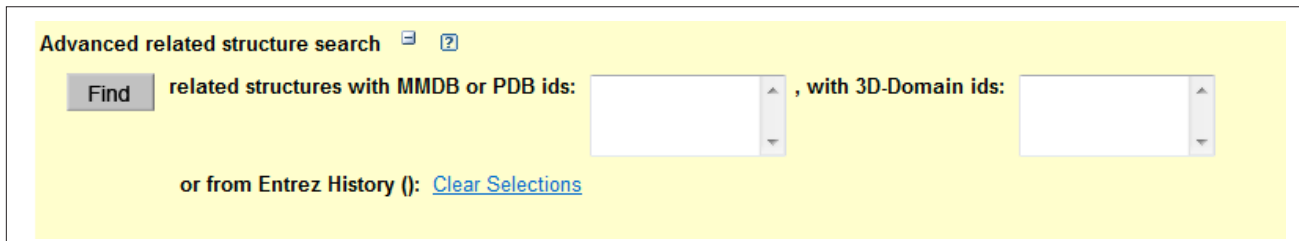


Figure 4: Enter your search term “1N5O” in the search box. Credit: NCBI Structure, Molecular Modeling Database (MMDB).

8. Check the box beside the first structure, **1N5O X**.
9. Click the gray **View Sequence Alignment** button near the top of the screen to see where the mutation is located (see **Figure 5**).

The format is similar to results of the BLAST in *Lesson Four*: the two sequences are shown in one color (red) in areas where they are the same, and a different color (blue) where they differ. Lori’s protein (1JNX) contains a methionine, while Deborah’s protein (1N5O) contains an arginine at the same position (the M1775R mutation).

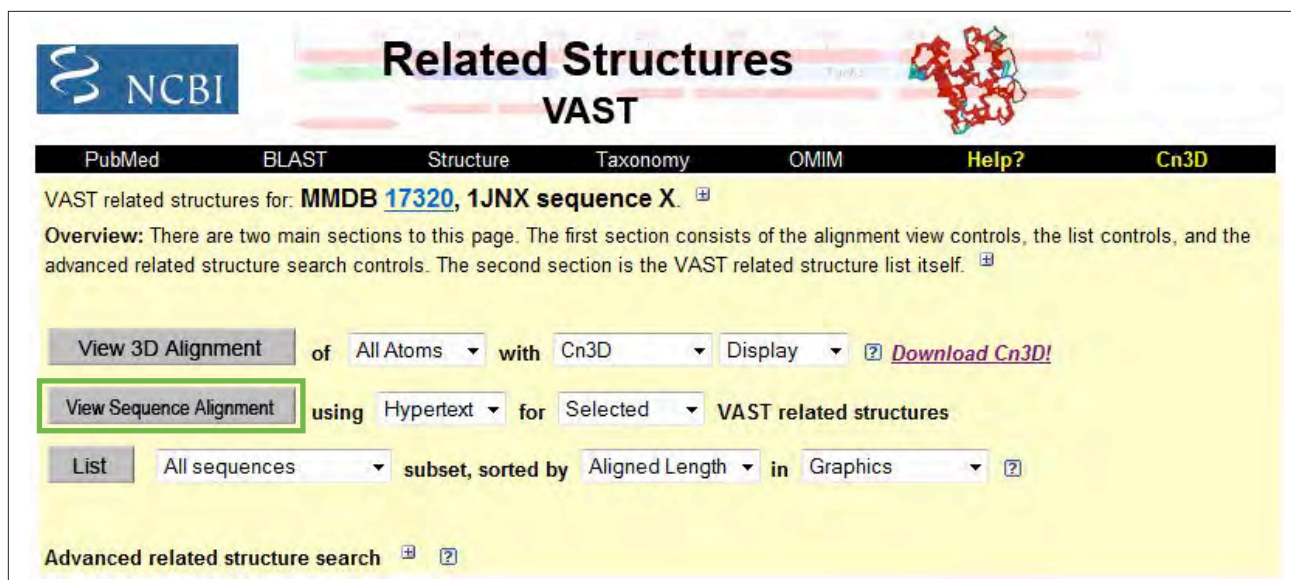


Figure 5: Viewing the Sequence Alignment will provide similar information as a BLAST alignment. Credit: NCBI Structure, Molecular Modeling Database (MMDB).

10. Click the **back** button on your browser to return to the previous window.

11. Click the **View 3D alignment** button (above the “**View Sequence Alignment**” button) to download a file containing the superimposed structures to your computer. Click “**Open with Cn3D**” to view the file directly with Cn3D, or save the file to your desktop and open it from there. The file may have a name like “vast.cgi” or “vast.cn3.”

The two structures are now superimposed. The mutation site appears in blue in the Sequence Viewer window while the regions of the three dimensional protein structures with identical amino acid sequences are shown in red. Amino acids shown in gray lowercase letters were not solved (or crystallized) as part of the protein structures.

12. **Click** in the structure window to activate the “**Structure**” menu.

13. Use the right and left arrow keys to switch between the views of the two protein structures.

14. From the **Style** menu, go to **Rendering Shortcuts** and select “**Space Filled.**”

Move the molecule around with the cursor, occasionally using the arrow keys to move from one structure to the next one. Find a view that you think best shows the change in structure between molecules.

15. From the **Style** menu, select “**Edit Global Style,**” and then **uncheck** the box in the “**Show**” column for the “**Heterogens**” row (see **Figure 6**). Click “**Apply**” and then “**Done.**” This will hide the heterogens. Heterogens are molecules that are neither RNA, DNA, nor proteins.

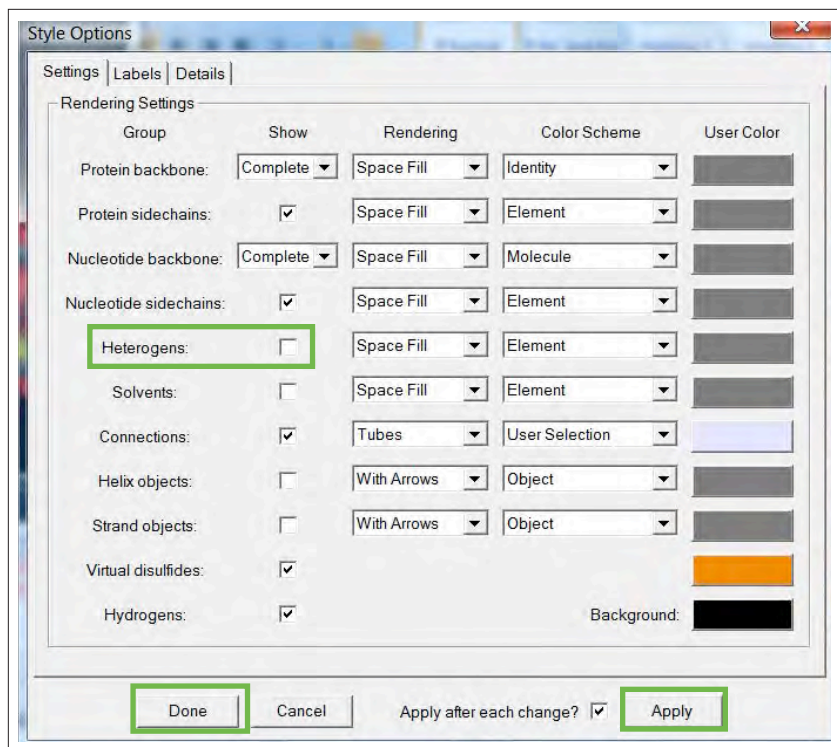
16. Look down to the Sequence/Alignment viewer. Move the cursor along the amino acid chain until you see a place where the amino acids differ.

When the cursor is directly over an amino acid, the bottom left corner of the screen shows the location of the cursor. Find “**PDB 1775**” to locate the mutation.

LESSON 5

RESOURCE

Figure 6: Hide the heterogens, which have no effect on the function of the proteins. These molecules are often artifacts of the protein crystallization process. Credit: Cn3D, Wang et al. 2000.



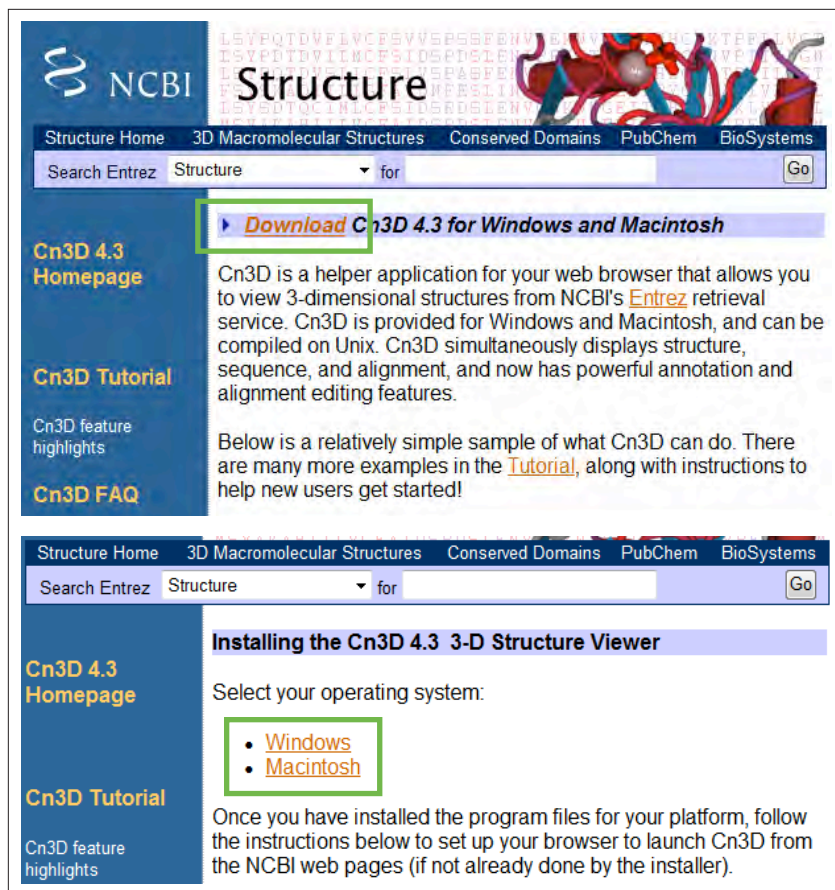
17. **Highlight the M** at 1775 in the 1JNX sequence by clicking on it.
Highlight the R in the 1N5O sequence by clicking on it and pressing “Ctrl” at the same time. This also highlights the amino acids in the structure viewer so they are more visible.
18. Experiment with using the different coloring styles to observe the impact of the mutation. Every time styles are changed, however, the heterogens associated with the 1N5O sequence will need to be hidden (repeat Step #15 to do this).
19. While toggling back and forth using the arrow keys, point out to students how the change in amino acids (from M to R) changes the protein structure.
 Methionine (M), found in the non-mutated version of the protein, is a non-polar amino acid. Non-polar (hydrophobic) amino acids fold to the inside of the protein structure, away from the aqueous environment. Arginine (R), found in the mutated version of the protein, is a polar amino acid. Polar (hydrophilic) amino acids tend to rotate to the outside of the protein structure. Students can see arginine “pop” to the outside of the mutated structure, which affects the shape of the entire structure in a subtle—yet important—way.

5

Installing Cn3D

The Cn3D software allows you to view the three-dimensional structures of macromolecules like proteins and DNA. If Cn3D has not already been downloaded to your computer, follow the instructions below.

1. To download the Cn3D program onto your computer, visit the Cn3D homepage at: <http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml>.
2. Click **"Download."**
3. Select your Operating System (Windows, Macintosh).
4. To install Cn3D on a Mac, click the link to download the file. When the file has been downloaded, double-click the icon to install Cn3D. To install Cn3D on a computer with Windows, click the link to **"Download the Cn3D 4.3 installer here."**
5. Close all internet browsers and then double-click on the .msi file or select **"Run."** This will launch the Windows Installer application if you have it already. If so, enter the information at the prompts, and you are done. If not, or if Windows tells you that you need a newer version of the installer software, you can download the latest Windows Installer from Microsoft® by following the appropriate links listed on the download page for your operating system.
6. Once Cn3D is installed, you can click the **"View Structure in Cn3D"** link from the Structure summary pages to download and view structures from the NCBI.



Credit: NCBI Structure, Molecular Modeling Database (MMDB).

5 From Sequence to Structure

Introduction

This lesson extension uses music as an analogy for protein structure and function. *Part I* compares primary, secondary, tertiary, and quaternary protein structures to a musical composition. *Part II* relates the idea of conserved domains in proteins to the song “Happy Birthday.” All mp3 music files for this optional lesson extension can be found under the Resources tab on the Introductory curriculum webpage at: <http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing>.

PART I: Primary, Secondary, Tertiary, and Quaternary Protein Structure

A. Primary Structure

Music is made up of a sequence of notes, and proteins are made up of a sequence of amino acids. Knowing only the **order** of the amino acids in a protein tells us the **primary structure** for that protein. This does not, however, tell the whole story of the protein. In a musical composition, knowing the **order** of the notes is of course important but also does not tell the whole story. For example, the musical notes for the beginning of a piece of music are:

DABADABAAABAAABAABAGF

If we could search a large database containing music for individual notes (iTunes® is a large database containing music, but it cannot search for sequences of notes), we might be able to find out more about our piece of music. Are there many other songs that contain the same sequence? A musician might be able to make an educated guess that this piece is in the key of D. Does that musical key link it to other musical pieces? Of course, since this is a piece of music, we can actually listen to it.



And it would sound like this:
Play the “Primary Structure Song.”

It doesn’t sound like much, does it?

Now, let’s take a look at the beginning sequence of amino acids from the *BRCA1* reference sequence that we used for the BLAST alignment in *Lesson Four*. The beginning sequence of amino acids for the *BRCA1* gene is:

MDLSALRVEEVQNVINAMQKILECPICLE

Again, knowing the **order** of the amino acids is important but does not tell the whole story. Using the NCBI, we can search a database that might compare this sequence with other sequences, which could tell us how common the sequence is, or perhaps even whether it is linked to other proteins with similar sequences. But, again, this does not tell us very much about what the protein **does**.

B. Secondary Structure

The basic shape of a protein begins to emerge when we look at the **secondary structure** that is formed as the **alpha helices** and **beta sheets** take shape.

Let's return to the music analogy. The order of the notes gives us an indication of what the music should sound like, but we need more information to make the music take shape. Music is given shape by rhythm, dynamics (how loudly or softly sections are played), accents, the key in which it is written, and many other factors. If we add rhythm, some tone, and accents to the string of notes we heard earlier, it begins to take shape and becomes much more recognizable:



Play the "Secondary Structure Song."

How does the BRCA1 protein take shape? It has to do with the interactions between the different amino acids and their functional groups that constitute the protein—interactions such as hydrogen bonding, **non-polar** interactions, ionic bonding, and even **covalent bonding**. If you take a closer look at the string of amino acids from our primary structure—look at the chemical structure and composition of each amino acid—you will see that some of the amino acids have **polar (hydrophilic, or water loving)** side groups and some have non-polar (**hydrophobic, or water fearing**) side groups. The non-polar side groups shy away from the watery solution the molecule is in, and bend the protein so that they can be gathered together on the inside of the structure. Hydrogen bonds also form between the amino acids themselves, which twists the protein into an **alpha helix**. Sometimes the interactions between the amino acids cause the string to fold back and forth in a flattened zigzag, which is called a **beta sheet**.

Just as rhythm, tone, and accent give shape to music, these chemical interactions twist and fold our amino acid chain into a basic three-dimensional structure.

C. Tertiary Structure

Tertiary structure is completed when the attractions between alpha helices and beta sheets cause the protein to fold back on itself and make "bridges" between sections of the protein. This gives the protein its final, folded, and compacted shape.

Using the music analogy, we have seen the music take shape from a string of single notes to, perhaps, a recognizable tune. At the level of tertiary structure, additional connections and bridges are made within the tune itself. The original sequence showed (and you have only heard) the first 21 notes of a much longer composition. If the sequence were written out in its entirety, there may be places that bridge to and interact with the beginning sequence. It might sound like this:



Play the "Tertiary Structure Song."

Non-polar: A substance that repels water. A synonym for hydrophobic.

Covalent bonds: A type of chemical bond that is characterized by the sharing of a pair of electrons between atoms.

Polar: A substance that is attracted to water. A synonym for hydrophilic. The opposite of hydrophobic or non-polar.

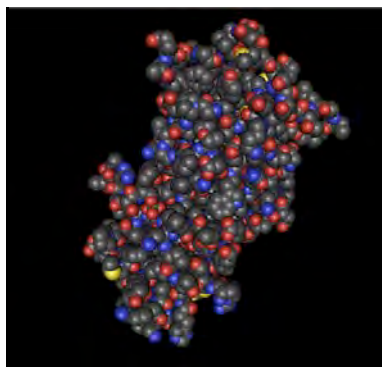
Hydrophilic: A substance that is attracted to water. From the Greek "hydro" which means water, and "philos" meaning love. A synonym for polar. The opposite of hydrophobic or non-polar.

Hydrophobic: A substance which repels water. From the Greek "hydro" which means water, and "phobos" which means fear. A synonym for non-polar. The opposite of hydrophilic or polar.

Alpha helix (Plural: "alpha helices"): A common structure of proteins, characterized by a single, spiral chain of amino acids stabilized by hydrogen bonds.

Beta sheet: A structure that occurs in many proteins and consists of two or more parallel adjacent polypeptide chains arranged so that hydrogen bonds can form between the chains.

The BRCA1 protein, likewise, has its basic shape but requires additional infrastructure to be fully functional. For many proteins, their structures are dictated by additional folding that occurs when important covalent bonds form between the sulfur-containing amino acid cysteine. The final structure looks something like this:



Credit: Varma et al. 2005.

This final shape, or **structure**, allows the BRCA1 protein to perform its function. It is bent, folded, and twisted to make it just the right size and shape so that it fits along the DNA double helix and does its job of repairing broken strands of DNA.

D. Quaternary Structure

Some proteins have an additional level of structure, the **quaternary structure**. If the fully functional end-protein is composed of more than one protein chain, the assembly of this larger unit is the fourth level of structure.

In the music analogy, we need to look at the end product. The composition was not written for a single violin, but for orchestra and choir. The composer built additional musical “bridges” between the different instruments and choir to create one cohesive piece of music. When it is all put together, it sounds like this:



Play the “Quaternary Structure Song.”

Something to think about: There are many levels of organization in making a “functional” musical composition and in folding functional proteins. This humble protein may not look like the equivalent of the “Hallelujah Chorus,” but BRCA1 is found in many species and performs a crucial function in DNA repair. In fact, George Frideric Handel would not have existed to write the “Hallelujah Chorus” without functional BRCA1 proteins.

Sources:

3-D Molecular Designs

<http://www.3dmoleculardesigns.com/news2.php>

Survey and Summary: Structural Classification of Zinc Fingers

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC140525/?tool=pubmed>

Wikipedia: Zinc Finger

http://en.wikipedia.org/wiki/Zinc_finger

PART II: Conserved Domains

Domain: Specific area of a protein that performs a particular function.

Nature has versions of its own “greatest hits” collection. These are the sequences of amino acids that code for proteins or regions of proteins that have crucial functions for many organisms. If many organisms require the same function (i.e., DNA replication and repair, energy metabolism, cell division) they will need similar proteins, made from similar sequences of amino acids. These related sequences are said to be **conserved** sequences, or conserved **domains**.

Note that these conserved sequences are **similar**, not **identical**. They need to be similar enough that the resulting protein can do the required job for that organism, yet organisms have a certain amount of genetic diversity. How can living things make proteins to do a specific job, yet allow for genetic diversity?

Back to the music analogy: You need a song with a specific function. Let’s say your friend is having a birthday and you would like to celebrate it with a song. We have, of course, a song just for this function.



Play the “Happy Birthday” song.

This is perhaps the most well-known song in the world—if it were a protein, it would be **highly conserved**. Would your friend, however, recognize this version?



Play the “Happy Birthday Improvisation” song.

Perhaps we do not need the entire original song to perform our function of wishing your friend a happy birthday—maybe the first six notes are crucial to the song, and then some variation can occur. Perhaps, throughout our improvisations, we need to have a few notes along the way that anchor us back to the original melody. One can also imagine that too much improvisation could make the melody totally unrecognizable and your friend would not know that you are trying to wish him or her a happy birthday. A song like that would not fulfill its function, and would probably not become known by too many people.

In a similar way, amino acid sequences that code for a functional protein can vary from one organism to another, as long as the conserved portion of the gene continues to code for the crucial elements that make up that protein.

6

Evaluating Genetic Tests: A Socratic Seminar Discussion

Introduction

In this lesson, students apply the ethical skills and scientific knowledge they have acquired over the previous lessons to: 1) determine whether BRCA1 testing meets the standards of a useful genetic test, or 2) participate in a discussion of the potential risks and benefits of direct-to-consumer (DTC) genetic testing. Students or teachers may choose from one of two readings, after which students participate in a Socratic Seminar to deepen their understanding of genetic testing. Through the seminar discussion of the first reading, students become familiar with a framework for considering genetic tests in terms of their clinical validity and the availability of effective treatment. Through the seminar discussion of the second reading, students learn about the concerns some groups have about the risks of DTC genetic testing, the response of some people in the DTC industry, and preliminary data on what consumers think of DTC genetic testing. After the seminar, students are supported in developing an individual position about genetic testing through the integration of scientific facts, stakeholder viewpoints, and ethical considerations. In Lesson Six, students learn how bioethicists might use bioinformatics tools in their career.

Learning Objectives

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

- Genetic tests differ in their clinical validity and usefulness.
- There are some conditions for which there are genetic tests but no effective treatment.
- Medical conditions differ in their penetrance and the number of genes involved.

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

- Identify ethical issues related to genetic tests.
- Compare and contrast genetic tests with respect to their clinical validity and the efficacy of treatments.
- Discuss the ethical issues related to providing different types of tests to patients.

Key Concepts

- Genetic tests can be characterized by their clinical validity and by the availability of effective treatments.
- Considerations related to the ethical, legal, and social implications of a genetic test are influenced by the characteristics of the test and the nature of the disorder.
- Bioinformatics tools are used by people in many careers, including bioethicists.

Class Time

One class period (50 minutes). If the article and supporting handouts are assigned as homework, class time could be decreased by 15-20 minutes, or more time would be available for discussion.

Prior Knowledge Needed

- A basic understanding of Mendelian genetics.
- An understanding of how mutations in genes can result in disease.
- How to have a classroom discussion in a way that is respectful of others.
- Helpful, but not required: background in ethical theories and perspectives.

Common Misconceptions

- Genetic tests are all basically the same.
- Genetic tests always provide useful information.

Materials

Materials	Quantity
Copies of Student Handout— <i>Careers in the Spotlight</i> (handed out in Lesson One)	1 per student
Student Handout— <i>Categorizing Genetic Tests</i>	1 per student -OR- 1 each for half of the students
Student Handout— <i>Weighing the Risks and Benefits of Direct-to-Consumer Genetic Testing: Who Should Decide?</i>	1 per student -OR- 1 each for half of the students
Student Handout— <i>Critical Reasoning Analysis Form</i> (optional, see Procedure)	1 per student
Student Handout— <i>Open-Ended Questions for a Socratic Seminar</i> (optional, see Procedure)	1 per student
Student Handout— <i>Socratic Seminar Discussion Partner Evaluation</i> (optional, see Procedure)	1 per student
Teacher Resource— <i>Socratic Seminar Rubric</i>	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 Six PowerPoint Slides— <i>Evaluating Genetic Tests: A Socratic Seminar Discussion</i> . 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 .

[**Note:** It is recommended that students read one of the Socratic Seminar readings (Student Handout—*Categorizing Genetic Tests* or Student Handout—*Weighing the Benefits of Direct-to-Consumer Genetic Testing*) as homework in advance of class.]

Teacher Preparation

- Load the classroom computer with the Lesson Six PowerPoint slides.
- Make copies of the Student Handout, one per student.
- Divide the number of classroom chairs in half and set up two circles – an inner circle and an outer circle.

Procedure

WARM UP

1. As students enter the classroom, show **Slide #1**, which highlights bioethicist Kelly Edwards, PhD

Evaluating Genetic Tests: **Slide #1**



BIOETHICIST
KELLY EDWARDS, PhD

Place of Employment:
University of Washington

Specialties and Interests:
Community-based research practices, environmental justice, and integrating ethics into training programs and public policy.

"Genetics researchers often work with distinct communities. To take moral account of how their research affects these communities, they need a richer conception of justice and they need to make those communities equal participants in decision-making about how the research is conducted and what is produced and published out of it."

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 bioethicist might do (Bioethicist Question #1). This will be revisited at the end of the lesson, including how a bioethicist might use bioinformatics in her or her job.
4. Tell students to keep their *Careers in the Spotlight* handout available for a future lesson.

PART I: Background on Reading Content

5. Explain to students the **aim of this lesson**. Some teachers may find it useful to write the aim on the board.

Lesson Aim:

- To understand the factors involved in evaluating genetic tests.

Teachers may also wish to discuss the *Learning Objectives* of the lesson, which are listed at the beginning of this lesson plan.

6. Introduce the lesson activities by saying ***“What if a rare genetic disorder ran in your family and a company was offering a genetic test for it? What would you want to know about the test before taking it?”***

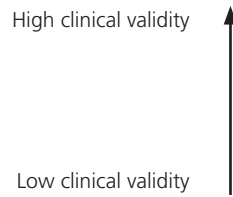
Students may cite expense, what kind of sample they would have to give, and privacy concerns. Direct student responses towards the concerns of **clinical validity** and **effective treatment**.

7. Help frame this conversation for students by first defining both terms:
 - **Clinical Validity:** How accurately a test predicts whether a person will get a particular disease or symptom (known as the “clinical outcome”).
 - **Effective Treatment Availability:** Whether there are treatment options available for a particular disease or condition. This is sometimes thought of as a “cure” although most genetic conditions are “treated” rather than “cured.”
8. Discuss with students the terms **penetrance** and **polygenic traits**.
 - **Penetrance:** The probability that individuals with a specific genotype will express a specific phenotype. For example, as students saw in Lesson Three, approximately 85% of women with particular BRCA1 mutations will develop breast or ovarian cancer.
 - **Polygenic:** A polygenic trait is one that involves multiple genes. For example, cat coat color is known to involve multiple genes, including ones for white, orange, and black coloring. Many common diseases, like heart disease and diabetes, are thought to be polygenic.

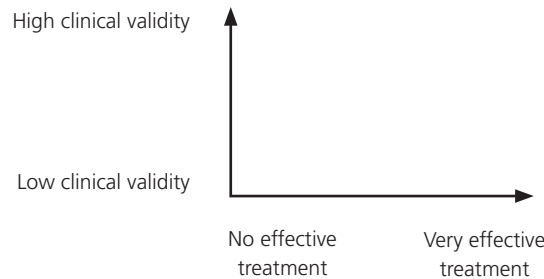
Penetrance and polygenic traits are important components of clinical validity. There may be genetic tests available for a particular condition, but if the trait has low penetrance, the clinical validity of tests for particular mutations may be low. If the condition is polygenic, studying mutations in only one gene will not provide doctors, genetic counselors, and bioethicists with a complete assessment of patient risk.

9. In both of the Socratic Seminar readings, the authors discuss the **clinical validity** of genetic tests. In one of the readings, the authors also evaluate genetic tests based on whether there is **effective treatment available**.

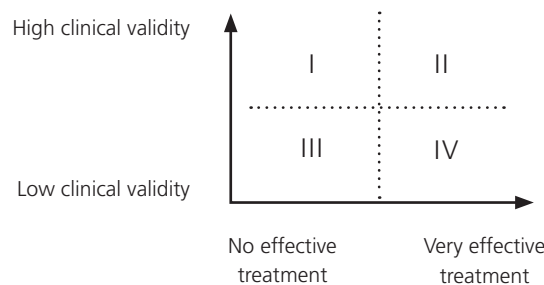
10. Draw a **y-axis** on the board, as shown below:



11. Ask students, **“What does it mean for a genetic test to have high clinical validity?”** [If a person who carries the genetic variant for a disease or condition has a 100% chance of developing that disease or condition (100% penetrance), then the genetic test for that condition has high clinical validity.]
12. Ask students, **“What does it mean for a genetic test to have low clinical validity?”** [A test with low clinical validity means that, even though a person may carry the genetic variant associated with a disease or condition, they may have a low chance of developing that disease or condition. Perhaps the environment plays an equal or greater part in developing the disease or condition than does genetics, the variant has very low penetrance, or the condition is polygenic.]
13. Add an **x-axis** to the graph, as shown below:



14. Ask students, **“What does it mean if there is no effective treatment for a disease or condition?”** [There is no “cure” or treatment. Nothing can be done to treat the disease or condition itself, although patients can still receive care to help alleviate the symptoms.]
15. Ask students, **“What does it mean if there is effective treatment for a disease or condition?”** [There are treatments, medications, or therapies that help treat the disease. Remember, most genetic conditions are treated rather than “cured.”]
16. This graph framework provides a way to think about genetic tests in different quadrants of the graph. A test in quadrant II would be ideal.



17. Tell students that this framework can help bioethicists evaluate whether a particular genetic test should be recommended for approval for use in patients. It can also help genetic counselors and patients make decisions about the validity of genetic tests. In addition, different ethical issues arise in each quadrant. These are explored in the reading on Student Handout—*Categorizing Genetic Tests* and can be discussed further in the seminar.
18. It is possible that genetic testing may have negative effects, such as anxiety, or positive effects, such as increased exercise or decreased fat intake. These issues are explored in the reading on Student Handout—*Weighing the Risks and Benefits of Direct-to-Consumer Genetic Testing* and can be discussed further in the seminar.

PART II: Before the Socratic Seminar

19. Introduce the seminar and its purpose: to facilitate a deeper understanding of the ideas and values in the text through shared discussion.
20. Have students read the article from Student Handout—*Categorizing Genetic Tests* or Student Handout—*Weighing the Risks and Benefits of Direct-to-Consumer Genetic Testing*. It is important that every student read the text, since the quality of the discussion depends on contributions from each participant. It may be helpful to allow time in class for students to read the article(s).
21. Students may use one of several formats to process the information. Student Handout—*Critical Reasoning Analysis Form* and/or Student Handout—*Open-Ended Questions for a Socratic Seminar* can be used to help students understand the content. If students have been given the reading as homework, the completed handouts can be used as the “entry ticket” to participate in the seminar.

Suggested scoring for Student Handout—*Critical Reasoning Analysis Form*: +2 points per question for a total of 16 possible points.

Suggested scoring for Student Handout—*Open-Ended Questions for a Socratic Seminar*: +2 points per question for a total of 10 possible points.

22. In addition to the classroom discussion norms you may have already set, it is important to include the following norms:
 - Do not raise hands.
 - Talk to (and look at) each other, not the teacher.
 - Listen carefully.
 - Address one another respectfully.
 - Base any opinions on the text.
 - Monitor your airtime so that everyone can share.
23. Encourage students to pose questions as well as build on the comments of others.

PART III: During the Socratic Seminar Discussion

24. To create the discussion groups, divide the class in half and form two circles (an inner circle and an outer circle). The inner circle will be engaged in the discussion, and the students in the outer circle will be listening to the inner circle discussion. If the class contains fewer than 20 students, a single discussion group may also be possible.
25. Students in the outer circle will take notes and write down ideas or comments on what they hear in the inner circle discussion. After approximately ten minutes (or another appropriate time period), flip the circles so that students in the inner circle and outer circle trade places. Teachers can use Student Handout—*Socratic Seminar Discussion Partner Evaluation* to help focus students during the discussion, if needed (see *Assessment*). Different questions can be asked in each half of the seminar.
26. To begin the discussion, the teacher/facilitator may pose the starting question(s) or the participants may agree upon questions to begin the discussion. If students completed Student Handout—*Open-Ended Questions for a Socratic Seminar*, many of the questions generated could be used as guiding questions for the discussion. Some teachers may want to start with a few factual questions such as ***“What is penetrance?”*** and ***“What is clinical validity?”***

Recommended starting questions:

Interpretive/supported by both articles:

(Students need to refer to specific parts of the article in their answers.)

- Why was this article written? What was the need for the article?
- Who is the audience for this article?
- What are the authors’ concerns regarding genetic tests?
- What do the authors recommend regarding genetic tests?
- What do the authors consider to be the most important ethical considerations related to testing?
- What are some assumptions the authors make?
- Which is the most important paragraph and why?

Interpretive/supported by *Categorizing Genetic Tests*:

- Do the authors believe that the framework applies equally well to all genetic tests?
- What do the authors believe to be the most challenging aspect of evaluating genetic tests?
- Does this framework relate to direct-to-consumer genetic tests? If so, how?
- What are the implications of this framework for health care?

Interpretive/supported by *Direct-to-Consumer Genetic Testing*:

- What do the authors consider to be the most important risks of direct-to-consumer genetic testing?

[**Caution:** Use evaluative questions once the discussion of interpretive questions is finished. These questions tend to lead the discussion off into student opinions, so the conversation must be managed more carefully. If this is your first seminar, you may choose not to use these kinds of questions at all.]

- What do the authors consider to be the most important benefits of direct-to-consumer genetic testing?
- What are some of the limitations of the studies cited in the article, and what effect could they have on the conclusions reached?
- What are the implications of this article for health care?

Sample questions to move the discussion along:

- Where do you find evidence for that in the text?
 - Who has not yet had a chance to speak?
 - Is there something in the text that is unclear to you?
 - Who can summarize the main ideas we have discussed so far?
 - Who has a different point of view?
27. You can choose to facilitate the discussion by asking clarifying questions, summarizing comments, and highlighting understandings and misunderstandings. You can restate the opening question if the conversation gets off track or ask for different ideas if it stalls.
28. Later in the discussion, “evaluative” questions that refer to students’ experiences and their own judgments can also be used. For example, ***“Is it right that....?”*** ***“Do you agree with the author?”*** or ***“Has anyone changed his or her mind?”*** These do not require reference to the text for an answer.

Evaluative/personal position questions:

- Do you agree with the authors’ conclusions? Why or why not?
- Who should decide how much information should be provided to patients?
- What do you think has been left out of the article?
- Do you think that clinical validity or the possibility of treatment is more important in determining whether to use a test?

PART IV: After the Seminar

29. Ask the whole group questions such as: ***“Do you think that you understand the article at a deeper level?”***, ***“How did we do in meeting our goals and norms?”*** or ***“What was one thing you noticed about the seminar?”***
30. Share the experience you had facilitating the seminar.

PART V: Reflection

31. After the seminar, ask students to write one or two paragraphs about the “big ideas” from the Socratic Seminar discussion, regarding either the paper they were assigned to read or both articles. In their reflection, students should discuss whether they believe that direct-to-consumer genetic testing companies should be required to offer genetic counseling to their patients. In addition, ask them to write one paragraph about how the ideas from the Seminar apply to *BRCA1* testing, and how those ideas relate to their initial views about *BRCA1* testing. This may also be completed as homework.

Closure: Careers in the Spotlight

32. Return to the picture of the bioethicist from the Careers in the Spotlight in **Slide #1**.

Evaluating Genetic Tests: **Slide #1**



BIOETHICIST
KELLY EDWARDS, PhD


Place of Employment:
University of Washington

Specialties and Interests:
Community-based research practices, environmental justice, and integrating ethics into training programs and public policy.

"Genetics researchers often work with distinct communities. To take moral account of how their research affects these communities, they need a richer conception of justice and they need to make those communities equal participants in decision-making about how the research is conducted and what is produced and published out of it."

33. Show **Slide #2**, which provides job information for a bioethicist. Review this information with students.

Evaluating Genetic Tests: **Slide #2**



CAREERS IN SPOTLIGHT: BIOETHICIST

What do they do?
Bioethicists study the ethical controversies that arise from advances in biology and medicine. They advise patients, researchers, public policy makers, and/or medical doctors about a wide range of ethical issues, from genetic testing and medical treatment to medical privacy and stem cell research.

What kind of training is involved?
Training varies widely from a certificate program completed during or after a Bachelor's degree in another subject to Doctoral (PhD) programs specifically in Bioethics.

What is a typical salary for a Bioethicist?
Depending upon training, anywhere from \$50,000/year (\$24/hour) to over \$100,000/year (\$48/hour).

34. Ask students, **"How does a bioethicist fit into today's lesson?"**

Point out that:

- The initial decision to approve genetic tests for use in patients is made by committees of medical doctors, genetic counselors, and bioethicists who weigh issues of clinical validity, availability of effective treatment, and the effect of the test results on the well-being of patients.
- The decision about whether an individual patient should have a particular genetic test is often made under the guidance of someone with bioethics training, like a medical doctor or a genetic counselor.
- Bioethicists often consult public policy makers about genetic testing issues and regulations, including whether to regulate direct-to-consumer genetic testing companies, and whether these companies should be required to offer genetic counseling to their clients.

35. Ask students to answer Bioethicist Question #2 on their *Careers in the Spotlight* handout, which has students explain how this lesson has changed their understanding about the kind of work a bioethicist does.
36. Ask students to also answer Bioethicist Question #3 on their *Careers in the Spotlight* handout, which has students explain how a bioethicist might use bioinformatics in his or her work.
37. Tell students to keep their *Careers in the Spotlight* handout available for future lessons.

Homework

See *Part V: Reflection*, above.

Adaptations

Some teachers ask students who are observing the discussion to take note of who said what, and then to incorporate these ideas into their reflection. Student Handout—*Critical Reasoning Analysis Form* can also be used as homework after the seminar instead of a writing assignment in advance. Teacher Resource—*Socratic Seminar Rubric* could also be adapted for student self-assessment, peer-assessment, or for teachers' own assessment of student participation. ***Suggested scoring for Student Handout—Socratic Seminar Rubric: +10 points per Dimension for “Exemplary” performance, +8 for each “Proficient” performance, +6 for each “Partially Proficient,” and +3 for each “Developing,” for a total of 40 possible points.***

Assessment Suggestions

The students' *Critical Reasoning Analysis Form* can be used as a formative assessment to prepare for the Socratic Seminar. ***Suggested scoring for Student Handout—Critical Reasoning Analysis Form: +2 points per question for a total of 16 possible points.***

To help engage students in the Socratic Seminar discussion, you can have them evaluate another student's participation. This can be done by pairing each student in the inner circle with a student in the outer circle and using Student Handout—*Socratic Seminar Discussion Partner Evaluation* to help students evaluate each other. Student Handout—*Socratic Seminar Discussion Partner Evaluation* can also be modified so that students reflect on their own participation.

Teacher Background: Socratic Seminar

In a Socratic Seminar, the participants bear responsibility for the quality of the discussion. Good discussions occur when participants study the text closely in advance, listen actively, share their ideas and questions in response to the ideas and questions of others, and search for evidence in the text to support their ideas. The discussion is not about right answers; it is not a debate. Students are encouraged to think out loud and to exchange ideas openly while examining ideas in a rigorous, thoughtful manner.

In a Socratic Seminar, there are several basic elements:

- A text containing important and powerful ideas that is shared by all participants. It is helpful to number the paragraphs in a text so that participants can easily refer to passages, as seen in the readings for this lesson.
- A distinctive classroom environment: seating students in a circle and using name cards helps facilitate discussion. The students should have a clear understanding of the discussion norms, which should be prominently posted.
- An opening question that requires interpretation of the text and is genuine (with no predetermined answer). For example, ***“What is the most important passage?”*** or ***“What is the author driving at in the text?”*** Recommended questions can be found in the Procedure section.

Glossary

Alzheimer’s disease: A brain disease that causes problems with memory (such as difficulty remembering people and events), thinking, and behavior. It is most common in people over 65, but up to five percent of people with the disease have early-onset Alzheimer’s (also known as younger-onset), which can appear in the patient’s 40s or 50s. Alzheimer’s worsens over time, and there is no cure, though some treatment options are available that appear to reduce the speed with which the condition worsens.

Carrier test: A genetic test to determine whether an individual carries a particular gene but may not show any conditions themselves. For example, a carrier test may be used to determine whether two parents both carry a copy of a recessive trait, which may be passed on to their child(ren).

Clinical validity: How accurately a test predicts whether or not a person will get a particular disease or symptom (known as the “clinical outcome”). This is often related to the penetrance of the gene involved, and whether or not the condition is polygenic.

Diagnostic test: A genetic test used to identify (or rule out) a particular genetic condition. For example, diagnostic genetic testing is used to determine whether a baby has Phenylketonuria (PKU).

Effective treatment availability: Whether or not there are treatment options available for a particular disease or condition. This is sometimes thought of as a “cure” although most genetic conditions are “treated” rather than “cured.”

Hemochromatosis: An autosomal recessive genetic disease that results in the body absorbing too much iron from food. This extra iron is stored in the body, including in organs like the liver and pancreas. The extra iron results in pain, organ damage, cancer, heart problems, and in some cases, death. Symptoms usually begin around age 30 to 40, but may begin in childhood. There is no cure, but the condition can be controlled with a specific diet, removal of blood (to remove the extra iron), and medication.

Huntington’s disease (HD): An autosomal dominant genetic disease causing nerve cells to waste away gradually, resulting in uncontrolled movements, severe problems with balance, clumsiness, emotional distress, problems swallowing, and loss of mental function. The condition usually begins when a person is in their 40s, and gets worse with age. The condition is ultimately fatal, and there is no cure.

In vitro fertilization: A process in which egg cells are fertilized by sperm outside the body. This is often used to help couples who have difficulty getting pregnant.

Penetrance: The frequency that individuals with a specific genotype will express a specific phenotype. For example, as seen in *Lesson Three*, approximately 85% of women with particular *BRCA1* mutations will develop breast or ovarian cancer. *BRCA1* is said to have “high penetrance” because an individual with a cancer-causing mutation in *BRCA1* has a large chance of developing breast cancer.

Phenylketonuria (PKU): A rare autosomal recessive genetic disease in which the body does not make an enzyme necessary to convert the amino acid phenylalanine to the amino acid tyrosine, causing phenylalanine to build up in the body to unsafe levels. This can cause mental retardation, brain damage, and seizures during infancy and early childhood. While a modified diet, including special protein supplements, can reduce the severity of PKU’s symptoms, new research suggests that diet alone is not enough to prevent symptoms.

Pre-implantation Genetic Diagnosis (PGD): Performing a genetic test on an embryo created by *in vitro* fertilization before placing it in the mother.

Prenatal: Before birth. For example, some genetic tests are performed on a developing fetus while s/he is still in the mother’s womb.

Polygenic: A polygenic trait is one that involves multiple genes. For example, cat coat color is known to involve multiple genes, including ones for white, orange, and black coloring. Many common diseases, like heart disease and diabetes, are thought to be polygenic.

Resources

The Genetic Information Nondiscrimination Act of 2008 (“GINA”) is a federal law that prohibits discrimination in health coverage and employment based on genetic information. A handout prepared by the Department of Health and Human Services (HHS) with information about GINA for researchers and health care professionals is provided in the *Appendix*. This handout is designed to provide a brief overview of what legal protections are now in place regarding genetic testing, genetic privacy, and genetic discrimination.

Credit

The Socratic Seminar procedures are based on materials shared by Walter Parker, PhD, University of Washington; Paula Fraser, Bellevue PRISM program, Bellevue, WA; Jodie Spitze and Dianne Thompson, Kent Meridian High School, Kent, WA. We also gratefully acknowledge the influence of the Coalition of Essential Schools and the National Paideia Center.

Categorizing Genetic Tests was adapted with permission from: Burke, W., Pinsky, L., and Press, N. (2001). Categorizing genetic tests to identify their ethical, legal, and social implications. *American Journal of Medical Genetics*, 106:233-240.

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Name _____ Date _____ Period _____

6

Categorizing Genetic Tests

Based on Burke, W., Pinsky, L., and Press, N. (2001). "Categorizing genetic tests to identify their ethical, legal, and social implications." *American Journal of Medical Genetics*, 106:233-240. Adapted with permission.

- 1 What if you could go to the doctor and present a card that contained all your genetic information? Your doctor could scan the card and see whether you were more likely to have an allergic reaction to a particular drug, or whether you were at increased risk for a disease or disorder. Every year, scientists come closer to making this scenario a reality. More than 1,500 genetic tests are currently available¹, and the number of tests available has more than doubled in the past eight years. Genetic testing companies are making it increasingly possible for consumers to have portions of their DNA sequenced, and to receive some information about what the results might mean. The growth of these "direct-to-consumer" testing companies has resulted in the ability of individuals to learn unprecedented amounts of information about their own genes.
- 2 There are a variety of genetic tests available. For example, some tests are performed on early embryos formed by **in vitro fertilization** (in which egg cells are fertilized by sperm outside the body) to determine whether they are disease-free and should be implanted in a woman (**Pre-implantation Genetic Diagnosis, or PGD**). **Prenatal** genetic tests test cells from a developing fetus before birth. Newborn screening tests are provided to children immediately upon birth. **Diagnostic tests** are used to identify (or rule out) a particular genetic condition and **carrier tests** are used to identify whether an individual who may not show any condition him/herself carries a particular gene.
- 3 Genetic tests have tremendous value in helping doctors diagnose and treat diseases. They can also predict who may develop a disease in the future, helping patients take a proactive role in their health care. Knowing an individual's genetic makeup may also help doctors identify the best treatments, as some drugs might be more effective for patients with particular genetic variations. They can also provide information to individuals that may affect their choices about having children in the future.
- 4 However, genetic tests also raise some challenging issues. For example, patients might encounter discrimination in various forms, or might experience stress as a result of knowing the outcome of a test. While the federal Genetic Information Non-Discrimination Act (GINA) of 2008 sets a baseline for patient protection with regard to insurance and employment, there are areas it does not cover (for example, companies with fewer than fifteen employees are exempt). GINA also does not prohibit health insurers from obtaining and using genetic test results to determine who should receive health insurance payments. Another concern is that the results of genetic tests impact not only the individual taking the test but entire families, who often share much of the same genetic information. Even a test that shows that a person does not have a genetic disorder might cause stress in the form of "survivor guilt" if other family members are affected by the disorder.
- 5 Genetic tests can be characterized according to their **clinical validity** and the availability of effective treatments. The term "clinical validity" means how accurately a test predicts a certain clinical outcome (such as getting a particular disease or symptom). Different types of tests raise different ethical issues and require different types of genetic counseling.

High Clinical Validity – Lack of Effective Treatment
- 6 Traditionally, genetic counselors have been guided by the view that recommendations should be "non-directive;" in other words, people should be provided with information and then allowed to make their own choices. This view acknowledges that many decisions regarding health care are currently driven

by personal preference. For example, the decision of whether to terminate a pregnancy because of a genetic disorder is viewed as a private matter. Non-directive approaches also apply to some genetic testing situations that do not involve reproductive choices. For example, an individual whose parent had **Huntington's disease (HD)** might want to find out if he or she carries the mutation that results in the disease. Because HD has a high penetrance (in other words, an individual with the mutation has a large chance of developing the disease), an individual who tests positive receives information that might be helpful in planning for his or her life. However, there are currently no effective treatments to delay or prevent the disease, so an individual who tests positive for the HD mutation cannot use this information to make decisions about medical treatments that might help them.

- 7 In addition, there is the possibility that a person with a positive test may face discrimination or harmful psychological effects (including the stress of knowing that they have the mutation). A counselor might explain the different concerns and issues related to taking the genetic test, but the decision to test is ultimately left to the patient. Ethical issues often focus on making sure that people consider the kind of information the test will provide and the lack of treatment options.

High Clinical Validity – Effective Treatment

- 8 Newborn screening tests, by contrast, are required by all states. Newborns are screened for a variety of disorders. In some states, parents may choose not to have their children screened (for example, for religious reasons). A classic example of a newborn screening test is the test for a disease called **Phenylketonuria (PKU)**. If a child who has the PKU mutation is diagnosed early in life, a modified diet can be given and mental retardation prevented. There is broad agreement that testing for PKU is extremely beneficial because a highly successful treatment—a modified diet—exists. Ethical concerns related to such genetic tests often focus on making sure that eligible people have access to the tests and treatment.
- 9 These examples show that the availability of an effective treatment makes a big difference in thinking about the implications of a genetic test, whether the use of that test is justified, and how health care providers should counsel families. In fact, health care providers have a duty (supported by court cases) to clearly tell patients if there are tests available in cases where successful treatments exist and non-treatment can lead to serious harm. If there are no effective treatments, non-directive counseling provides an appropriate framework for talking to patients about the possibility of testing.

Low Clinical Validity – Lack of Effective Treatment

- 10 Clinical validity is affected not only by the penetrance of the mutation, but also by how good a test is at predicting whether someone will eventually get the disease. In other words, if a patient receives a positive test, how high is the likelihood that they will eventually become ill with that disease? In some cases, such as testing for the ApoE4 genotype (which may result in an increased risk for **Alzheimer's disease**), a positive result may show an increased risk, but the actual lifetime risk for the disease is uncertain. People with two ApoE4 alleles are ten times more likely to have Alzheimer's disease than those with other versions of the gene, but because Alzheimer's can occur late in life, someone might have two ApoE4 alleles and die of something else before Alzheimer's sets in. No treatment is available to reduce the risk.
- 11 As with testing for HD, the main risks are related to psychological effects on those who are tested, as well as discrimination. However, the HD test provides a highly accurate prediction about future risk. The risk associated with the ApoE4 test is less certain. Many experts recommend not testing for ApoE4, based on the ethical obligations for health care providers to avoid harm. Many genes that contribute to human disease have been identified. However, since the corresponding genetic tests may not be clinically valid, the real impacts of a positive test may be difficult to interpret, and few treatments may be available. Many direct-to-consumer tests fall into this category; they provide information related to disease risk that is difficult to evaluate due to uncertainty about the validity of the test as well as a lack of effective treatment.

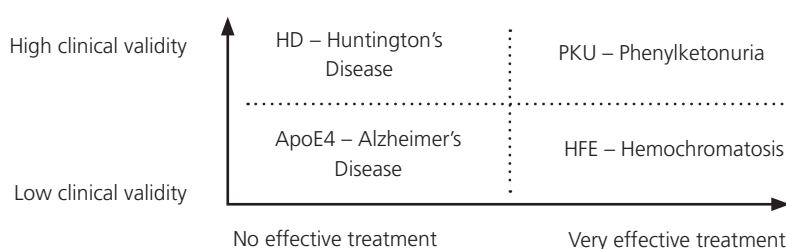
Low Clinical Validity – Effective Treatment

- 12** So far, the examples presented have been ones that either predict diseases well (HD and PKU), but differ as to the treatments available, or that do not predict the disease well and also do not have an effective treatment (Alzheimer's – ApoE4). A fourth case is when there is an effective treatment, but the test is not clinically valid. For example, mutations in the HFE gene can lead to susceptibility to a disease called **hemochromatosis**. This disease causes iron overload and has potentially life-threatening consequences. However, only a small proportion of individuals with mutations in both copies of their HFE gene actually show symptoms of disease (low penetrance); therefore, the clinical validity of the test is low. Periodic blood draws, however, can help prevent dangerous complications such as liver cancer. So, in the case of HFE, the clinical validity of the test is low, but the treatment is minimal and beneficial. The benefit to the patient in terms of health outcomes may outweigh the potential psychological effects of testing or the potential social stigma of being labeled a carrier of a genetic disease. Ethical discussions about these types of tests, therefore, tend to be framed in terms of balancing potential harms and benefits. Tests that do not predict outcomes very well might be acceptable when the "label" associated with the disease has little social stigma (for example, hypertension).
- 13** *BRCA1* and *BRCA2* are interesting to analyze using this framework. There is uncertainty about how penetrant the *BRCA1* and *BRCA2* mutations are. The lifetime risk of breast cancer associated with *BRCA1* or *BRCA2* mutations ranges from 36%-85%, with a wide variation in how the cancer manifests itself (as breast cancer, ovarian cancer, or both). The penetrance is probably determined by the exact type of mutation (many *BRCA1* and *BRCA2* mutations are known) as well as environmental and other genetic factors. In a "high risk" family (four or more relatives affected by breast/ovarian cancer before age 60), females with mutations in *BRCA1* or *BRCA2* are estimated to have a lifetime risk of 85% for breast cancer. The effectiveness of the different treatments offered to carriers of *BRCA1* or *BRCA2* mutations is also subject to debate. The options include early mammograms, ovarian cancer screening, and surgery before any cancer appears. Most women with *BRCA1* or *BRCA2* mutations do not opt for such preventative surgery, especially if they do not have other risk factors in their history. However, those in a "high risk" family might consider the test to be highly predictive and the treatment effective.
- 14** A genetic test, therefore, should be evaluated based on both the test's clinical validity and the treatments available for those individuals with positive results. It may take many years for researchers to gauge how accurate a test is at predicting a disease outcome. The development of treatments and tests of their effectiveness in patients also requires time. This framework can help guide researchers in making decisions about which kinds of information to seek about tests, and can help patients think about the characteristics of the tests they are considering. It also explains why some tests have become widely accepted while others have not. As more genetic tests with limited clinical validity and predictive value become available, and more direct-to-consumer tests are marketed to the general public, it will be increasingly important to consider carefully how those tests are used.

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Genetic Test Categories



Genetic Conditions Glossary

Alzheimer's disease: A brain disease that causes problems with memory (such as difficulty remembering people and events), thinking, and behavior. It is most common in people over 65, but up to five percent of people with the disease have early-onset Alzheimer's (also known as younger-onset), which can appear in the patient's 40s or 50s. Alzheimer's worsens over time, and there is no cure, though some treatment options are available that appear to reduce the speed with which the condition worsens.

Carrier test: A genetic test to determine whether an individual carries a particular gene but may not show any conditions themselves. For example, a carrier test may be used to determine whether two parents both carry a copy of a recessive trait, which may be passed on to their child(ren).

Clinical validity: How accurately a test predicts whether a person will get a particular disease or symptom (known as the "clinical outcome"). This is often related to the penetrance of the gene involved, and whether or not the condition is polygenic.

Diagnostic test: A genetic test used to identify (or rule out) a particular genetic condition. For example, diagnostic genetic testing is used to determine whether a baby has Phenylketonuria (PKU).

Effective treatment availability: Whether or not there are treatment options available for a particular disease or condition. This is sometimes thought of as a "cure" although most genetic conditions are "treated" rather than "cured."

Hemochromatosis: An autosomal recessive genetic disease that results in the body absorbing too much iron from food. This extra iron is stored in the body, including in organs like the liver and pancreas. The extra iron results in pain, organ damage, cancer, heart problems, and in some cases, death. Symptoms usually begin around age 30 to 40, but may begin in childhood. There is no cure, but the condition can be controlled with a specific diet, removal of blood (to remove the extra iron), and medication.

Huntington's disease (HD): An autosomal dominant genetic disease causing nerve cells to waste away gradually, resulting in uncontrolled movements, severe problems with balance, clumsiness, emotional distress, problems swallowing, and loss of mental function. The condition usually begins when a person is in their 40s, and gets worse with age. The condition is ultimately fatal, and there is no cure.

In vitro fertilization: A process in which egg cells are fertilized by sperm outside the body. This is often used to help couples who have difficulty getting pregnant.

Penetrance: The frequency that individuals with a specific genotype will express a specific phenotype. For example, as seen in *Lesson Three*, approximately 85% of women with particular *BRCA1* mutations will develop breast or ovarian cancer. *BRCA1* is said to have "high penetrance" because an individual with a cancer-causing mutation in *BRCA1* has a large chance of developing breast cancer.

Phenylketonuria (PKU): A rare autosomal recessive genetic disease in which the body does not make an enzyme necessary to convert the amino acid phenylalanine to the amino acid tyrosine, causing phenylalanine to build up in the body to unsafe levels. This can cause mental retardation, brain damage, and seizures during infancy and early childhood. While a modified diet, including special protein supplements, can reduce the severity of PKU's symptoms, new research suggests that diet alone is not enough to prevent symptoms.

Pre-implantation Genetic Diagnosis (PGD): Performing a genetic test on an embryo created by *in vitro* fertilization before placing it in the mother.

Prenatal: Before birth. For example, some genetic tests are performed on a developing fetus while s/he is still in the mother's womb.

Name _____ Date _____ Period _____

6

Weighing the Risks and Benefits of Direct-to-Consumer Genetic Testing: Who Should Decide?

Concerns about Direct-to-Consumer Genetic Testing

- 1 Direct-to-consumer (DTC) genetic testing has offered personal genetic data to consumers since 2006, without the need for—or potential benefit of—medical doctors or genetic counselors. However, medical providers and those in government have been concerned that the risks of DTC genetic testing may outweigh the benefits.
- 2 What if the estimates for how likely someone is to develop a disease prove to be incorrect, either overestimating or underestimating a person's risk? If someone found out that their risk for a disease like Alzheimer's was well above average, would they become depressed? Would they alter their behavior for better, for worse, or perhaps not at all?
- 3 These concerns have led some states – including New York and California – to either ban DTC genetic testing, or strictly limit it. According to a report by the Genetics and Public Policy Center in June 2007, only about half the states in America permitted DTC genetic testing with no restrictions.¹
- 4 The US Food and Drug Administration (FDA) does not currently provide oversight of the DTC genetic testing industry. In March 2011, the Molecular and Clinical Genetics Panel, an advisory committee to the FDA, suggested more oversight. They expressed concern that consumers may misunderstand genetic results without medical counseling, or that the disease risk estimates provided in those results may be incorrect, as there is currently no standard about the level of evidence needed by DTC genetic test manufacturers to make claims about their genetic tests. The FDA panel noted that, while DTC genetic tests seem similar to other at-home medical tests like those for blood sugar or pregnancy, “many DTC clinical genetic tests often carry a disclaimer stating that they are intended for ‘educational and informational’ purposes, and that the individual receiving the test results may wish to take them to their clinician for follow-up.”²
- 5 A member of the FDA panel noted that companies have a right to sell their products to the public, but the FDA has an obligation to compare the risks and benefits of these products, set product standards, and make sure information is understandable by the public.

Response from a DTC Company: 23andMe

- 6 23andMe is a DTC company. According to the creators of the company, “23andMe was founded on the belief that individuals have a right to access their own genetic information, and this conviction is still as firmly held as ever,” but 23andMe is assured that the FDA will take a “reasoned approach to integrating the feedback it received from the panel.”³ However, they hope that feedback will come from all involved parties, as 23andMe notes that the FDA panel had a panel member who represented the consumer and the patient, but they did not “hear directly from consumers and others who have first-hand experience with the information provided by direct-access genetic testing services.”³ 23andMe encourages consumers to make their voices heard about their experiences with DTC genetic testing.
- 7 In addition, DTC genetic testing companies like 23andMe use the genetic information of their customers to further our understanding of human genetics, if customers consent. In a study published in the scientific journal PLoS Genetics, 23andMe used customer data and web-based surveys to evaluate genetic variation in a number of common human genetic traits, such as hair color, eye color, and freckling.⁴ This may not seem like a giant scientific breakthrough, but it makes an important contribution to our understanding of **how** we can conduct genetic research. Genetic studies often require a great deal of time and money to recruit study participants, perform genotyping, document phenotypes, and perform genetic analyses. The 23andMe approach offers a potentially powerful new way to conduct these types of studies with willing DTC genetic testing customers, using less time and at lower cost.

Challenges to Understanding Genetic Test Results

- 8 According to the American Medical Association (AMA), “[t]he results of genetic tests (whether DTC or ordered by a physician) can be challenging to interpret. A positive result does not necessarily indicate a clinical diagnosis. Often, a positive result indicates an increased risk for developing a disease or condition.”⁵ The AMA goes on to say that the same mutation in different people can mean different things, based on penetrance, environment, and other factors. “Also, since only a fraction of testable mutations are identified for genetically based diseases, a genetic test with a negative result is not indicative of the absence of disease risk.” The AMA recommends that any patient undergoing genetic testing (DTC or otherwise), do so “under the guidance of a qualified health care provider.”⁵
- 9 The American Society for Human Genetics (ASHG) noted in a report released in September 2007 that the federal government currently has limited oversight of the “analytic validity” of genetic tests (the ability of the test to correctly detect a particular genetic variant), and no oversight of the “clinical validity” of genetic tests (the ability of the test to correctly predict whether someone will develop a particular disease).⁶ The ASHG recommends that all DTC genetic testing companies provide consumers with information about genetic testing accuracy, including the strength of the scientific evidence about genetic test results, and that the federal government improve regulation of DTC genetic testing companies, to ensure the accuracy of the information provided to consumers.
- 10 However, some are concerned that, without direct government oversight, the DTC genetic testing industry may not do a good job regulating themselves. The Government Accountability Office (GAO) testified in 2010 before the US House of Representatives about their 2006 investigation of DTC genetic testing companies. They obtained 10 genetic tests from four DTC genetic testing companies, using DNA from two donors, and compared the results.⁷ According to the GAO report, “GAO’s fictitious consumers received test results that are misleading and of little or no practical use.” One fictitious donor received contradictory results from each of the companies: below average, average, and above-average risks of developing hypertension and prostate cancer. Many of the estimates of genetic disease risks are based on scientific studies, but often these studies contain too few African American or Asian participants to make meaningful conclusions about these groups. In addition, “follow-up consultations offered by three of the companies failed to provide the expert advice that the companies promised.”⁷ There were also examples of deceptive marketing, in which two companies claimed that donor genetic information could be used to create personalized supplements to “repair damaged DNA” or cure disease, or predict which sports donors’ children would do well in. Experts say these claims lack scientific evidence, and the GAO has referred all four of the companies “for appropriate action” to the FDA and the Federal Trade Commission (which regulates marketing of products).⁷

What Do Consumers Think about DTC Genetic Testing?

- 11 But is all of this concern really necessary? If these tests are truly for “educational and informational purposes only,” do we really need government agencies to regulate them as they would regulate genetic tests in a doctor’s office? What do patients think? Has anyone asked them? Two studies provide insights about what DTC genetic testing consumers think about these products.
- 12 A paper published in the *New England Journal of Medicine* in February 2011 describes preliminary results from the Scripps Genomic Health Initiative, which measures the psychological and behavioral effects of DTC genetic testing on subjects recruited from health and technology companies.⁸ Study subjects purchased genetic tests using the Navigenics Health Compass (Navigenics is a DTC genetic testing provider) at a discounted price—\$225 instead of \$400 to \$2,000. Researchers then followed the subjects for three months using web-based surveys to measure anxiety level, diet, exercise, whether the DTC genetic tests caused subjects any distress, and whether subjects used more medical screening tests after receiving their genetic test results.

- 13** What did these researchers find? About half of the study subjects said that they intended to use more medical screening tests after receiving their DTC genetic test results, and about half did not. There was no clear increase in anxiety level, dietary fat intake, and exercise behavior among these subjects—who were all in general good health at the beginning of the study. About 10% of the subjects said they discussed their test results with the Navigenics board-certified genetic counselor, and about 26% said they discussed their results with their doctor. In fact, most of the study subjects did not do anything different after obtaining their study results: they did not talk to their doctor, they did not change their diet or exercise, and they did not seem to be upset by any of their test results.
- 14** In another report published in *Health Economics* in 2010, researchers studied how much, if anything, people would be willing to pay for DTC genetic tests that predicted their risk of future diseases.⁹ The study included 1,463 people randomly chosen to participate through web-based surveys. They were asked about their willingness to pay for testing for Alzheimer's disease, arthritis, breast cancer, or prostate cancer, using tests that were "perfect" or "not perfectly accurate." Between 70-88% of study participants said they would pay for these genetic tests, with rates lower for Alzheimer's or "not perfectly accurate tests" and higher for prostate cancer or "perfect" tests, even if there were no direct impact on the person's medical treatment options.
- 15** The costs of DNA sequencing and analysis technologies continue to go down, making genetic testing available to more people at lower costs. Consumers, DTC genetic testing companies, and the US government will have to decide how best to move forward – balancing the rights of companies to sell their products, the rights of individuals to have access to their own genetic information through accurate and scientifically valid genetic testing, and the obligations of the federal government to protect its citizens.

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LESSON 6

HANDOUT

Name _____ Date _____ Period _____

6

Critical Reasoning Analysis Form



<p>Point of View</p> <p>What is the point of view of the authors, and how does that particular perspective show through</p>	
<p>Purpose</p> <p>Why was this material written?</p>	
<p>Questions</p> <p>What questions are addressed by the authors? What questions do you have about the material?</p>	
<p>Information</p> <p>What are some of the most important facts included?</p>	

LESSON 6

HANDOUT

Concepts What are the main ideas and concepts addressed?	
Implications What is the larger meaning? What are the consequences of the decision to be made?	
Assumptions What assumptions are the authors making? Are any of these assumptions questionable?	
Inferences What can you infer and conclude based on the material?	

Credit: Foundation for Critical Thinking, <http://www.criticalthinking.org>, and Paula Fraser, Bellevue School District PRISM Program.

Name _____ Date _____ Period _____

6

Open-Ended Questions for a Socratic Seminar



When preparing for a Socratic Seminar, write questions using these sentence frames to stimulate your thinking about the article you read. Choose and complete five of the following:

- What puzzles me is... _____

- I'd like to talk with people about... _____

- I'm confused about... _____

- Don't you think this is similar to... _____

- Do you agree that the big ideas seem to be... _____

- I have questions about... _____

- Another point of view is... _____

- I think it means... _____

- Do you think... _____

- What does it mean when the author says... _____

Name _____ Date _____ Period _____

6

Socratic Seminar Discussion Partner Evaluation



Name of person you are observing _____

1. Record a check for each time your partner contributed in a meaningful way:

2. On a scale of 1–5, with 5 being the highest, how well did your partner do at the following?

Analysis & Reasoning Points: _____	Did your partner... <ul style="list-style-type: none"> • Cite reasons and evidence for his/her statements with support from the text? • Demonstrate that he/she had given thoughtful consideration to the topic? • Provide relevant and insightful comments? • Demonstrate organized thinking? • Move the discussion to a deeper level? 	Notes/Comments:
Discussion Skills Points: _____	Did your partner... <ul style="list-style-type: none"> • Speak loudly and clearly? • Stay on topic? • Talk directly to other students rather than to the teacher? • Stay focused on the discussion? • Invite other people into the discussion? • Share “air time” equally with others (didn’t talk more than was fair to others)? 	Notes/Comments:
Civility Points: _____	Did your partner... <ul style="list-style-type: none"> • Listen to others respectfully? • Enter the discussion in a polite manner? • Avoid inappropriate language (slang, swearing)? • Avoid hostile exchanges? • Question others in a civil manner? 	Notes/Comments:

6

Socratic Seminar Rubric

Dimension	Exemplary	Proficient	Partially Proficient	Developing	Comments
Analysis & Reasoning	Clearly references text to support reasoning.	Occasionally references text to support reasoning.	Rarely references text; may reference text incorrectly.	Does not reference text.	
	Demonstrates thoughtful consideration of the topic.	Demonstrates consideration of the topic.	Demonstrates awareness of the topic but little reflection on it.	Demonstrates little or no consideration of the topic.	
	Provides relevant and insightful comments; makes new connections.	Provides relevant comments.	Comments are mostly relevant.	Comments are off-topic or irrelevant.	
	Demonstrates exceptionally logical and organized thinking.	Thinking is clear and organized.	Thinking is mostly clear and organized.	Thinking is confused, disorganized, or stays at a very superficial level.	
	Moves the discussion to a deeper level.				
Discussion Skills	Speaks loudly and clearly.	Speaks at an appropriate level to be heard.	Mostly speaks at an appropriate level but may need to be coached.	Cannot be heard, or may dominate the conversation.	
	Stays on topic and brings discussion back on topic if necessary.	Stays on topic and focused on the discussion.	Sometimes strays from topic.	Demonstrates inappropriate discussion skills.	
	Talks directly to other students (rather than the teacher).	May occasionally direct comments to teacher.	Talks directly to teacher.	Does not talk.	
	Shares "air time" equally with others.	Aware of sharing "air time" with others.	Occasionally dominates the conversation.	Dominates the conversation.	

LESSON 6

RESOURCE

Dimension	Exemplary	Proficient	Partially Proficient	Developing	Comments
Discussion Skills (Continued)	Invites other people into the discussion.	May invite other people into the discussion.		Does not invite other people into the discussion.	
	References the remarks of others.			Does not reference the remarks of others.	
	Stays focused on the discussion.			May be distracted or not focused on the conversation.	
Civility	Listens to others respectfully by making eye contact with the speaker, and waiting their turn to speak.	Listens to others respectfully.	Listens to others respectfully, but may not always look at the speaker or may sometimes interrupt.	Interrupts frequently.	
	Remarks are polite and demonstrate a high level of concern for the feelings of others.	Remarks demonstrate a concern for the feelings of others.	Remarks demonstrate an awareness of feelings of others.	Remarks demonstrate little awareness or sensitivity to the feelings of others.	
	Addresses others in a civil manner, using a collegial and friendly tone.	Uses appropriate language and tone.	Uses inappropriate language or tone.	Uses an aggressive, threatening, or otherwise inappropriate tone.	

7

An Introduction to Bioinformatics Careers

Introduction

In this lesson, students explore more deeply the information they have learned throughout the unit about people in various careers that use bioinformatics. Students choose one career they would like to learn more about. They further explore that career by reading a series of in-depth questions asked of the person highlighted in that career, as well as provided internet resources. Students then respond to a job posting for a summer internship in their chosen field, developing a resume for that position. Optional activities include peer-editing of resumes and socializing in a professional environment.

Learning Objectives

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

- Bioinformatics tools are used by people in many careers.
- Different careers require different skills and education.
- Jobs in many fields require submission of a resume specific to that job.

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

- Identify a career path for at least one career that uses bioinformatics.
- Research a scientific field using reliable internet resources.
- Develop a resume that describes their jobs skills.

Key Concepts

- Although bioinformatics is a career choice in itself, there is a wide variety of careers that use the tools of bioinformatics.
- Different career paths require different amounts of education and training.
- Resumes summarize one's job-related skills.

Class Time

Two class periods of 50 minutes each (up to 100 minutes total) and one homework assignment between Days 1 and 2. If completing the resume peer-editing assignment, and/or the optional Open House event, a third day may be required.

Common Misconceptions

- All science-related careers require the same basic skill set and education.
- The only relevant job-related skills required to work in the sciences involve performing lab experiments.

Materials

Materials	Quantity
Copies of Student Handout— <i>Careers in the Spotlight</i> (handed out in Lesson One)	1 per student
Copies of Student Handout— <i>Spotlight On My Career</i>	1 per student
Copies of Student Handout— <i>Making a Resume</i>	1 per student
Copies of Student Handout— <i>Resume Peer-Editing Form</i> (Optional: See Part II of lesson plan)	1 per student
Class set of Student Handout— <i>Career Interview 1: Bioengineer Adrienne R. Minerick, PhD</i> [Note: Also available online at: http://www.nwabr.org/students/exploring-careers.]	6 -OR- Have students read online
Class set of Student Handout— <i>Career Interview 2: Veterinarian Deborah Tegarden, DVM</i> [Note: Also available online at: http://www.nwabr.org/students/exploring-careers.]	6 -OR- Have students read online
Class set of Student Handout— <i>Career Interview 3: Genetic Counselor Robin Bennett, MS</i> [Note: Also available online at: http://www.nwabr.org/students/exploring-careers.]	6 -OR- Have students read online
Class set of Student Handout— <i>Career Interview 4: Laboratory Technician Zane Kraft, MS</i> [Note: Also available online at: http://www.nwabr.org/students/exploring-careers.]	6 -OR- Have students read online
Class set of Student Handout— <i>Career Interview 5: 3D Animator Beth Anderson</i> [Note: Also available online at: http://www.nwabr.org/students/exploring-careers.]	6 -OR- Have students read online
Class set of Student Handout— <i>Career Interview 6: Bioethicist Kelly Edwards, PhD</i> [Note: Also available online at: http://www.nwabr.org/students/exploring-careers.]	6 -OR- Have students read online
Name badges and pens (Optional: see Part III of lesson plan)	1 per student

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 Seven PowerPoint Slides— <i>An Introduction to Bioinformatics Careers</i> . Available for download at: http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing.
All career interviews and resources found in the <i>Career Interview</i> Student Handouts are also available on the NWABR Exploring Science Careers webpage at: http://www.nwabr.org/students/exploring-careers. [Note: This web page contains additional careers not featured in the <i>Introductory</i> curriculum.]
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.
Computer lab with internet access for students. Students will also need access to a text editing program such as Notepad or Microsoft Word® to write their cover letters and resumes.

Teacher Preparation

- Load the classroom computer with the *Lesson Seven* PowerPoint slides.
- Make copies of the following Student Handouts:
 - *Spotlight On My Career*
 - *Making a Resume*
 - *Resume Peer-Editing Form* (optional)
- If students will not be reading the career interviews and job postings online, also make copies of *Career Interviews 1-6*. These handouts are designed to be re-used as Class Sets.
- Have name badges and pens available if completing the optional Open House activity (see *Part III* of lesson plan).

Procedure

Day 1

PART I: Researching a Career

1. Explain to students the **aims of this lesson**. Some teachers may find it useful to write the aims on the board.

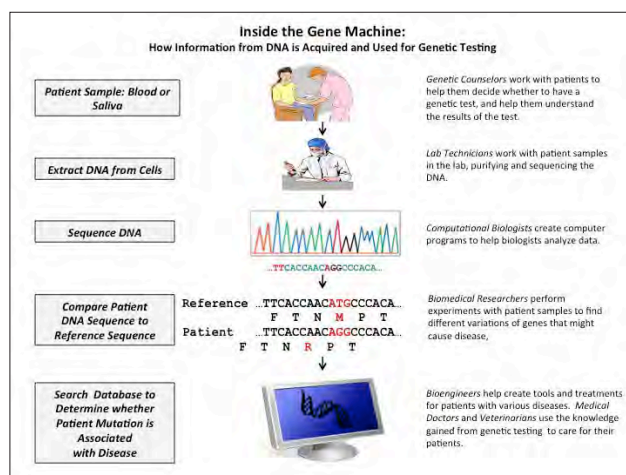
Lesson Aims:

- a. To learn more about one of the careers highlighted in *Lessons One through Six*.
- b. To learn how to research careers using credible internet resources.
- c. To develop a resume.

Teachers may also wish to discuss the *Learning Objectives* of the lesson, which are listed at the beginning of this lesson.

2. Have students use Student Handout—*Careers in the Spotlight* given out on the first day of the unit as a reference. Review all six of the *Careers in the Spotlight* PowerPoint slides that have been shown to students during the *Warm-up* portion of each lesson.
3. Show the PowerPoint for *Lesson Seven*, beginning with **Slide #1**, which returns students to the *Gene Machine*. Remind students of the general steps involved in genetic research, from collecting patient samples, to extracting DNA from the cells, to sequencing the DNA and analyzing it to determine whether it contains mutations known to be associated with disease. Help students make connections between each of the highlighted careers and the role they play in making genetic testing possible.

[**Note:** Career slides are also included in the Career Interview handouts used later in this lesson.]



Bioinformatics Careers: **Slide #1**

4. Show **Slide #2**, which highlights bioengineer Adrienne R. Minerick, PhD. Take a moment to review the information on the slide.

Bioinformatics Careers: **Slide #2**



BIOENGINEER
ADRIENNE R. MINERICK, PhD

Place of Employment:
Michigan Technological University

Type of Research:
Biomedical Microdevices
For example, tiny devices to measure and manipulate living cells

"If a person is determined to learn, there will always be opportunities or resources for that person to pursue an education in science and engineering... There is a *real* demand for scientists and engineers whose contributions advance knowledge, technology, and the economic foundation of our society. I chose my career because I wanted to be a part of advancing knowledge and facilitating others to gain knowledge."

5. Show **Slide #3**, and remind students that bioengineers use the principles and tools of bioengineering to create products and tools, like DNA sequencing machines, medical devices, and perhaps someday, even a "Gene Machine"!

Bioinformatics Careers: **Slide #3**

CAREERS IN SPOTLIGHT:
BIOENGINEER


What do they do?
A bioengineer uses the principles and tools of engineering to address problems in biology and medicine, creating usable products. These include designing medical devices, diagnostic equipment, renewable bioenergy, and genetically modified organisms.

What kind of training is involved?
A bachelor's degree in engineering is required for almost all entry-level engineering jobs. Sometimes a graduate degree is also required, such as a Master's degree or PhD

What is a typical salary for a Bioengineer?
The average salary is about \$55,000/year (\$26/hour), with a range from \$45,000/year (\$22/hour) to more than \$120,000/year (\$58/hour).

6. Show **Slide #4**, which highlights veterinarian Deborah Tegarden, DVM. Take a moment to review the information on the slide.

Bioinformatics Careers: **Slide #4**



VETERINARIAN
DEBORAH TEGARDEN, DVM

Place of Employment:
Elliot Bay Animal Hospital
Seattle, Washington

"While we often think of genetic testing in humans, vets are seeing more and more tests being developed for animal patients. This is the most exciting time I can imagine in veterinary medicine, when things are getting more and more cutting edge and technology is developing at lightning speed."

7. Show **Slide #5**, and remind students that veterinarians and their animal patients also benefit from genetic testing. Many genetic tests are now available for cats and dogs to help diagnose or even prevent certain congenital conditions, such as those found in particular dog or cat breeds. In addition, much of what we learn about genetics by studying animals can also be used to help humans.

CAREERS IN SPOTLIGHT:
VETERINARIAN

What do they do?
Veterinarians diagnose and treat animals. Some veterinarians specialize in a particular area (such as oncologists who treat cancer), and some perform research to improve animal and human health. Veterinary technicians assist veterinarians in their work.

What kind of training is involved?
Veterinarians complete a Bachelor's degree and a DVM (Doctor of Veterinary Medicine) degree, which requires four years. Veterinary technician training is usually a two year (Associate's) program.

What is a typical salary for a Veterinarian?
Veterinarians: \$45,000/year (\$22/hour), up to \$140,000/year (\$67/hour). Veterinary Technicians: \$20,000/year (\$10/hour), up to \$45,000/year (\$22/hour).

Bioinformatics Careers: **Slide #5**

8. Show **Slide #6**, which highlights genetic counselor Robin Bennett. Take a moment to review the information on the slide.

GENETIC COUNSELOR
ROBIN BENNETT, MS

Place of Employment:
University of Washington

Specialties:
Huntington's disease, neurogenetics, cancer genetics, genetic family history, and ethical issues in genetic counseling and genetic testing

"I feel privileged to be a part in some small way with each of my patients and that hopefully I have helped them with some difficult decisions and with making choices that work for them within their belief systems."

Bioinformatics Careers: **Slide #6**

9. Show **Slide #7**, and remind students that it can be difficult for an individual to decide whether to get a genetic test. It can also be challenging for a patient to understand the test results. Genetic counselors help patients by reviewing their medical and family histories, while working with them to come to a decision about testing that fits with their ethics and beliefs.

Bioinformatics Careers: **Slide #7**

CAREERS IN SPOTLIGHT:
GENETIC COUNSELOR

What do they do?


- Review a patient's medical and family history.
- Advise patients and their families about the benefits and consequences of genetic testing, and about the nature of genetic disorders.
- Offer counseling consistent with the patient's belief system.

What kind of training is involved?
Bachelor's degree and a two to three year Master's degree.

What is a typical salary for a Genetic Counselor?
Start at \$60,000/year (\$29/hour), and may make up to \$120,000/year (\$58/hour).

10. Show **Slide #8**, which highlights laboratory technician Zane Kraft. Take a moment to review the information on the slide.

Bioinformatics Careers: **Slide #8**



LABORATORY TECHNICIAN
ZANE KRAFT, MS

Place of Employment:
Seattle Biomedical Research Institute

Type of Work:
HIV Vaccine Research

"We do a lot of DNA alignments of HIV Envelope sequences. Another fun thing that we now do more of is computer modeling of HIV Envelope proteins with different mutations."

11. Show **Slide #9**, and remind students that "lab techs" do much of the "behind the scenes" work for genetic testing. They often help isolate the DNA from the patient's sample, as well as sequence the DNA and report the results to the patient, the patient's doctor, and the genetic counselor.

Bioinformatics Careers: **Slide #9**


CAREERS IN SPOTLIGHT:
LABORATORY TECHNICIAN

What do they do?
Laboratory technicians, sometimes called "lab techs" or research technicians, do much of the day-to-work in the lab, including purifying and sequencing DNA, caring for laboratory machinery, and analyzing samples.

What kind of training is involved?
Some laboratory technicians have a two year Associate's degree or a four year Bachelor's degree. Some, like Zane Kraft, also have a Master's degree (additional two year training).

What is a typical salary for a Laboratory Technician?
Ranges with experience from \$30,000/year (\$14/hour) up to \$60,000/year (\$29/hour) or more.

12. Show **Slide #10**, which highlights 3D animator Beth Anderson. Take a moment to review the information on the slide.



3D ANIMATOR
BETH ANDERSON

Place of Employment:
Arkitek Studios

Type of Work:
Develops 3D animations that help scientists and students visualize biological processes.

"Bioinformatics interfaces in many ways with our work, from data that clients give us to visualize representationally, to creating actual visual data."

Bioinformatics Careers: **Slide #10**

13. Show **Slide #11**, and remind students that 3D animators work with scientists, students, educators, and the public to help communicate scientific information. Part of their job is to help make difficult concepts easier to understand. 3D animator Beth Anderson developed the *BRCA1* animation used in this curriculum. She and other animators also help scientists develop new ways to visualize their data, including by making scientific illustrations.



CAREERS IN SPOTLIGHT:
3D ANIMATOR

What do they do?
3D animators are visual communicators and designers. The animations and illustrations they create help communicate scientific content and ideas to scientists, students, and educators.

What kind of training is involved?
Associate of Arts degree. Some also have training in biology, illustration, graphic design, and computer animation.

What is a typical salary for a 3D Animator?
Ranges with experience from very little when people are first starting out to \$100,000/year (\$48/hour) or more.

Bioinformatics Careers: **Slide #11**

- Show **Slide #12**, which highlights bioethicist Kelly Edwards, PhD. Take a moment to review the information on the slide.

Bioinformatics Careers: **Slide #12**



BIOETHICIST
KELLY EDWARDS, PhD


Place of Employment:
University of Washington

Specialties and Interests:
Community-based research practices, environmental justice, and integrating ethics into training programs and public policy.

"Genetics researchers often work with distinct communities. To take moral account of how their research affects these communities, they need a richer conception of justice and they need to make those communities equal participants in decision-making about how the research is conducted and what is produced and published out of it."

- Show **Slide #13**, and remind students that bioethicists help us understand some of the many controversies that arise with new biomedical advances. Just because we **can** test for a genetic condition does not mean that we **should**, as we consider issues like clinical validity and effective treatments. Bioethicists consult with doctors, patients, genetic counselors, scientists, and policy makers about many ethical issues.

Bioinformatics Careers: **Slide #13**



CAREERS IN SPOTLIGHT:
BIOETHICIST

What do they do?
Bioethicists study the ethical controversies that arise from advances in biology and medicine. They advise patients, researchers, public policy makers, and/or medical doctors about a wide range of ethical issues, from genetic testing and medical treatment to medical privacy and stem cell research.

What kind of training is involved?
Training varies widely from a certificate program completed during or after a Bachelor's degree in another subject to Doctoral (PhD) programs specifically in Bioethics.

What is a typical salary for a Bioethicist?
Depending upon training, anywhere from \$50,000/year (\$24/hour) to over \$100,000/year (\$48/hour).

- Explain to students that each of the featured individuals was interviewed about their work so that students could learn more about their careers. Included in each *Career Interview* handout are online resources that students can use to learn more about each career, as well as an example of a job posting for a summer internship related to that career.
- Ask students to think about a career they would like to know more about, referring to Student Handout—*Careers in the Spotlight* if needed. Pass out the corresponding Career Interview handouts. Alternatively, students may read the *Career Interviews* online at: <http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing>.

18. After students have read their chosen interview, tell students that they will perform their own research about their chosen career. Pass out copies of Student Handout—*Spotlight On My Career* and allow time for students to complete the handout using the internet and the links found in the *Resources* section at the end of each interview.

Tell students that many of these resources, such as the Bureau of Labor and Statistics, as well as the suggested key word searches, can be used to find information about a number of different careers. More information about different types of careers in each field, as well as all of the resources listed after each interview, are available on NWABR's Student Career Center at: <http://www.nwabr.org/students/student-resource-center/career-center>.

19. Give students 25-30 minutes to work through the handout on their own before asking students to share some of the interesting things they found in the interviews and during their own internet research.
20. Ask students how they think each featured person got their current positions. Students may say things like, "veterinarians like working with animals," or "lab techs like working in the lab."
21. Explain to students that each of the featured people needed to apply for their jobs, which included submitting a **resume** that described their skills, experience, and what made them a good match for the position.
22. As homework for Day One, tell students to use the career interview they read, the job posting found at the end of the interview, and the research they completed on Student Handout—*Spotlight On My Career* to develop a list of at least ten key words, skills, and knowledge needed for a summer internship in their chosen career. Urge them to consider not only the skills and knowledge that they have acquired during this genetic testing unit, but also skills that they have learned during the rest of their class time, as well as outside activities. Students will use this list on Day Two when they write a mock resume to apply for their internship.

[**Note:** Suggested point values are included after each question, and are intended to provide general guidelines for the weight each question could be given. The suggested point value for this handout is 10-15 points.]

[**Note:** This assignment may be graded, with 0.5 points for each relevant key word listed.]

Day 2

PART II: Writing a Resume

23. Remind students that adults often must apply for jobs and internships, which includes submitting a resume that describes the knowledge, skills, and experience they have that would make them good at that particular job.
24. Pass out copies of Student Handout—*Making a Resume*, and tell students that they should use their key word list and the Student Handout to write a resume to apply for the summer internship position described at the end of their career interview.
25. Explain to students that a resume should be no more than two pages (and is often only one page); therefore, students should not list all of their knowledge and skills, but should focus instead on the knowledge and skills asked for in the job posting on the *Career Interview* handout.
26. Have students work on their resume independently at the computer.

[**Note:** This assignment may be graded, with a suggested total value of 10-20 points.]

27. **Peer Editing (optional):** If time permits, either on Day 2 or as an extension into Day 3, students may benefit from peer editing one another's resumes. Explain to students that one should **always** ask someone to review their resume before giving it to a potential employer to be sure that the resume is well-organized, specific to the job posting, and free of any typos or grammatical errors.
28. Pass out copies of Student Handout—*Resume Peer-Editing Form*. Ask students to exchange resumes with a classmate sitting next to them (such as everyone passing their resume to the person sitting on their right). Tell students to review, comment on, and grade the resume of one of their classmates. 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 **five points**.
29. Emphasize to students that this process is meant to be helpful, and that students will be graded on the quality of their editing and review.

PART III: Putting it All Together (Optional Extension) **Seattle Research University Open House**

30. Explain to students that not all professional interactions happen on the job or during job interviews. One important skill that all professionals in any career must learn is how to talk about themselves and their work in a more social setting. This is also relevant to a number of new, and sometimes uncomfortable, social situations students may encounter, such as starting a new job or beginning college.
31. Ask students how many of them enjoy parties and experiences where they have to meet a number of people that they don't know. If few students raise their hands, tell them that it is very common for people to feel uncomfortable in new situations. Brainstorm with the class about the challenges of these types of situations, what kind of language they might use to introduce themselves and ask others about themselves, as well as how to politely disengage from a conversation.
32. **Set the Stage for the Open House Event:** A professional colleague (your teacher) is having a party with all of his/her professional friends. Each student will assume the role of the type of professional they studied in the career interview and Student Handout—*Spotlight On My Career*. Encourage students to create a name tag with their name (or the name of the person in their career interview, or a name that they make up), as well as their profession. Tell students that they are free to change the names, hobbies, where their character came from, etc. Encourage students to mingle, and to learn as much as possible about each others' careers. This is an adaptation of a jigsaw activity, in which students from "like" and "mixed" careers will have opportunities to learn more about each other, and to realize that there are many different jobs in each career field.
33. After 15-20 minutes, review with students what strategies they found effective for learning more about other professionals, engaging in conversations with individuals from similar or different careers, and how to transition from one group or conversation to another.

34. As an assessment, ask students to introduce some of the people they met, or write a short paragraph about two or three of the people they met.

Closure

35. At the end of the lesson, review with students how each career either directly uses the tools of bioinformatics or benefits from the knowledge gained from bioinformatics. You may ask students to provide examples of their answers to Question #6 on Student Handout—*Spotlight On my Career*:

- Given what you have learned about your career and the field of bioinformatics, **formulate at least one question that you could answer using the tools of bioinformatics in the career you chose.**

36. Emphasize that the skills they have learned in this lesson are applicable to any job or career, including: researching career information using reliable sources like interviews with professionals in that field and the Bureau of Labor and Statistics; writing a resume; and (if applicable), peer-editing and socializing in a professional environment. Whether students pursue careers in the sciences, humanities, law, or other professions, they will need to know how to find out about required education and training, future employment projections, which colleges and universities offer programs in their field, and how to write a good resume when applying for a job.

Extension

- Teachers may wish to have students find a job posting online in their career of choice, beyond those included in this lesson.
- Additional resume-building activities, including writing and peer-editing a cover letter and enhancing skills needed during job interviews, can be found in the Bio-ITEST Advanced curriculum, *Using Bioinformatics: Genetic Research*.

Resources

See also the *Resources* section at the end of each Career Interview.

For more information about different types of jobs in each career field, including what you can do with different degrees (two year Associate's degree, four year Bachelor's degree, graduate, or professional degrees) visit NWABR's Student Career Center at: <http://www.nwabr.org/students/student-resource-center/career-center>. The site also includes descriptions of and links to different types of degree programs, various career paths, resources on writing a resume and cover letter, evaluating online resources, and tips for successful job interviews. All of the links found after each career interview can also be accessed from NWABR's Student Career Center.

The Occupational Outlook Handbook (OOH), 2010-11 Edition. Bureau of Labor and Statistics: <http://www.bls.gov/oco/>.

National Human Genome Research Institute "Genomic Careers" Center: <http://www.genome.gov/GenomicCareers/careers.cfm>.

Sloan Career Cornerstone Center: Career Planning Resources in Science, Technology, Engineering, Mathematics, Computing, Health care: <http://www.careercornerstone.org/index.htm>.

Virginia Jobs: Career Development Career Guides: http://jobs.virginia.gov/cd_guidesList.html#elist.

Credit

Anderson, Beth. Personal Interview. 2 July 2010.

Bennett, Robin. Personal Interview. 18 March 2010.

Edwards, Kelly. Personal Interview. 11 November 2010.

Kraft, Zane. Personal Interview. 30 September 2010.

Minerick, Adrienne. Personal Interview. March 2010.

Tegarden, Deborah. Personal Interview. 11 November 2009.

Photo of Beth Anderson provided by Doug Huff.

The authors wish to thank Adam Waltzer of Eastside Preparatory School for his “cocktail party mixer” adaptation to this career lesson.

Name _____ Date _____ Period _____

Spotlight On My Career



My Career is: _____

Often in bioinformatics, we refer to **Tool Makers** and **Tool Users**.

Tool Makers ask: What kinds of new bioinformatics tools can I make? How can I make the existing tools better?

Tool Users ask: What new questions can I answer using bioinformatics tools?

Use the information found in your *Career Interview* and the links found in the *Resources* section of the *Career Interview* to answer the questions below. If you need additional sources of information, use your search engine and the keywords in bold below to find out more about your career.

1. All career paths require **education and training**. What are the common requirements for your chosen career?
2. What are the **employment projections** in your field for the next five to ten years (i.e., after **you** would graduate from college or professional training)?
3. List three **colleges or universities** that offer programs in your field of study:
 - A.
 - B.
 - C.

LESSON 7

HANDOUT

4. Many researchers and professionals join **organizations or associations** with others in their field to set guidelines for career paths and to provide mentorship and guidance to people new to the field. These organizations are also reliable sources of information about that particular career. **List at least one professional organization or association with members in your chosen career.**

5. Based on the information you have read so far about your career, complete the following sentences:

a. I was surprised to learn that _____
_____.

b. I was confused by _____
_____.

c. I would like to learn more about _____
_____.

6. Given what you have learned about your career and the field of bioinformatics, **formulate at least one question that you could answer using the tools of bioinformatics in the career you chose.**

Name _____ Date _____ Period _____

7

Making a Resume

You are now ready to develop a resume based on your knowledge about bioinformatics and the skills you have used while learning about genetic testing. Use the following format to create your resume. Create headings, and put examples below each heading in bullets. Resumes are often customized to meet the requirements of the job for which you are applying. Be sure to note any special qualifications you have that your potential employer is looking for.

Name
Address
Contact Information

Job Objective/Career Interest: Describe the career you are most interested in that relates to bioinformatics. You should choose from the careers you learned about during the *Genetic Testing* unit.

Knowledge/Understanding: Review each of the lessons you studied about genetic testing, as well as other lessons you have studied in class that may relate to this career, and use those skills to fill out this section. For example:

- Understanding of the genes involved in breast cancer (*BRCA1* and *BRCA2*), and why mutations in those genes can cause cancer
- Understanding of the type of information gained from family trees and Punnett squares

Skills: Review the skills you have learned and practiced from the *Genetic Testing* lessons and other exercises and lessons from your class(es). For example:

→ **Laboratory Skills:** List any “wet lab” skills you have acquired. For example:

- DNA purification
- Polymerase Chain Reaction (PCR)

→ **Bioinformatics Skills:** List each general **type** of bioinformatics program that you have used, the specific program(s) in parentheses, and then what you can use those programs to do. For example:

- Molecular Structure Visualization Software (Cn3D): Use Cn3D to identify sites of mutations in proteins, and relate those mutations to impacts on protein function.

→ **Professional Skills:** List any additional skills you have acquired that are beneficial for this career. For example:

- Writing a research report
- Creating scientific posters
- Teamwork
- Microsoft Office (Word®, Excel®, Powerpoint®)

Research Experience: Describe your experience designing an experiment to answer a testable question. Describe your research project(s).

- Designed a research experiment to test....

Name _____ Date _____ Period _____

7

Resume Peer-Editing Form

When submitting a resume for a job, it is always a good idea to ask someone else to review it before you show it to your potential new boss. You will be grading your classmate's resume, and using this as an opportunity to offer valuable feedback. Follow the instructions below to review and assign points to your peer's resume. You can circle or check off below each item that is correct in the resume, and enter the point total on the lines provided. This sheet and your classmate's resume will be returned to your classmate after your teacher reviews them both.

[**Note:** You will be graded on the quality of your review and comments (*up to five points possible*).]



Name of the person whose resume you are reviewing: _____

Instructions and Grading:

1. Did the resume contain all of the required sections found on Student Handout—*Making a Resume*?

- + 0.5 points for Name and Contact Information at the top of page 1
- + 0.5 points for Job Objective/Career Interest
- + 0.5 points for a list of Knowledge/Understanding
- + 0.5 points for a list of Skills
- + 1.0 points if the skills listed are subdivided into separate categories, such as Laboratory Skills, Bioinformatics Skills, Professional Skills, or as appropriate for the job (such as Artistic Skills, Animal Care Skills, Research Experience, etc.)

(3.0 points possible) Total for #1: _____

2. Using the summer internship job postings found in the career handout or online, circle each key word, skill, or knowledge required on the resume that also appears in the job posting.

(5 points possible, +0.5 points for each key word included) Total for #2: _____

3. Note and correct any misspelled words or grammatical errors.

[**Note:** Your final draft of your resume should not have any of these errors.]

- +2 points if there were no grammatical errors, misspelled words, or other typos
- +1 point if there were 1–4 grammatical errors, misspelled words, or other typos
- +0 points if there were 5 or more grammatical errors, misspelled words, or other typos


(Up to 2.0 points possible) Total for #3: _____

4. List below any other comments you wish to make about your classmate's resume. Be positive and helpful, noting things that they did well, or things you think they could improve:

Total Points for Resume: _____

7

Adrienne R. Minerick, PhD Bioengineer

 <p>BIOENGINEER ADRIENNE R. MINERICK, PhD</p> <p>Place of Employment: Michigan Technological University</p> <p>Type of Research: Biomedical Microdevices <i>For example, tiny devices to measure and manipulate living cells</i></p> <p>"If a person is determined to learn, there will always be opportunities or resources for that person to pursue an education in science and engineering... There is a real demand for scientists and engineers whose contributions advance knowledge, technology, and the economic foundation of our society. I chose my career because I wanted to be a part of advancing knowledge and facilitating others to gain knowledge."</p>	<p>CAREERS IN SPOTLIGHT: BIOENGINEER</p> <p>What do they do? A bioengineer uses the principles and tools of engineering to address problems in biology and medicine, creating usable products. These include designing medical devices, diagnostic equipment, renewable bioenergy, and genetically modified organisms.</p> <p>What kind of training is involved? A bachelor's degree in engineering is required for almost all entry-level engineering jobs. Sometimes a graduate degree is also required, such as a Master's degree or PhD</p> <p>What is a typical salary for a Bioengineer? The average salary is about \$55,000/year (\$26/hour), with a range from \$45,000/year (\$22/hour) to more than \$120,000/year (\$58/hour).</p>
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1. Where did you grow up?

Alamosa, CO – it is a small college town in the center of a high altitude mountain valley.

2. What do you do (i.e., what career or field are you in; what is the title of your position)?

I'm an associate professor of chemical engineering at Michigan Technological University in Houghton, MI. My job is a combination of research with graduate and undergraduate students, and teaching bio-focused chemical engineering courses. My research is on biomedical microdevices. We use electric fields to distinguish ABO blood types that will help change medical diagnostics.

3. How did you choose your career? When did you first know this was the career you wanted?

Education is a very valuable asset that transcends all socio-economic classes. If a person is determined to learn, there will always be opportunities or resources for that person to pursue an education in science and engineering. On the other side of the college experience, there is a real demand for scientists and engineers whose contributions advance knowledge, technology, and the economic foundation of our society. I chose my career because I wanted to be a part of advancing knowledge and facilitating others to gain knowledge.

4. Did your family support your decision to pursue your career?

Yes, mostly. Some did not understand what an advanced degree (MS/PhD) really was. They didn't understand that it wasn't just coursework. Now that I'm a professor, they think that all I do is teach and don't really understand what goes into writing research proposals, managing a lab of researchers/equipment, and publishing papers to disseminate knowledge to others.

5. What is the highest level of education you have?

PhD in Chemical Engineering.

6. What is the highest level of education reached by other members of your family?

Mother – PhD in Vertebrate Paleontology.

Father – PhD in Mathematics.

7. What is the salary range for a person in your position?

\$85,000 - \$95,000 over nine months (professors aren't paid for three months in the summer unless they secure research funding to pay those three months). [That's about \$41-\$46/hour.]

8. What do you like most about your job?

The freedom and the variation. When an exciting research problem presents itself, I have the ability to seek resources to pursue and explore that. No day is the same and I'm continuously learning and challenging myself.

9. What do you like least about your job?

Sometimes students are just after a grade and not truly interested in learning. I don't like having to discuss allocations of points with students like that.

10. What's an abbreviated day in the life of your job?

This varies so much. I'll describe my favorite day. I come in, catch up on email and note the important tasks I need to do for others that day. I meet with one of my graduate students, see the data they collected over the last week. We discuss what it means and make plans for experiments for the next week. I study material and prepare lecture notes. Search for nice visuals online or in books/other resources. Go to class and lecture/work problems with students. Meet with undergraduate senior unit operations team who is doing a lab experiment under my supervision (we use pilot scale equipment in our curriculum). Read/review research article and provide feedback to editors on whether it should be published. File away this information for later (knowledge of the literature is important to continue learning and advance knowledge). This type of information will later be used to develop creative ideas to solve real research problems.

11. In one to two sentences, how would you say you use bioinformatics in your work? If you don't use bioinformatics directly in your work, how has bioinformatics impacted your career field?

Biomedical engineering is a broad field. Basically any area of engineering that uses its knowledge to solve biological problems qualifies. Bioinformatics in the traditional sense is using mathematical tools to compile and understand large amounts of biological data (DNA sequences, protein folding, etc.). I don't do this traditional work. Instead, I explore how chemical expression in biological cells impacts behaviors within microchannels the width of a human hair.

12. Do you have any recommendations for students who are interested in entering your field?

Yes! There is no traditional educational path to this type of research. Follow your interests and look for the links between the different areas. This approach will give you a unique background and thus unique insights to be able to solve problems.

13. What are your favorite hobbies?

Gardening, my two kids, my husband, cooking, our two dogs, camping, equestrian riding, remodeling our house, woodworking, boating. I also want to get my pilot's license and learn to quilt.

Resources:

In the **field of bioengineering**, there are many different types of jobs available, depending upon what type of education and experience you have. For more information about different types of jobs in this field, including what you can do with different degrees (two year Associate's degree, four year Bachelor's degree, graduate, or professional degrees), visit NWABR's Student Career Center at: <http://www.nwabr.org/students/student-resource-center/career-center>.

The site also includes descriptions of and links to different types of degree programs, various career paths, resources on writing a resume and cover letter and evaluating online resources, and tips for successful job interviews.

All of the links below can also be accessed from NWABR's Student Career Center.

To learn about **job prospects**, **salary information**, and **job skills** ("qualifications") required for engineering in general, and biomedical engineers in particular, visit the US Bureau of Labor Statistics: <http://www.bls.gov/oco/ocos027.htm>.

Find information on careers in biomedical engineering at the National Human Genome Research Institute, including information about **career outlook**, working conditions, and **salary**. Scroll through the career listings until you reach "Biomedical Engineer" one page 1: <http://www.genome.gov/GenomicCareers/careers.cfm>.

To learn more about careers in bioengineering, visit the Biomedical Engineering Society's Frequently Asked Questions page at: http://www.bmes.org/aws/BMES/pt/sp/be_faqs.

You can also visit the Sloan Career Cornerstone Center's webpage on Bioengineering at: <http://www.careercornerstone.org/bioeng/bioeng.htm>.

Dr. Minerick is the Director of the Medical Micro-Device Engineering Research Lab (MD-ERL) at Michigan Tech: http://www.mderl.org/all_projects.php.

Some of the Resources above may also be used to research other careers that may be of interest to you in the future, including the Bureau of Labor and Statistics and the National Human Genome Research Institute.

Job Posting: Biomedical Engineering Summer Internship

The laboratory of Dr. Maynard Smith is looking for motivated individuals interested in learning about the development of small biological microdevices that can be used to detect disease-causing mutations in small samples of patients' blood. Interns will work closely with lab members and help develop and evaluate microdevices. Prior lab experience is not necessary, but an understanding of molecular biology, including the role of DNA in encoding human traits, is required. Applicants familiar with genetic testing and bioinformatics tools used to detect mutations are encouraged to apply. Additional computer skills, including Microsoft Office (Word®, Excel®, PowerPoint®), are preferred. Applicants must be hard-working, responsible, and able to work in a team environment. Address all inquiries to Dr. Maynard Smith, Seattle Research University, Biomedical Devices Department, Suite 100, Seattle, WA

LESSON 7

CLASS SET

7 Deborah Tegarden, DVM Veterinarian

 <p>VETERINARIAN DEBORAH TEGARDEN, DVM</p> <p>Place of Employment: Elliot Bay Animal Hospital Seattle, Washington</p> <p>"While we often think of genetic testing in humans, vets are seeing more and more tests being developed for animal patients. This is the most exciting time I can imagine in veterinary medicine, when things are getting more and more cutting edge and technology is developing at lightning speed."</p>	<p>CAREERS IN SPOTLIGHT: VETERINARIAN</p> <p>What do they do? Veterinarians diagnose and treat animals. Some veterinarians specialize in a particular area (such as oncologists who treat cancer), and some perform research to improve animal and human health. Veterinary technicians assist veterinarians in their work.</p> <p>What kind of training is involved? Veterinarians complete a Bachelor's degree and a DVM (Doctor of Veterinary Medicine) degree, which requires four years. Veterinary technician training is usually a two year (Associate's) program.</p> <p>What is a typical salary for a Veterinarian? Veterinarians: \$45,000/year (\$22/hour), up to \$140,000/year (\$67/hour). Veterinary Technicians: \$20,000/year (\$10/hour), up to \$45,000/year (\$22/hour).</p>
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1. Where did you grow up?

Near Portland, Oregon.

2. What do you do (i.e., what career or field are you in, what is the title of your position)?

I am a veterinarian in Seattle, Washington.

3. How did you choose your career? When did you first know this was the career you wanted?

I think I always wanted to be a veterinarian. I remember talking about it as early as my third grade essay contest, in which I described my love of animals.

4. Did your family support your decision to pursue your career?

Very much so! It was always expected that I would go to college and my parents always supported my dreams.

5. What is the highest level of education you have?

I obtained a Bachelor's degree from Portland State before joining the joint degree program in Veterinary Medicine offered through Oregon State and Washington State Universities. I now have a DVM (a Doctor of Veterinary Medicine).

6. What is the highest level of education reached by other members of your family?

I had eight half brothers and sisters, and I was the first of them to go to college. My dad went to college, however, and really valued education. I'm the youngest of my siblings.

7. What is the salary range for a person in your position?

It ranges depending upon where you are and what kind of animals you see. Most veterinarians start around \$40,000 per year [\$19/hour]; the average is about \$60,000-\$100,000 per year [\$29-\$48/hour].

8. What do you like most about your job?

I love that nearly every day, I learn something new at work. I feel like the variety of the job keeps it exciting and constantly evolving.

9. What do you like least about your job?

This job, at least at a very busy, fast-paced clinic like mine, can be extremely emotionally and physically draining. It has taught me to really value my time outside of work and create boundaries between the two so that I can fill back up!

10. What's an abbreviated day in the life of your job?

Since I've had a baby, I work three days a week instead of four. We all alternate between roles in the clinic.

If I'm seeing appointments, I arrive at 8:00 am. I see about 15-16 appointments in the morning – one every 20-30 minutes. It's fast-paced. I have to think on my feet and make quick decisions and recommendations. I really enjoy the social interaction with clients during appointments. Some days I take a lunch break of an hour, some days the appointments spill into lunch. Then I make phone calls answering client questions for about an hour. In the afternoon, I see about four to six appointments. Then I make phone calls and go home around 7-7:30 pm.

Emergency days are challenging. I come in at 7:00 am. I examine and make plans for any pets in the hospital. Then I see things that need to be seen that day. Some are huge, life-threatening emergencies and others are more urgent care appointments (i.e., ear infections, pets that are vomiting, etc.). These days are unpredictable. Usually there is very little time for a break and I usually don't leave the hospital until 9:00 pm or later.

Surgery days are fun! I find surgery very relaxing. I arrive at 7:30 am to meet with all the clients leaving their pets for surgery. Then I'm in surgery from 9:00 am to about 1:00 or 2:00 pm. I love just listening to music and not really talking in surgery. Then I usually do eat lunch and work on my charts, call clients back, and leave by 6:00 or 6:30 pm! Get to see my son!

11. In one to two sentences, how would you say you use bioinformatics in your work? If you don't use bioinformatics directly in your work, how has bioinformatics impacted your career field?

While we often think of genetic testing in humans, we are seeing more and more tests being developed for my animal patients, and much of the research is being done at my *alma mater*, the Washington State University (WSU) College of Veterinary Medicine. For example, WSU's Veterinary Cardiac Genetics Laboratory (VCGL) has developed a test for hypertrophic cardiomyopathy, the most common form of heart disease in cats. The test for MyBPC mutations in Ragdoll and Maine Coon cats cannot prevent the disease, which often strikes without warning when cats are three or four years old, but it can prepare cat owners for what is happening to their pet.

I'm also excited about genetic testing for drug sensitivities in dogs. This has tremendous clinical use. Some breeds such as collies and their relatives, shelties, Australian shepherds, and long-haired whippets, are known to be potentially sensitive to particular drugs, and they are not used on them in the clinic because of fear of a fatal reaction. However, genetic testing for mutations in Multi-Drug Resistance 1 (*MDR1*) gene can distinguish between those dogs that are sensitive and those that are not, informing both breeding programs and treatment options for those dogs.

I think bioinformatics research will have profound effects on breeding programs, but we have to proceed with caution. There is the example of the Cavalier King Charles spaniels, who have a genetic predisposition to a deadly congenital heart condition called Mitral Valve Disease (MVD). In the 1980s, when dog breeders tried to select for animals without MVD, they did not appreciate the small Cavalier Spaniel gene pool which inadvertently led to selection for an equally deadly congenital spinal cord defect, *Syringomyelia*. As we learn more about these genetic conditions, and cross and develop new breeds with increased genetic diversity and fitness, hopefully these problems will be reduced.

12. Do you have any recommendations for students who are interested in entering your field?

Getting into college and veterinary school is becoming more and more competitive. Really focus on academics and grades while trying to still take in some extracurricular activities to present a well-rounded candidate.

13. What are your favorite hobbies?

I love being a mom. I enjoy exercise, such as running and hiking. I love cooking and eating. Being with people I love doing anything is what it's all about. I love to travel but haven't done much as of late.

Resources:

In the **field of veterinary medicine**, there are many different types of jobs available, depending upon what type of education and experience you have. For more information about different types of jobs in this field, including what you can do with different degrees (two year Associate's degree, four year Bachelor's degree, graduate, or professional degrees), visit NWABR's Student Career Center at: <http://www.nwabr.org/students/student-resource-center/career-center>.

The site also includes descriptions of and links to different types of degree programs, various career paths, resources on writing a resume and cover letter and evaluating online resources, and tips for successful job interviews.

All of the links below can also be accessed from NWABR's Student Career Center.

To learn about **job prospects**, **salary information**, and **job skills** ("**qualifications**") required for veterinarians, specifically those that research ways to treat human and animal problems, visit the US Bureau of Labor Statistics: <http://www.bls.gov/oco/ocos076.htm>.

For more information about genetic testing and research with animals, visit the Veterinary Genetics Laboratory at the University of California (UC) Davis. To see the tests available for different species, click the "Test Ordering and Information" button and then select your animal of interest. To learn more about the research being done at UC Davis using genetic techniques, click on the "Research" button and then click the name of the "Investigator" who works on your species of interest: <http://www.vgl.ucdavis.edu/>.

See Dr. Deborah Tegarden at Elliott Bay Animal Hospital: <http://elliottbayah.aahavet.org/web1/veterinarians.aspx>.

For more information about the Veterinary Cardiac Genetics Laboratory at Washington State University, visit: <http://www.cvm.ncsu.edu/vhc/csds/vcgl/index.html>.

About.com offers a Career Brief for Veterinarians: <http://careerplanning.about.com/od/occupations/p/veterinarian.htm>.


Some of the Resources above may also be used to research other careers that may be of interest to you in the future, including the Bureau of Labor and Statistics and the National Human Genome Research Institute.

Job Posting: Veterinary Genetic Research Summer Internship

The laboratory of Dr. Harriet Atman is looking for motivated individuals interested in learning about genetic risk factors for hip dysplasia in dogs. Hip dysplasia is the leading cause of painful arthritis in dogs, and is known to be a polygenic trait (involving multiple genes). Interns will work closely with lab members and help identify dogs to include in our research studies, analyze dog DNA samples, and help present research findings to other members of the veterinary genetic testing community. Prior lab experience is not necessary, but an understanding of molecular biology, including the role of DNA in encoding physical traits, is required. Applicants familiar with genetic testing and bioinformatics tools used to detect mutations are encouraged to apply. Additional computer skills, including Microsoft Office (Word®, Excel®, PowerPoint®), are preferred. Demonstrated experience of working closely with animals in the home, animal shelters, or other areas is particularly valuable. If applicants are interested in genetic research but do not enjoy working with animals, we suggest applying for an internship in the lab of Dr. Leo Frankos in the Department of Applied Genetic Research. Applicants must be hard-working, responsible, and able to work in a team environment. Address all inquiries to Dr. Harriet Atman, Seattle Research University, Veterinary Research Department, Suite 200, Seattle, WA.

7

Robin Bennett, MS Genetic Counselor

 <p>GENETIC COUNSELOR ROBIN BENNETT, MS</p> <p>Place of Employment: University of Washington</p> <p>Specialties: Huntington's disease, neurogenetics, cancer genetics, genetic family history, and ethical issues in genetic counseling and genetic testing</p> <p>"I feel privileged to be a part in some small way with each of my patients and that hopefully I have helped them with some difficult decisions and with making choices that work for them within their belief systems."</p>	<p>CAREERS IN SPOTLIGHT: GENETIC COUNSELOR</p> <p>What do they do?</p> <ul style="list-style-type: none"> • Review a patient's medical and family history. • Advise patients and their families about the benefits and consequences of genetic testing, and about the nature of genetic disorders. • Offer counseling consistent with the patient's belief system. <p>What kind of training is involved? Bachelor's degree and a two to three year Master's degree.</p> <p>What is a typical salary for a Genetic Counselor? Start at \$60,000/year (\$29/hour), and may make up to \$120,000/year (\$58/hour).</p>
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1. Where did you grow up?

I grew up on Mercer Island in Washington State and attended Mercer Island High School.

2. What do you do (i.e., what career or field are you in, what is the title of your position)?

I am a Senior Genetic Counselor and Co-Director of the Genetic Medicine Clinic at the University of Washington.

3. How did you choose your career? When did you first know this was the career you wanted?

I really hadn't cared too much for science until I had a wonderful biology teacher in tenth grade, Mr. Bill Tougaw. He taught science by telling stories and I was fascinated. He taught a course in Embryology and I was hooked. The field of genetic counseling was just starting. Once I learned about genetic counseling I knew it was the perfect fit because I could work in the fields of medicine and psychology but have more flexibility with my schedule to raise a family. I loved that the field was changing so rapidly that there were always new things to learn. This is still what I love most about being a genetic counselor almost 30 years later. I am honored to be among the first genetic counselors in the world and to have been a part of the growth of genetic counseling as a career.

4. Did your family support your decision to pursue your career?

Some days I feel that they still don't understand exactly what I do, but they were very supportive of my education and continue to support me in my career.

5. What is the highest level of education you have?

Currently, genetic counseling is a two year Master's Degree which is required from a program that is accredited by the American Board of Genetic Counseling. In the next five years there are likely to be Clinical Doctorate programs in genetic counseling which will probably be three years in duration. This change will allow genetic counselors more flexibility in seeing complex patients and reflects how complicated it has now become to provide genetic counseling because of the range of genetic tests now available.

6. What is the highest level of education reached by other members of your family?

My family is well-educated in unusual things! My father has a Master's in engineering and was a test pilot. My paternal grandfather was a family physician and my paternal grandmother was the nurse in his home office. My mother has a PhD in folklore. My sister has a PhD in mathematics and my brother has a Master's in plastic engineering.

7. What is the salary range for a person in your position?

It varies with experience but usually starts at about \$60,000 annually [\$29/hour] and some genetic counselors make at least \$120,000 annually [\$58/hour], plus benefits.

I suggest that you look at the professional salary survey for the National Society of Genetic Counselors for salary information at <http://www.nsgc.org>.

8. What do you like most about your job?

I learn so much from the people I counsel. I am inspired by the ways that they cope with such difficult problems that influence not only them but their whole family. I feel privileged to be a part in some small way with each of my patients and that hopefully I have helped them with some difficult decisions and with making choices that work for them within their belief systems. I love learning something new every day. I have never once had a boring day at work! I find genetic counselors to be very inquisitive and caring people. Usually I attend a genetics meeting once or twice a year for a few days; not only is it fun to see a new city but I love to be with my genetic counseling colleagues and learning from each other.

9. What do you like least about your job?

I am very sad when a patient dies, but even then I usually have learned something from the experience. All health care positions have too much paperwork and processes. I have trouble with administrators who seem more focused on their processes than the big picture of what helps patients and their families. Although genetic counselors are becoming more recognized on the health care team, it is still a relatively small profession. It can be hard to explain that you are not a generic counselor or a geriatric counselor.

10. What's an abbreviated day in the life of your job?

Most genetic counselors see patients two to three days a week, with four to eight patients daily. At a patient visit, we obtain family history information and draw a family pedigree. The pedigree is analyzed for patterns of inheritance and psychosocial clues to help the patient (for example, a patient may be particularly anxious about developing a disease like breast or colon cancer because he or she is now the age at which a relative also developed cancer). We would discuss genetic testing options, implications of the disease, ethical issues, and if testing is done, make a plan for disclosing test results (either by a return visit or a telephone appointment). Usually a summary letter is sent that includes recommendations to see other relatives. The rest of the time is spent following up on test results, researching genetic diseases, and often attending lectures to keep up in the field. Many genetic counselors give talks in the community about their work and often participate in research studies.

These studies might involve disease registries, looking at psychological issues, performing patient surveys, etc. I have been fortunate in being able to write many book chapters and even my own book. I have been able to give talks in Australia, Saudi Arabia, and Europe. I also volunteer in many ways for my profession including serving on various boards related to my profession (such as for the National Society of Genetic Counselors, the American Board of Genetic Counseling, and the American Society of Human Genetics).

11. In one to two sentences, how would you say you use bioinformatics in your work? If you don't use bioinformatics directly in your work, how has bioinformatics impacted your career field?

Genetic counselors are totally dependent on databases that help interpret genetic testing results, risk models for disease prediction, and electronic reviews in the medical and psychological literature. All medical records are or will soon be electronic. There are unique issues around electronic medical records in genetics because they may interface with records of other relatives.

12. Do you have any recommendations for students who are interested in entering your field?

Currently, the number of genetic counseling programs is still limited and thus they are quite competitive. It is good to get some experience (volunteering is fine) making sure you like working with people, especially people who can have quite severe problems. Working or volunteering in a hospital or other medical setting is important (it doesn't have to be with people with genetic diseases). Ideas include Planned Parenthood, a crisis phone line, a hospice, patient escort, even volunteering to do paperwork in a genetic clinic office. Most genetic counselors are happy to speak to you about what they do. It is good to talk to genetic counselors who work with different populations, such as those in prenatal genetics (working with pregnant patients), cancer genetics, and pediatric genetics. The National Society of Genetic Counselors has excellent materials on their website about genetic counseling including salary/job surveys (<http://www.nsgc.org>). The American Board of Genetic Counseling has information about the schools accredited to provide genetic counseling and the competencies needed to be a genetic counselor (<http://www.abgc.net>).

13. What are your favorite hobbies?

Is eating chocolate a hobby? I play the harp and piano, sew, swim, do needlework, garden, walk my dog, and hang with my family and friends.

Resources:

In the **field of genetic counseling**, there are many different types of jobs available, depending upon what type of education and experience you have. For more information about different types of jobs in this field, including what you can do with different degrees (two year Associate's degree, four year Bachelor's degree, graduate, or professional degrees), visit NWABR's Student Career Center at: <http://www.nwabr.org/students/student-resource-center/career-center>.

The site also includes descriptions of and links to different types of degree programs, various career paths, resources on writing a resume and cover letter and evaluating online resources, and tips for successful job interviews.

All of the links below can also be accessed from NWABR's Student Career Center.

The Commonwealth of Virginia has a "Career Guide for Genetic Counselors" that details the **skills, knowledge, abilities, and tasks** required to be a genetic counselor, as well as information on **education and training**. For more information, visit: <http://jobs.virginia.gov/careerguides/geneticcounselor.htm>.

Find information on careers in genetic counseling at the National Human Genome Research Institute, including information about **career outlook, working conditions, and salary**. Scroll through the career listings until you reach "Genetic Counselor" on page 2: <http://www.genome.gov/GenomicCareers/careers.cfm>.

The US Bureau of Labor and Statistics offers a little information about genetic counselors: <http://www.bls.gov/oco/ocos047.htm>.

Visit the National Society of Genetic Counselors for more information about genetic counseling and salaries:
<http://www.nsgc.org>.

Robin Bennett's homepage: http://depts.washington.edu/medgen/faculty/Robin_Bennett.shtml.

Some of the Resources above may also be used to research other careers that may be of interest to you in the future, including the Bureau of Labor and Statistics, and the National Human Genome Research Institute.

Job Posting: Genetic Counselor Assistant Summer Internship

The genetic counseling clinic of Dr. Margaret Parry-Perkins is looking for motivated individuals interested in learning about genetic counseling and testing for genetic conditions. Our clinic works with patients who have a history of genetic disease in their family. We help patients understand the genetic testing process and what their results mean. Interns will work closely with counselors and laboratory technicians who analyze the patient samples. Prior lab experience is not necessary, but an understanding of molecular biology, including the role of DNA in encoding physical traits, is required. Applicants familiar with genetic testing and bioinformatics tools used to detect mutations are encouraged to apply. Additional computer skills, including Microsoft Office (Word®, Excel®, PowerPoint®), are preferred. Demonstrated ability to communicate well with others, listen to the needs of others, and explain complex topics in an easy-to-understand way is particularly valuable. If applicants are interested in genetic research but do not enjoy working with patients, we suggest applying for an internship in the lab of Dr. Leo Frankos in the Department of Applied Genetic Research. Applicants must be hard-working, responsible, good communicators, and able to work in a team environment. Address all inquiries to Dr. Margaret Parry-Perkins, Seattle Research University, Genetic Counseling Clinic, Suite 300, Seattle, WA.

7 Zane Kraft, MS

Laboratory Technician

 <p>LABORATORY TECHNICIAN ZANE KRAFT, MS</p> <p>Place of Employment: Seattle Biomedical Research Institute</p> <p>Type of Work: HIV Vaccine Research</p> <p>"We do a lot of DNA alignments of HIV Envelope sequences. Another fun thing that we now do more of is computer modeling of HIV Envelope proteins with different mutations."</p>	<p>CAREERS IN SPOTLIGHT: LABORATORY TECHNICIAN</p> <p>What do they do? Laboratory technicians, sometimes called "lab techs" or research technicians, do much of the day-to-work in the lab, including purifying and sequencing DNA, caring for laboratory machinery, and analyzing samples.</p> <p>What kind of training is involved? Some laboratory technicians have a two year Associate's degree or a four year Bachelor's degree. Some, like Zane Kraft, also have a Master's degree (additional two year training).</p> <p>What is a typical salary for a Laboratory Technician? Ranges with experience from \$30,000/year (\$14/hour) up to \$60,000/year (\$29/hour) or more.</p>
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1. Where did you grow up?

I grew up in the Seattle area, in Kent, Washington to be exact. I went to Kentwood High School and for college I went to Central Washington University.

2. What do you do (i.e., what career or field are you in, what is the title of your position)?

I am a research technician, also called a laboratory technician, in an HIV vaccine lab at Seattle Biomed, a non-profit research institution in Seattle. I do a lot of different things in the lab – molecular biology, protein analyses, and cell-based assays.

3. How did you choose your career? When did you first know this was the career you wanted?

I think it all started in undergrad. Like so many others, I wanted to be an MD, and took lots of biology courses. I was in microbiology and went to talk to the professor and asked her what kind of work she was doing. I ended up liking it so much, I decide I wanted to keep on doing it!

4. Did your family support your decision to pursue your career?

Yes, definitely. The whole MD thing may have seemed much sexier, but overall they were very supportive.

5. What is the highest level of education you have?

I have a BS in biology with an emphasis in microbiology, and a Master's in biology. Both are from Central Washington University.

6. What is the highest level of education reached by other members of your family?

Bachelor's degrees. I don't think we have any PhDs in the family that I can think of.

7. What is the salary range for a person in your position?

For the non-profits, which is what I can attest to, the starting salary is at about \$30,000 [\$14/hour] and the upper limit is about \$65,000 [\$31/hour] – a pretty wide range, depending on educational background and years of experience.

8. What do you like most about your job?

Well, obviously, every lab is a little different, but my boss is pretty flexible, and lets us venture out on our own ideas and test some of them. The flexibility of the hours is common with most labs, as long as you get the work done. You come in when you want and leave when you need to. And you're always learning new stuff.

9. What do you like least about your job?

Obviously I wish it paid more. Being in the HIV vaccine field, there are also a lot of disappointments. I think I have a thick skin now.

10. What's an abbreviated day in the life of your job?

This is always such a tough question, because every day is so different. On the average day, I come in, set up some PCR reactions [polymerase chain reaction], and then do a cell-based assay. Later on I'll probably do some data analysis, some number crunching and basic statistics, and prepare graphs of my data.

11. In one to two sentences, how would you say you use bioinformatics in your work? If you don't use bioinformatics directly in your work how, has bioinformatics impacted your career field?

We do a lot of DNA alignments of HIV Envelope sequences. Another fun thing that we now do more of is computer modeling of HIV Envelope proteins with different mutations. With the new grant from the Gates Foundation, we're looking at protein scaffolds that mimic specific structures of Envelope that we could use as vaccines, and how they react to monoclonal antibodies we have in the lab. It's all done with computer models.

12. Do you have any recommendations for students who are interested in entering your field?

One thing I always hammer into all of our interns is, if they are interested in science, when they get into college, they should seek out labs that have research opportunities. Whether you're working for free or if you're lucky enough to be with a lab that will pay you, the experience helps you immensely later on. When we hire, that's one thing we screen for—what type of research they did as an undergrad. It's amazing how many students coming out of college have no research experience.

13. What are your favorite hobbies?

I like to rock climb in my spare time; that's my biggest hobby, and hiking – outdoor things. I would love for traveling to be a bigger hobby. The flexibility with my work schedule helps a lot with my hobbies – sometimes I can sneak out at 3:00 pm to go to the gym.

Resources:

In the **field of laboratory research**, there are many different types of jobs available, depending upon what type of education and experience you have. For more information about different types of jobs in this field, including what you can do with different degrees (two year Associate's degree, four year Bachelor's degree, graduate, or professional degrees), visit NWABR's Student Career Center at: <http://www.nwabr.org/students/student-resource-center/career-center>.

The site also includes descriptions of and links to different types of degree programs, various career paths, resources on writing a resume and cover letter and evaluating online resources, and tips for successful job interviews.

All of the links below can also be accessed from NWABR's Student Career Center.

To learn about **job prospects** and **salary information** for science technicians, visit the US Bureau of Labor Statistics: <http://www.bls.gov/oco/ocos115.htm>.

The Commonwealth of Virginia has a "Career Guide for Biological Technicians" which details the **skills, knowledge, abilities**, and **tasks** required to be a biological or laboratory technician, as well as sample career path for a technician, beginning with a Laboratory Aide and ending with a Laboratory Manager. For more information, visit: <http://jobs.virginia.gov/careerguides/BiologicalTechs.pdf>.

To read a Seattle Times article about the work Zane Kraft does, visit: http://seattletimes.nwsources.com/html/localnews/2003139170_aids20m.html.

About.com offers a Career Brief for Laboratory Technicians, which includes short summaries about required **education, job outlooks, salary**, and "**a day in the life**" of a Laboratory Technician: <http://careerplanning.about.com/od/occupations/p/laboratory-technician.htm>.

Some of the Resources above may also be used to research other careers that may be of interest to you in the future, including the Bureau of Labor and Statistics and the National Human Genome Research Institute.

Job Posting: Genetic Research Summer Internship

The laboratory of Dr. Leo Frankos is looking for motivated individuals interested in learning about genetic risk factors for bone cancer in young people and applied genetic research. Cancer is a complex disease, and our lab is working to identify genes that contribute to a higher risk for bone cancer, as well as genes that appear to protect people from developing bone cancer. We also develop cancer treatments, also called cancer therapeutics, in which we try to apply our genetic research findings directly to patient care. Interns will work closely with lab members and help analyze DNA samples, including DNA sequence analysis, and help present research findings to other members of the bone cancer research community. Prior lab experience is not necessary, but an understanding of molecular biology, including the role of DNA in encoding physical traits, is required. Applicants familiar with genetic testing and bioinformatics tools used to detect mutations are encouraged to apply. Additional computer skills, including Microsoft Office (Word®, Excel®, PowerPoint®), are preferred. Demonstrated experience of commitment and a willingness to learn new things is particularly valuable. Applicants must be hard-working, responsible, and able to work in a team environment. Address all inquiries to Dr. Leo Frankos, Seattle Research University, Department of Applied Genetic Research, Suite 400, Seattle, WA.


LESSON 7

CLASS SET

7

Beth Anderson

3D Animator

 <p>3D ANIMATOR BETH ANDERSON</p> <p>Place of Employment: Arkitek Studios</p> <p>Type of Work: Develops 3D animations that help scientists and students visualize biological processes.</p> <p>"Bioinformatics interfaces in many ways with our work, from data that clients give us to visualize representationally, to creating actual visual data."</p>	<p>CAREERS IN SPOTLIGHT: 3D ANIMATOR</p> <p>What do they do? 3D animators are visual communicators and designers. The animations and illustrations they create help communicate scientific content and ideas to scientists, students, and educators.</p> <p>What kind of training is involved? Associate of Arts degree. Some also have training in biology, illustration, graphic design, and computer animation.</p> <p>What is a typical salary for a 3D Animator? Ranges with experience from very little when people are first starting out to \$100,000/year (\$48/hour) or more.</p>
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1. Where did you grow up?

Oak Ridge, Tennessee, Atomic City, USA.

2. What do you do (i.e., what career or field are you in, what is the title of your position)?

3D Animator and business owner. Officially I'm CEO and co-founder of Arkitek Studios, a visual communications design group specializing in content development for the science, technology, and education communities.

3. How did you choose your career? When did you first know this was the career you wanted?

When I was working for my father in his biotech company, building biotech equipment, I learned AutoCad, knew that I wanted to make things move.

4. Did your family support your decision to pursue your career?

Yes, but I was stubborn. I put myself through school (and am still paying for it!).

5. What is the highest level of education you have?

AA – Associate of Arts, Art Institute of Seattle.

6. What is the highest level of education reached by other members of your family?

Father – PhD in Biochemistry, Duke University. Brother Leigh – PhD in X-Ray Crystallography from Cambridge, under Max Perutz.

7. What is the salary range for a person in your position?

Oh boy, that's a loaded one. Anywhere from nothing when people are first starting out, building their business, to six figures and beyond. I'm sad to say I am not in that category.

8. What do you like most about your job?

It's different every day.

9. What do you like least about your job?

It's different every day. Sometimes I would like a job I didn't have to think about so hard.

10. What's an abbreviated day in the life of your job?

Check email around 7:00 am, get into work around 9:00 am, check email and work with clients for an hour, then hop onto any administrative work that needs to be done, then have lunch. Then work on production until it's time to go home, around 6:30 pm. I'm very proud to say that we now actually **stop** working around 6:30 pm; it used to be 10:00-11:00 pm before we stopped.

11. In one to two sentences, how would you say you use bioinformatics in your work? If you don't use bioinformatics directly in your work, how has bioinformatics impacted your career field?

Bioinformatics, or list biology, as it was originally termed, interfaces in many ways with our work, either from data that clients give us to visualize representationally, or to create actual visual data.

12. Do you have any recommendations for students who are interested in entering your field?

- a. You'll never learn anything you won't find you use later on, no matter how esoteric or boring you think it is now. I have proven that to myself countless times.
- b. Technology and technique are cool things, but they will be useless if you can't communicate well with other people, or if you don't actually **care** about how others see the world. I've seen way too many talented people who don't play well with others sabotage themselves personally and professionally by not thinking about the other guy. Compassion trumps all.

13. What are your favorite hobbies?

Motorcycles, dogs, books, learning something new every day no matter how insignificant, travel, making people I don't know smile.

Resources:

In the **field of animation**, there are many different types of jobs available, depending upon the type of education and experience you have. For more information about different types of jobs in this field, including what you can do with different degrees (two year Associate's degree, four year Bachelor's degree, graduate, or professional degrees), visit NWABR's Student Career Center at: <http://www.nwabr.org/students/student-resource-center/career-center>.

The site also includes descriptions of and links to different types of degree programs, various career paths, resources on writing a resume and cover letter and evaluating online resources, and tips for successful job interviews.

All of the links below can also be accessed from NWABR's Student Career Center.

To learn about **job prospects** and **salary information** for multi-media artists and animators, visit the US Bureau of Labor Statistics: <http://www.bls.gov/oes/current/oes271014.htm>.

Find information on medical illustration at the National Human Genome Research Institute, including information about **career outlook**, **working conditions**, and **salary**. Scroll through the career listings until you reach "Medical Illustrator" on page 3: <http://www.genome.gov/GenomicCareers/careers.cfm>.

About.com offers a Career Brief for Animators, including **employment facts**, **job requirements**, and **salary information**: <http://careerplanning.about.com/od/occupations/p/animation.htm>.

AllArtSchools also offers information about careers in animation, including **job skills needed to be an animator**, and assistance in **finding a school that offers programs in animation**. Visit: <http://www.allartschools.com/faqs/animation-career>.

Beth Anderson's company is called Arkitek Studios. Visit their homepage at: <http://www.arkitek.com/>.

Some of the Resources above may also be used to research other careers that may be of interest to you in the future, including the Bureau of Labor and Statistics and the National Human Genome Research Institute.

Job Posting: Biological Animation Summer Internship


The studio of Ms. Anne Gottsling is looking for motivated individuals interested in learning how to develop 3D animations to help high school students learn about biology. Research on education and learning supports the theory that students learn better by doing biology instead of just reading about it in textbooks. However, not all science classes have the facilities to perform all types of laboratory experiments. Ms. Gottsling is developing "virtual laboratories" for students to perform experiments using computers. The current project explores laboratory experiments that are performed with patient samples and genetic testing, including DNA purification, DNA sequencing, and bioinformatics analyses. Prior lab experience is not necessary, but an understanding of molecular biology, including the role of DNA in encoding physical traits, is required. Applicants familiar with genetic testing and bioinformatics tools used to detect mutations are encouraged to apply. Additional computer skills, including Microsoft Office (Word®, Excel®, PowerPoint®), are preferred. Demonstrated artistic experience or ability, as well as a willingness to learn new things, is particularly valuable. Applicants must be hard-working, responsible, and able to work in a team environment. Address all inquiries to Ms. Anne Gottsling, Seattle Research University, Biological Animations Studio, Suite 500, Seattle, WA.

LESSON 7

CLASS SET

7

Kelly Edwards, PhD Bioethicist

 <p>BIOETHICIST KELLY EDWARDS, PhD</p> <p>Place of Employment: University of Washington</p> <p>Specialties and Interests: Community-based research practices, environmental justice, and integrating ethics into training programs and public policy.</p> <p>"Genetics researchers often work with distinct communities. To take moral account of how their research affects these communities, they need a richer conception of justice and they need to make those communities equal participants in decision-making about how the research is conducted and what is produced and published out of it."</p>	<p>CAREERS IN SPOTLIGHT: BIOETHICIST</p> <p>What do they do? Bioethicists study the ethical controversies that arise from advances in biology and medicine. They advise patients, researchers, public policy makers, and/or medical doctors about a wide range of ethical issues, from genetic testing and medical treatment to medical privacy and stem cell research.</p> <p>What kind of training is involved? Training varies widely from a certificate program completed during or after a Bachelor's degree in another subject to Doctoral (PhD) programs specifically in Bioethics.</p> <p>What is a typical salary for a Bioethicist? Depending upon training, anywhere from \$50,000/year (\$24/hour) to over \$100,000/year (\$48/hour).</p>
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1. Where did you grow up?

I grew up in Federal Way, Washington. My family is fourth generation Washington State, and we would spend summers visiting our family cabin in the Blue Mountains outside of Walla Walla or my family's property up the Teanaway River Valley outside of Cle Elum. I love this state!

2. What do you (i.e., what career or field are you in, what is the title of your position)?

I am on faculty in the Bioethics Department in the School of Medicine and also the School of Public Health. I am in a grant-funded faculty position, which means I am involved with a number of research projects and teaching efforts which makes for a really interesting mix of issues in ethics of medicine and science.

3. How did you choose your career? When did you first know this is the career you wanted?

It really surprised me. I went to Occidental College in Los Angeles and they emphasized building writing and critical thinking skills. They asked us to sign up for "freshman writing seminars" by choosing from a list of topics the faculty there were passionate about teaching. Not knowing anything, I selected "Personal Identity, Immortality, and the Meaning of Life." It turned out to be taught by a philosophy professor and was all about death and dying. This was 1985 and I had never heard of "bioethics" – it was hardly yet a field – but some major landmark cases were going on in that decade that were pushing the issues of when should we withdraw or withhold life-sustaining treatment from someone. Medicine and technology had progressed to the point where we had to ask not just can we do this, but should we. I was captivated by these questions, as it was the first time I had been faced with questions where there was no clear right answer. That hooked me. I ended up majoring in philosophy (which my dad said would only set me up for being a bartender ☺), but did not realize bioethics could actually be a career to pursue until four years after college, when I was encouraged to look at the MA in Bioethics program at the University of Washington.

4. Did your family support your decision to pursue your career?

Absolutely. Despite the funny remark from my dad above, they have always supported me and my sister in pursuing our passions and interests, as long as we find our own way to make a contribution to the world. (My sister is in environmental education and ecotourism in Alaska.)

5. What is the highest level of education you have?

PhD in Philosophy of Education.

6. What is the highest level of education reached by other members of your family?

My Dad has a MBA, and my mom had a BA until she went back for her BSN in her 40s. Her father was a general surgeon. My family has always valued education and appreciated there were a variety of ways to contribute to the world.

7. What is the salary range for a person in your position?

It depends on the university and geographic region, and your seniority and experience. Starting salary for a junior faculty in bioethics can be \$60,000-\$80,000 [\$29-\$38/hour] and our more senior faculty at UW (who are PhDs but not MDs) are in the range of \$90,000-120,000 [\$43-\$58/hour].

8. What do you like most about your job?

I feel very lucky for my job and I like a number of things about it. One is the flexibility to work on a number of different projects and issues. The work is always evolving and emerging along with issues in science and medicine so we are always learning. I also love the people that I work with – all my work occurs in interdisciplinary teams.

9. What do you like least about your job?

It can be stressful to be a grant-funded faculty member, as you have to be constantly on the lookout for the next funding opportunity. In the current funding climate, federal funds are very competitive. This means you can spend a lot of your time writing grants with no guarantee of being successful. Our group has been very lucky and I've been fully funded for the ten years I have been in this position. Because the work never ends, you have to be willing and able to draw boundaries to make sure you are still taking care of yourself and making time for exercise, friends, and family.

10. What's an abbreviated day in the life of your job?

Every day is really different (hence the variety I like), but here's an example: Get up at 6:00 am and respond to email for about an hour or two. Bike to my first meeting, which could be in one of three research buildings around the city. Have a series of meetings with research teams, or some of our clinical teachers at the medical school about teaching approaches for ethics and professionalism with the medical students, or with hospital staff and leadership about if and how we should use clinical data for research purposes. I might give a guest lecture on ethics to one of several graduate seminars or medical groups, then bike home, sometimes via the yoga studio. Often my days are filled with meetings so I need to do my own writing in the evenings. And then get up and see what's on the calendar for the next day!

11. In one to two sentences, how would you say you use bioinformatics in your work? If you don't use bioinformatics directly in your work, how has bioinformatics impacted your career field?

I collaborate actively with colleagues in our Biomedical Health Informatics Core within our Institute for Translational Health Sciences. We are interested in the use of health information in research, how to do data sharing effectively and ethically, and how technical systems can help support ethical biobanking practices. For example, using computer interfaces to manage participant preferences and information flow in a research project.

12. Do you have any recommendations for students who are interested in entering your field?

Be creative and curious. All fields have ethical dimensions to their work, so you can have a subspecialty in ethics even if you are focusing on a different area of work. As an emerging field, you can create your own path in bioethics (there is not a set career path). We come from many different core disciplines: philosophy, theology, health services, anthropology, sociology, biology, public health, law, etc.

13. What are your favorite hobbies?

I love being outside in any form. I love backpacking in our Cascade Mountains and skiing or snowshoeing in the winter. My sister is teaching me how to telemark ski so I can get into the backcountry. I also enjoy finding interesting urban spaces for picnics and listening to live music.

Resources:

In the **field of bioethics**, there are many different types of jobs available, depending upon what type of education and experience you have. For more information about different types of jobs in this field, including what you can do with different degrees (two year Associate's degree, four year Bachelor's degree, graduate, or professional degrees), visit NWABR's Student Career Center at: <http://www.nwabr.org/students/student-resource-center/career-center>.

The site also includes descriptions of and links to different types of degree programs, various career paths, resources on writing a resume and cover letter and evaluating online resources, and tips for successful job interviews.

All of the links below can also be accessed from NWABR's Student Career Center.

Find information on careers in bioethics and genomics at the National Human Genome Research Institute, including **career outlooks, working conditions, and salary information**. Scroll through the career listings until you reach "Bioethicist Using Genomics" on page 1: <http://www.genome.gov/GenomicCareers/careers.cfm>.

To learn about **careers in bioethics**, visit: <http://bioethics.virginia.edu/careers.html>.

For more information about reliable bioethics career resources visit "Bioethics Resources of the Web" at: <http://bioethics.od.nih.gov/careers.html>.

Dr. Kelly Edwards's homepage: http://depts.washington.edu/bhdept/facres/kfe_bio.html.

Some of the Resources above may also be used to research other careers that may be of interest to you in the future, including the Bureau of Labor and Statistics and the National Human Genome Research Institute.

Job Posting: Bioethics Summer Internship

The Bioethics Group at Seattle Research University, under the leadership of Dr. MaryEllin Sundberg, is seeking motivated individuals interested in bioethical and policy issues related to direct-to-consumer (DTC) genetic testing. Direct-to-consumer genetic testing refers to genetic tests that are marketed directly to consumers, such as through the internet, without involving a doctor or insurance company. While advocates claim that this allows consumers greater access to their own genetic information, critics are concerned that the public is not fully educated about the risks such information can bring, or the clinical validity of DTC genetic testing results. The Bioethics Group will be hosting focus groups with community groups on issues related to DTC genetic testing, and will develop a report summarizing our findings, which will be submitted to the United States Congress to inform future public policy debates. Prior lab experience is not necessary, but an understanding of molecular biology, including the role of DNA in encoding physical traits, is required. Applicants familiar with genetic testing, DTC genetic testing, and bioinformatics tools used to detect mutations are encouraged to apply. Additional computer skills, including Microsoft Office (Word®, Excel®, PowerPoint®), is preferred. Interest in bioethics and familiarity with bioethical principles is required. Demonstrated ability to communicate well with others, listen to the needs of others, and explain complex topics in an easy-to-understand way is particularly valuable. Applicants must be hard-working, responsible, and able to work in a team environment. Address all inquiries to Dr. MaryEllin Sundberg, Seattle Research University, Bioethics Group, Suite 600, Seattle, WA.

LESSON 7

CLASS SET

8

Genetic Testing Unit Assessment: *ALAD* and *SOD1*

Introduction

As an assessment of the unit, students revisit some of the bioinformatics tools they have used in prior lessons to locate a mutation in a protein associated with a harmful genetic condition. Students also evaluate current genetic tests for the condition using the criteria of clinical validity and treatment options. Two conditions and their tests are presented: porphyria and amyotrophic lateral sclerosis (ALS).

Learning Objectives

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

- Genetic tests vary in terms of utility.
- Similar bioinformatics and bioethical tools can be used when assessing the utility of different genetic tests.

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

- Apply bioinformatics tools for comparing and visualizing information to a new genetic testing example.
- Evaluate a genetic test in terms of its clinical validity, available treatment options, and any related ethical considerations.

Key Concepts

- Bioinformatics tools can be used to compare sequences and identify differences.
- Visualization software can help researchers understand how genetic changes impact protein structure.
- Mutations can impact the ability of a protein to fulfill its function, which can in turn result in disease.
- Genetic tests vary in terms of clinical validity and available treatment options.

Class Time

One class period (50 minutes).

Prior Knowledge Needed

- Completion of *Lessons One* through *Six*.

Common Misconceptions

- All genetic tests are similar in their utility.
- All genetic disorders are similar in their modes of inheritance.

Materials

Materials	Quantity
Class set of Student Handout— <i>Porphyria</i>	1 per student for half of class (class set)
Class set of Student Handout— <i>Amyotrophic Lateral Sclerosis</i>	1 per student for half of class (class set)
“Amino Acid Abbreviations and Chemistry Resources” (Optional for Challenge Questions, found in the <i>Appendix</i>)	1 per student
Teacher Answer Key— <i>Porphyria</i>	1
Teacher Answer Key— <i>Amyotrophic Lateral Sclerosis</i>	1

Computer Equipment, Files, Software, and Media
Assessment protein sequences can be downloaded from http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing . Provide local copies for students, or ask them to download the sequences directly.
<i>Lesson Eight</i> PowerPoint® Slides—Bioinformatics and Genetic Testing. 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 .
Computer lab with internet access for students, the program Cn3D, and a word processing program such as Microsoft Word® or Google Docs for answering the assessment questions.

Teacher Preparation

- Make copies of the Student Handouts, one per student, with half of the class working on porphyria, and half of the class working on ALS. Alternatively, the entire class could work on the same condition, or pairs of students can be assigned to work on different conditions together. These handouts are designed to be reused as a class set.
- Teachers will need to provide the protein sequences for ALS and porphyria, both the reference sequence and the patient sequence for each disease. These sequences should be in an electronic format in a central location where students will have access to them during class, or they can be accessed directly from the NWABR website. These sequences can be found at: <http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing>.
- A PowerPoint® presentation for *Lesson Eight*, which includes the answer key for both activities, is posted on the curriculum website. This can be used to review students’ answers.

Procedure

1. Explain to students the **aim of this lesson**. Some teachers may find it useful to write the aim on the board.

Lesson Aim:

- Apply what you have learned about genetic testing to a new genetic disorder.

Teachers may also wish to discuss the Learning Objectives of the lesson, which are listed at the beginning of this lesson plan.

2. Remind students that they have learned about many aspects of genetic testing, including: 1) comparing patient DNA sequences to reference sequences to identify mutations; 2) viewing the three-dimensional structure of a protein to determine where a mutation is located and what effect a particular mutation may have on the function of the protein; and 3) considering the ethical issues involved in genetic testing.
3. Explain to students that in today's lesson, they will be applying each of these skills to the study of a different genetic disease: either porphyria or Amyotrophic Lateral Sclerosis (ALS).
4. Either assign students to one of the two diseases (porphyria or ALS), allow students to pick one of the diseases to study, or assign pairs of students to work on different conditions together.
5. Pass out Student Handout—*Porphyria* to the porphyria group(s) and Student Handout—*Amyotrophic Lateral Sclerosis* to the ALS group(s). Students will need to use computers with internet access.
6. Tell students to read the background information about their assigned genetic condition before beginning the activities in *Parts I, II* and *III*.
7. Tell students where to find the electronic versions of the patient and reference protein sequences for both genetic disorders, as well as the protein structure files.
8. Students will perform a BLAST alignment first using the protein sequences (*Part I*), then examine the protein structure and identify where the mutation is located (*Part II*), and last, discuss the ethical considerations of genetic testing for their assigned condition (*Part III*). For *Parts I* and *II*, students will document their work with screen capture images that they will save in a word processing document, along with their answers to the questions from Student Handout—*Porphyria* or Student Handout—*Amyotrophic Lateral Sclerosis*.
9. Students should turn in the word processing document with their completed answers, via email or print. The assignment could also be finished as homework.

[Note: Scientists use the one letter amino acid abbreviations for protein sequences. (See the *Appendix*, "Amino Acid Abbreviations and Chemistry Resources," for a list of the one letter amino acid codes and chemistries, if needed).]

Closure

10. Tell students that in today's lesson, they applied what they had learned in earlier lessons to a genetic condition that was new to them. All genetic testing involves comparing DNA or protein sequences obtained from patients to reference sequences, as students did with BLAST. If the structure of the protein is known, as in the case of porphyria and ALS, scientists and doctors can locate the mutation on the protein structure to try to determine how that mutation might affect the function of the non-mutated protein. It is important to realize that not all genetic tests are equally useful, and not all genetic disorders are inherited in the same way. For example, most cases of ALS are not hereditary, and there is no effective treatment. ALS is usually caused by a sporadic mutation, so genetic testing and family pedigrees are not likely to be useful. In contrast, porphyria is hereditary and there are effective treatments available, so genetic testing would be useful.

11. Students have seen throughout these lessons that many different people, representing many different types of careers, make genetic testing possible. These include:

- The **bioengineers** who design the machines like the DNA sequencer.
- The **genetic counselors** and **veterinarians** who work directly with human and animal patients.
- The **laboratory technicians** who collect and sequence patient samples.
- The **3D animators** who help us represent and understand massive amounts of biological data.
- The **bioethicists** who help us understand the ethical implications of genetic testing, for ourselves, our families, and our communities.

Direct-to-consumer genetic testing offers individuals greater access to their genetic information, but it is not without its challenges and risks. As science and technology continue to advance, today's high school students face a future full of increasing amounts of genetic information. What they choose to do with it will be up to them.

Extension

- Challenge students to use their understanding of amino acid chemistry and the program Cn3D to develop a hypothesis about how their particular mutation might impact protein binding or some other function.
- Challenge students to research nucleotide and protein sequences for a disease or disorder of their choosing, and try to find an associated protein structure. In some cases, the protein structure may have been solved for only part of the protein. As students saw with the BRCT domain of BRCA1, while only part of the BRCA1 protein structure is known, there is still much to learn about how mutations can impact the protein's function.
- Facilitate a discussion about the pros and cons of direct-to-consumer genetic testing. Ask students to reflect upon whether their views about direct-to-consumer genetic testing have changed since they were first exposed to the topic in *Lesson One*.

Glossary

Amyotrophic Lateral Sclerosis (ALS): A progressive, fatal neurodegenerative disease that results in the degradation of motor neurons.

Free radicals: Highly reactive molecules capable of causing tissue damage and enhancing the effects of aging.

Heme: An iron-containing molecule that forms the non-protein portion of hemoglobin and is essential for the transport of oxygen by hemoglobin.

Hemoglobin: A protein responsible for transporting oxygen in the blood of vertebrates.

Heterogen: Any molecule that is not part of a DNA, RNA, or protein chain, such as a metal ion.

Motor neurons: Nerve cells that form the pathways for signals that pass along the brain and spinal column.

Porphyria: A rare hereditary disease in which the blood pigment hemoglobin is abnormally metabolized. Porphyrins are excreted in the urine, which becomes dark. Other symptoms include mental disturbances and extreme sensitivity of the skin to light.

Credit

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8

Porphyria (Poor-fear-E-ah)

(From *porphyrus*, meaning purple, a reference to the darkly colored urine of patients with porphyria)



Photo Credit: Wikimedia Commons. http://en.wikipedia.org/wiki/File:George_III_in_Coronation_Robes.jpg.

Heme: An iron-containing molecule that forms the non-protein portion of hemoglobin and is essential for the transport of oxygen by hemoglobin.

Hemoglobin: A protein responsible for transporting oxygen in the blood of vertebrates.

Background

King George III, who ruled England at the time of the American Revolution, was termed “mad” because of his bizarre and unusual behavior. His symptoms (severe pain in his abdomen, delirium, confusion, dark red urine) were not understood at the time. Some historians believe that his medical condition – which was not diagnosed until the 1970s as porphyria – may have influenced his ability to rule the colonies. If so, porphyria may be one reason that the United States became a separate country!

Porphyria is related to a defect in the manufacture of **heme**, an important part of **hemoglobin** (the oxygen carrier in red blood cells). Each molecule of the protein hemoglobin contains four heme groups, which are the site of oxygen binding.

There are at least eight types of porphyria, and they are very different from each other. However, in all porphyrias molecules that are precursors to heme build up in the body. There are eight steps in making heme from the amino acid glycine, and each step requires a different enzyme. If there is a mutation in one of these enzymes, too many precursors could be made, and not enough heme. This is the case when there are mutations in the gene *ALAD*, the second step in the path to making heme.

Most of the symptoms of porphyria impact the nervous system or skin, and are not very specific (such as blistering or burning of areas of skin exposed to the sun). There is no cure for porphyria, but each type of the disease has treatments available (such as the heme therapy “Panhematin”). Mild attacks are treated with intravenous glucose, and a high carbohydrate diet is recommended for patients.

DNA testing for porphyria will detect most of the known disease-causing mutations. If mutations are detected, it can provide relief to families to know that the symptoms are related to porphyria rather than a mental illness.

Although labor-intensive and expensive, DNA testing is very reliable. However, many different genes could cause porphyria, so often multiple genes need to be tested. There are no common mutations so each possible gene must be completely sequenced in each new family.

PART I: BLAST Protein Alignment

ALAD is a gene that encodes one enzyme in the pathway to making heme. Take the reference *ALAD* protein sequence and use BLAST to align the amino acid sequences of the two proteins to determine where the mutation is (similar to Student Handout—*Aligning Sequences with BLAST Instructions* from Lesson Four).

LESSON 8

CLASS SET

1. Go to the NCBI BLAST page: <http://blast.ncbi.nlm.nih.gov/Blast.cgi>.
2. Select "**protein blast.**"
3. Check the box "**Align two or more sequences.**" A second text box will appear.
4. Copy the ALAD Reference sequence into the first box and the patient ALAD sequence into the second box. Be sure to include the ">" symbol.
5. Click "**BLAST.**"
6. Once your search is complete, click the "**Formatting options**" link, and use the drop-down menu under the "**Alignment View**" to select "**Query-anchored with dots for identities.**" Click the "**Reformat**" button in the upper right corner.
7. Open your word processing software and create a new document. You will use this document for recording your answers and saving screen images while you work. Label the document your LASTNAME_ALAD_NCBI. Type your name, class period, and date at the top of the blank page. Save the document once you have pasted in your alignment.
8. Capture an image of the aligned sequences from the BLAST results page and paste it in your document. Add a descriptive title to describe the image and save your document.
9. Answer the following questions under your screen shot in your document:

Are there any differences between the reference (query) sequence and the patient sequence?
If so, answer the following:

 - a. Specify where the change is by describing its location by number within the protein (for example, at position 181, etc.).
 - b. Use the one-letter amino acid abbreviations to describe which amino acid has changed, and what the new amino acid is. (For example, M has changed into R).
 - c. Describe whether the change is a substitution (one amino acid exchanged for another), an insertion (an amino acid where there was none before), a deletion, or some other kind of change.
10. Answer the following question in several complete sentences: How can tools such as BLAST help scientists study and treat genetic diseases?

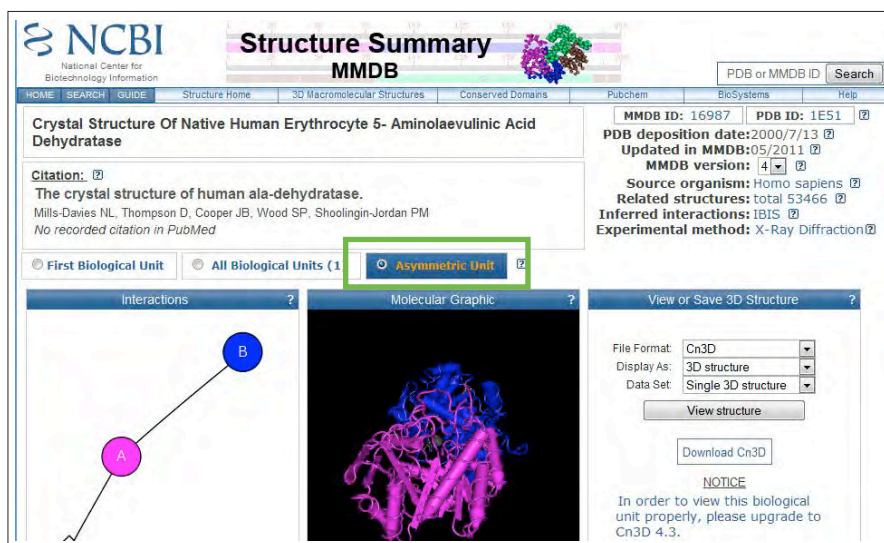


Figure 1: Select the "Asymmetric Unit."
Credit: NCBI Structure, Molecular Modeling Database (MMDB).

PART II: Protein Visualization Using Cn3D

In *Part II*, you will visualize both the reference protein and the location of the mutation in a mutant protein using the Cn3D program. First, we're going to investigate the structure of a functional protein (1E51). Then, we will look at a structure from a protein with the same mutation we identified in *Part I* (1PV8).

11. Go to the NCBI structure page: <http://www.ncbi.nlm.nih.gov/Structure/index.shtml>.
12. In the search box, type the structure accession number "**1E51**" and click "**Go.**"
13. Click on the picture of the structure or the structure title to go to the Structure Summary.
14. Select the option "**Asymmetric Unit**" in the middle of the screen (see **Figure 1**).
15. Click "**View structure**" and open the file in Cn3D.

[Note: depending on your browser, you may have to download the file and then open it in Cn3D.] Notice that there are two identical chains of amino acids that interact with each other (1E51_A and 1E51_B), one of which is colored pink and the other blue.

16. In the Sequence Viewer window, move your cursor until you come to the position of the mutation identified in *Part I: BLAST Protein Alignment*. (For example, if you found in *Part I* that the mutation was at position 8, you would move your cursor over the amino acids until you saw that you were over amino acid 8 in the bottom left window of the Sequence Viewer.) Be sure to confirm that you are looking at the correct amino acid by double-checking the sequence of the amino acids immediately before and after the mutation. There is often more than one of each type of amino acid in a protein sequence.
17. Hold down the "**CONTROL**" key ("**Command**" on Mac) and click to select the amino acid in that position in both chains in the Sequence Viewer. The individual amino acids that you just selected should be highlighted yellow.
18. Turn the structure around to see where the mutation is located relative to the two protein chains.



19. In your word processing document, in one to two sentences, describe where the mutation is located in the protein structure.

20. Experiment with different "**Style -> Rendering Shortcuts**" and zoom in and out until you find an image that you think shows the mutation location well relative to the overall shape of the protein (such as ball and stick or spacefill).



21. Capture the image of this protein structure with the location of the mutation highlighted, insert it into your word processing document, and type a descriptive title for your image.

22. Return to the NCBI structure page and find and open the structure "**1PV8**," making sure to select the asymmetric unit before opening the structure, as you did with the previous protein. 1PV8 is the structure of the mutant protein.

23. Use the same "**Rendering Shortcuts**" as you did with the reference protein structure.

24. Highlight the mutation location as you did with the reference protein structure.

25. Rotate the protein so that it is oriented in approximately the same way as the reference protein structure image, to see how the mutation affects the shape of the protein.



26. Capture the image of the mutated protein structure, insert it into your document, and type a descriptive title for your screen image.

LESSON 8

CLASS SET



27. Answer the following questions under your screen images of the reference and mutated protein structures in your document:

- In general, how can mutations impact the function of a protein?
- How might the specific mutation that you looked at impact the protein?
- Answer the following in a few complete sentences: How can tools such as Cn3D help scientists study and treat genetic diseases?



Challenge: Look up the full names of the amino acids involved in the mutation using the “Amino Acid Abbreviations and Chemistry Resources” showing one-letter abbreviations. How are they chemically different? How might this difference impact the protein’s function?



Optional: Use your drawing tools in Word® to use an **arrow** to point out the location of the mutation in the sequence alignment in the BLAST sequence window and in the Cn3D view. (“**Insert Shapes -> Arrow -> Color**”). If you prefer, you can draw **red circles** instead. (“**Insert Shapes -> Oval -> Shape Fill: no fill, Shape outline: red line**”). If you want to crop the image, you can use the **Crop** feature in Word®.

PART III: Genetic Testing

Use the information in the background section, as well as the knowledge you gained from the genetic testing lessons in this unit, to **answer the following questions in your word processing document:**



28. How clinically valid is the genetic test? (Low, Medium, or High) Explain why you chose that level.



29. Is there an effective treatment for this condition, whether medical or behavioral? Explain your answer.



30. If you were a genetic counselor, and this condition was found in your patient’s family, would you recommend that your patient be tested for this condition? Why or why not?



31. If you were a genetic counselor, and this condition was not found in your patient’s family, would you recommend that your patient be tested for this condition? Why or why not?



32. What are the characteristics of a good genetic test, in your opinion? To what extent do direct-to-consumer genetic tests that consumers can purchase on their own meet your criteria?

Turn in your Microsoft Word® document in the manner specified by your teacher.

8 Amyotrophic Lateral Sclerosis (ALS)

(ah-my-uh-tro-fik lah-tuh-rul skluh-ro-sis)

Background

Stephen Hawking, a highly regarded theoretical astrophysicist, has a condition called Amyotrophic Lateral Sclerosis (ALS). ALS causes progressive loss of neuromuscular control. Despite his physical challenges, Hawking served as Professor of Mathematics at the University of Cambridge for thirty years. He is best known for his influential theories on black holes and quantum gravity and his popular book, *A Brief History of Time*. Hawking was one of the youngest people elected to the British Royal Society, and won the 2009 US Presidential Medal of Freedom. Hawking uses a voice synthesizer that converts his typed messages into speech.



Photo Credit: Wikimedia Commons. http://en.wikipedia.org/wiki/File:Stephen_Hawking.StarChild.jpg.

Motor neurons: Nerve cells that form the pathways for signals that pass along the brain and spinal column.

Free radicals: Highly reactive molecules capable of causing tissue damage and enhancing the effects of aging.

Amyotrophic means that the muscles have lost their nourishment (from Greek, “a” = without, “myo” = muscle, “trophic” = nourishment). **Lateral** refers to the sides of the spinal cord, where the nerves that interact with muscles are found (“lateral” = side). **Sclerosis** means the diseased portion of the spinal cord becomes hardened or scarred (“sclerosis” = hardening or scarring).

ALS affects nerve cells in the brain and the spinal cord. The **motor neurons** that reach from the brain to the spinal cord and from the spinal cord to the muscles in the body degenerate and eventually die. As a result, brain signals can no longer start and control muscles. In later stages of the disease, patients may become paralyzed.

ALS is hereditary in only a small percentage of families; 90% of individuals with adult-onset ALS do not have a family history of the disease. Such individuals have “sporadic” ALS. There is a genetic component to this type of ALS but it is not inherited; it arises during an individual’s development. Because most individuals do not have hereditary ALS, ALS is usually diagnosed by a doctor who reviews a person’s symptoms and medical tests. When ALS is inherited (in 10% of cases), it is usually inherited in an autosomal dominant manner. Changes in a gene on chromosome 21, superoxide dismutase (*SOD1*), have been found in about 20% of families with inherited ALS. The *SOD1* protein normally detoxifies **free radicals**, molecules that are harmful to cells. Somehow, changes in *SOD1* result in harm to motor neurons. Both prenatal and pre-symptomatic tests for *SOD1* changes related to ALS exist. However, most inherited ALS (80%) is not due to changes in *SOD1*, and currently there are no other genetic tests to offer. Inherited (or “familial”) ALS is usually diagnosed based on family history. The two types of ALS do not differ in terms of symptoms. A



positive genetic test does not change the way that ALS is treated, but it may allow families to plan for the future more effectively. However, a positive genetic test does not indicate precisely when someone with ALS will begin to show symptoms. Other genes in addition to *SOD1* are involved in ALS, but tests have not been developed for these genes. So an individual who has a family history of ALS but tests negative for mutations in *SOD1* may still develop ALS.

Scientists have developed a way to study ALS using mice, based on changes in the *SOD1* gene. The mouse model helps them understand how mutations in *SOD1* can lead to the symptoms of the disease, and allows them to look for new treatments. Currently, there are no preventative treatments for ALS, only life-prolonging ones.


[**Note:** Amyotrophic Lateral Sclerosis is sometimes called “Lou Gehrig’s Disease,” although some evidence suggests that Lou Gehrig may have had a different condition.]

PART I: BLAST Protein Alignment

SOD1 is a gene that encodes the protein superoxide dismutase. Take the reference *SOD1* protein sequence and use BLAST to align the amino acid sequences of the two proteins to determine where the mutation is (similar to Student Handout—*Aligning Sequences with BLAST Instructions* from Lesson Four).

1. Go to the NCBI BLAST page: <http://blast.ncbi.nlm.nih.gov/Blast.cgi>.
2. Select “**protein blast.**”
3. Check the box “**Align two or more sequences.**” A second text box will appear.
4. Copy the *SOD1* Reference sequence into the first box and the patient *SOD1* sequence into the second box. Be sure to include the “>” symbol.
5. Click “**BLAST.**”
6. Once your search is complete, click the “**Formatting options**” link, and use the drop-down menu under the “**Alignment View**” to select “**Query-anchored with dots for identities.**” Click the “**Reformat**” button in the upper right corner.
7. Open your word processing software and create a new document. You will use this document for recording your answers and saving screen images while you work. Label the document your LASTNAME_ *SOD1*_NCBI. Type your name, class period, and date at the top of the blank page. Save the document once you have pasted in your alignment.
8.  Capture an image of the aligned sequences from the BLAST results page and paste it in your document. Add a descriptive title to describe the image and save your document.
9.  Answer the following questions under your screen shot in your document:

Are there any differences between the reference (query) sequence and the patient sequence? If so, answer the following:

 - a. Specify where the change is by describing its location by number within the protein (for example, at position 181, etc.).
 - b. Use the one-letter amino acid abbreviations to describe which amino acid has changed, and what the new amino acid is. (For example, M has changed into R).
 - c. Describe whether the change is a substitution (one amino acid exchanged for another), an insertion (an amino acid where there was none before), a deletion, or some other kind of change.
10.  Answer the following question in several complete sentences: How can tools such as BLAST help scientists study and treat genetic diseases?

PART II: Protein Visualization Using Cn3D

Visualize both the reference protein and the location of the mutation in a mutant protein using the Cn3D program. First, we're going to investigate the structure of a functional protein (1HL5). Then we will look at a structure from a protein with the same mutation we identified in *Part I* (1OEZ).

11. Go to the NCBI structure page: <http://www.ncbi.nlm.nih.gov/Structure/index.shtml>.
12. In the search box, type the structure accession number "**1HL5**" and click "**Go**."
13. Click on the picture of the structure or the structure title to go to the Structure Summary.
14. Click "**View structure**" and open the file in Cn3D.

[**Note:** depending on your browser, you may have to download the file and then open it in Cn3D.]

Notice that there are two chains of amino acids that interact with each other. **Also note the many metal ions that are important for SOD1 activity (Cu, copper and Zn, zinc).**

15. In the Sequence Viewer window, move your cursor along 1HL5_A until you come to the position of the mutation identified in *Part I: BLAST Protein Alignment*. (For example, if you found in *Part I* that the mutation was at position 8; you would move your cursor over the amino acids until you saw that you were over amino acid 8 in the bottom left window of the Sequence Viewer.) Be sure to confirm that you are looking at the correct amino acid by double-checking the sequence of the amino acids immediately before and after the mutation. There is often more than one of each type of amino acid in a protein sequence.
16. Hold down the "**CONTROL**" key ("**Command**" on Mac) and click to select the amino acid in that position in both chains in the Sequence Viewer. The individual amino acids that you just selected should be highlighted yellow.



17. In your word processing document, in one to two sentences, describe where the mutation is located in the protein structure.
18. Open "**Style -> Edit Global Style**" and click the "**Labels**" tab. Check the box beside "**Metal ion labels**," if it is not checked already, then click "**Apply**" and "**Done**."
19. Open "**Select -> Select by Distance**."

Heterogen: Any molecule that is not part of a DNA, RNA, or protein chain, such as a metal ion.

20. A window will appear with the default distance. Click the box near "**Select heterogens**" and click "**OK**."

21. Experiment with different "**Style -> Rendering Shortcuts**" and zoom in and out until you find an image that you think shows the mutation location well relative to the overall shape of the protein (such as ball and stick or spacefill).

22. Zoom in closer to the protein structure to look at the mutation site and the nearby metal ions, rotating the protein to see how a mutation at that site might impact the interaction of the protein with a metal ion. [**Hint:** Which metal ion is colored yellow?]



23. Capture the image of this protein structure with the location of the mutation highlighted, insert it into your word processing document, and type a descriptive title for your image.
24. Return to the NCBI structure page and find and open the structure "**1OEZ**," [the letter "O" not the number "zero"]. 1OEZ is the structure of the mutant protein.
25. Use the same "**Rendering Shortcuts**" as you did with the reference protein structure.

LESSON 8

CLASS SET

26. Highlight the mutation location as you did with the reference protein structure.
27. Rotate the protein so that it is oriented in approximately the same way as the reference protein structure image, to see how the mutation affects the shape of the protein.



28. Capture the image of the mutated protein structure, insert it into your document, and type a descriptive title for your screen image.



29. Answer the following questions under your screen images of the reference and mutated protein structures in your document:

- In general, how can mutations impact the function of a protein?
- How might the specific mutation that you looked at impact the protein?
- Answer the following in a few complete sentences: How can tools such as Cn3D help scientists study and treat genetic diseases?



Challenge: Look up the full names of the amino acids involved in the mutation using the “Amino Acid Abbreviations and Chemistry Resources” showing one-letter abbreviations. How are they chemically different? How might this difference impact the protein’s function?



Optional: Use your drawing tools in Word® to use an **arrow** to point out the location of the mutation in the sequence alignment in the BLAST sequence window and in the Cn3D view. (“**Insert Shapes -> Arrow -> Color**”). If you prefer, you can draw **red circles** instead. (“**Insert Shapes -> Oval -> Shape Fill: no fill, Shape outline: red line**”). If you want to crop the image, you can use the **Crop** feature in Word®.

PART III: Genetic Testing

Use the information in the background section, as well as your knowledge gained from the genetic testing lessons in this unit, to **answer the following questions in your word processing document:**



30. How clinically valid is the genetic test? (Low, Medium, or High) Explain why you chose that level.



31. Is there an effective treatment for this condition, whether medical or behavioral? Explain your answer.



32. If you were a genetic counselor, and this condition was found in your patient’s family, would you recommend that your patient be tested for this condition? Why or why not?



33. If you were a genetic counselor, and this condition was not found in your patient’s family, would you recommend that your patient be tested for this condition? Why or why not?



34. What are the characteristics of a good genetic test, in your opinion? To what extent do direct-to-consumer genetic tests that consumers can purchase on their own meet your criteria?

Turn in your Microsoft Word® document in the manner specified by your teacher.

8

Porphyria

Teacher Answer Key

[**Note:** Suggested point values are included for 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 **35 points plus three points for the Challenge questions.**]

PART I: BLAST Protein Alignment

(8 points possible)

7. (+1 point for including a screen capture image of the BLAST alignment with the mutation circled.)

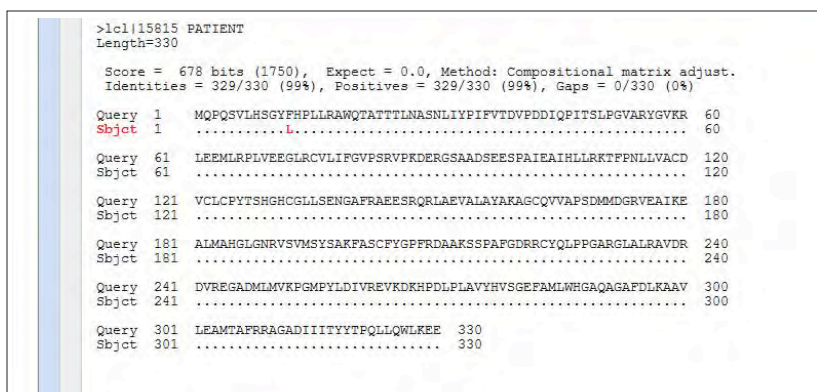


Figure 1: Screen image of BLAST alignment.
Credit: NCBI BLAST.

8. Type a descriptive title for your image.

Example Title: Comparison of Reference and Patient Amino Acid Sequences for ALAD.

(+1 point for including a descriptive title that refers to comparing a patient's sequence and a reference sequence.)

9. Are there any differences between the reference (query) sequence and the patient sequence?

Yes. (+1 point for noting that there is a difference between the two sequences.)

- a) Specify where the change is by describing its location by *number* within the protein (for example, at position 181, etc.).

Position 12. (+1 point for correctly noting the position of the change.)

- b) Use the one-letter amino acid abbreviations to describe which amino acid has changed, and what the new amino acid is. (For example, M has changed into R).

F has changed to L (F12L).

(+1 point for correctly using the one-letter amino acid abbreviation to note which amino acid (F) was changed to which (L).)

- c) Describe whether the change is a substitution (one amino acid exchanged for another), an insertion (an amino acid where there was none before), a deletion, or some other kind of change.

Substitution. (+1 point for correctly noting that the change is a substitution.)

10. Answer the following in a few complete sentences: How can tools such as BLAST help scientists study and treat genetic diseases?

Tools such as BLAST can help compare patient sequences to reference sequences to help determine whether disease-causing mutations are present in the patient.

(+2 points for noting at least two of the underlined phrases above; +1 if students only note that BLAST is used to compare patient sequences to reference sequences without explaining why, i.e., to determine whether mutations are present.)

[Note: BLAST can also be used to identify novel mutations associated with disease.]

PART II: Protein Visualization Using Cn3D

(12 points possible plus three possible Challenge points)

19. In one to two sentences, describe where the mutation is located in the protein structure.

The mutation is near where the two protein subunits interact.

(+1 point for noting that the mutation is near where the two subunits interact.)

21. Capture the image of this protein structure with the mutation highlighted, insert it into your word processing document, and type a descriptive title for your image.

Example Title: Location of mutation in reference ALAD protein structure. [A variety of descriptive titles are acceptable.]

(+1 point for inclusion of the image, with the region of the mutation circled (optional), and +1 point for a descriptive title that refers to the mutation and the reference protein structure.)

26. Capture the image of the mutated protein structure, insert it into your document, and type a descriptive title for your screen image.

Example Title: Comparison of mutant (left) and reference (right) ALAD structures. [A variety of descriptive titles are acceptable.]

(+1 point for inclusion of the image, with the region of the mutation circled (optional), and +1 point for a descriptive title that refers to the mutation in the protein structure.)

27. Answer the following questions under your screen image in your document.

- a) In general, how can mutations impact the function of a protein?

In general, changes/mutations can alter the protein's shape or chemical characteristics and thus impact the protein's function.

(+1 point for noting that mutations change a protein's shape and/or chemical characteristics and +1 point for noting that this change impacts protein function.)

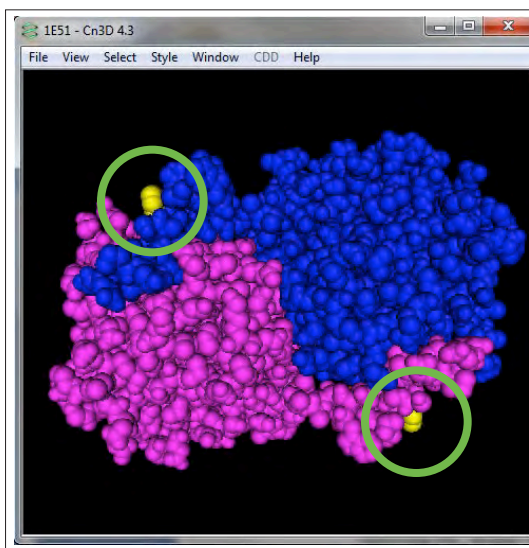


Figure 2: Structure 1E51.
Credit: Adapted from Mills-Davies et al., 2000.

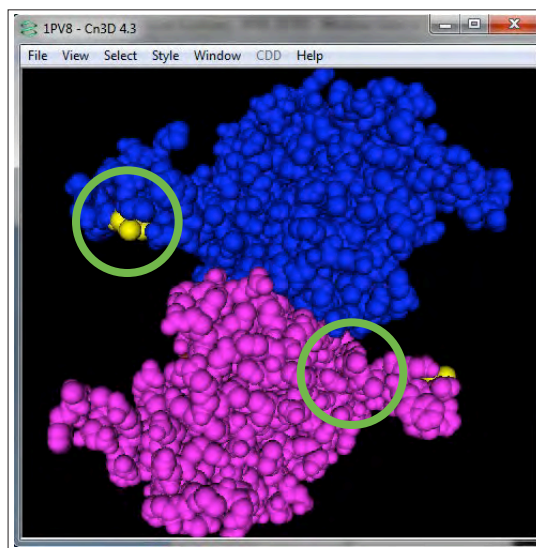


Figure 3: Structure 1PV8.
Credit: Adapted from Brenig et al., 2003.

b) How might the specific mutation that you looked at impact the protein?

The shape of the protein is drastically altered, with a loop swinging out of the main protein core in the mutant.
(+2 points for a clear explanation of the change in protein shape; +1 point for simply noting that the change in shape impacts protein function.)

c) Answer the following in a few complete sentences: How can tools such as Cn3D help scientists study and treat genetic diseases?

Tools such as Cn3D can help scientists visualize the location of mutations on important proteins associated with disease. This knowledge can help them understand how the protein's function might be impacted, and the role the protein might play in the development of disease.
(+3 points for inclusion of at least three of the underlined phrases, with an emphasis on demonstration of student understanding that being able to visualize protein shape aids understanding of protein function.)

[Note: understanding the structure of a protein associated with a disease-causing mutation might also aid in the development of treatments.]

Challenge: Look up the full names of the amino acids involved in the mutation using the "Amino Acid Abbreviations and Chemistry Resources" showing one-letter abbreviations. How are they chemically different? (1 point) How might this difference impact the protein's function? (2 points) **[Note:** This exercise is considered extra credit.]

F=Phenylalanine, L=Leucine
Both are hydrophobic, but phenylalanine is larger, with an [aromatic] ring.
(+1 point for noting that phenylalanine is larger than Leucine; +2 points for explaining that the size difference between the two amino acids can alter the protein's shape and thus impact the protein's function.)

PART III: Genetic Testing

(15 points possible)

28. How clinically valid is the genetic test? (Low, Medium, or High) Explain why you chose that level.

Clinical validity – middle/high (both acceptable). (+1 point) While the test is very reliable, many genes are involved and multiple genes must be tested and completely sequenced in each family.

(+1 point each for noting that the test is reliable and that multiple genes must be sequenced.)

29. Is there an effective treatment for this condition, whether medical or behavioral? Explain your answer.

Yes, effective treatment is available (+1 point), including heme therapy, intravenous glucose, and a high carbohydrate therapy.

(+ 1 point for listing at least one treatment option.)

30. If you were a genetic counselor, and this condition was found in your patient's family, would you recommend that your patient be tested for this condition? Why or why not?

Recommendations for testing if the condition was found in patient's family: yes, valid test and highly treatable.

(+1 point for recommending 'yes,' +1 point for each reason: valid test available (+1) and effective treatment available (+1).)

31. If you were a genetic counselor, and this condition was not found in your patient's family, would you recommend that your patient be tested for this condition? Why or why not?

Recommendations for testing if the condition was NOT found in patient's family: no, expensive and labor-intensive. However, if mental illness is a symptom the porphyria test may be informative.

(+1 point for recommending 'no;' +1 for explaining why it is not recommended (because it is expensive and labor intensive); +1 point for explaining the exception in the case of mental illness.)

32. What are the characteristics of a good genetic test, in your opinion? To what extent do direct-to-consumer genetic tests that consumers can purchase on their own meet your criteria?

A good genetic test should be clinically valid (the test accurately predicts a certain clinical outcome, such as getting a particular disease or symptom), and should have an effective treatment, whether through lifestyle modification or clinical invention (surgery, drug treatment, etc.). Many of the tests offered as direct-to-consumer (DTC) genetic tests do not meet these criteria. Some DTC tests have only preliminary scientific research to support their clinical validity, and many have no effective treatment, or treatment options are unclear. In addition, the lack of genetic counseling associated with many DTC tests makes it difficult to advise consumers about these potential shortcomings.

(+2 points for noting good tests are clinically valid (+1) and have effective treatment (+1); +2 points for explaining that many DTC tests do not meet these criteria because: research findings are preliminary (+1), there is no effective treatment (+1), and no genetic counseling is offered to advise patients about the risks.)

8 Amyotrophic Lateral Sclerosis (ALS) Teacher Answer Key

[**Note:** Suggested point values are included for 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 **35 points plus three points for the Challenge questions.**]

PART I: BLAST Protein Alignment

(8 points possible)

7. (+1 point for inclusion of the screen capture image of the BLAST alignment with the mutation circled.)

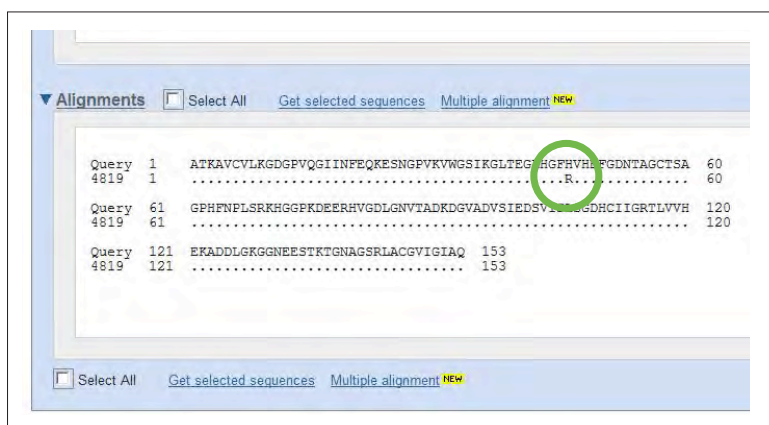


Figure 1: Screen image of BLAST alignment.
Credit: NCBI BLAST.

8. Type a descriptive title for your screen shot and answer the following questions under your screen shot in your word processing document.

Example Title: Comparison of Reference and Patient Amino Acid Sequences for SOD1.

(+1 point for including a descriptive title that refers to comparing a patient's sequence and a reference sequence.)

9. Are there any differences between the reference (query) sequence and the patient sequence?

Yes. (+1 point for noting that there is a difference between the two sequences.)

- a. Specify where the change is by describing its location by *number* within the protein (for example, at position 181, etc.).

Position 46. (+1 point for correctly noting the position of the change.)

- b. Use the one-letter amino acid abbreviations to describe which amino acid has changed, and what the new amino acid is. (For example, M has changed into R).

H has changed to R (H46R). (+1 point for correctly using the one-letter amino acid abbreviation to note which amino (H) was changed to which (R).)

- c. Describe whether the change is a substitution (one amino acid exchanged for another), an insertion (an amino acid where there was none before), a deletion, or some other kind of change.

Substitution. (+1 point for correctly noting that the change is a substitution.)

10. Answer the following in a few complete sentences: How can tools such as BLAST help scientists study and treat genetic diseases?

Tools such as BLAST can help compare patient sequences to reference sequences to help determine whether disease-causing mutations are present in the patient.

(+2 points for noting at least two of the underlined phrases above; +1 if students only note that BLAST is used to compare patient sequences to reference sequences without explaining why, i.e., to determine whether mutations are present.)

[Note: BLAST can also be used to identify novel mutations associated with disease.]

PART II: Protein Visualization Using Cn3D

(12 points possible plus three Challenge points)

17. In one to two sentences, describe where the mutation is located in the protein structure.

The mutation is located within each protein subunit.

(+1 point for noting that the mutation is located within each protein subunit.)

23. Capture the image of this protein structure with the location of the mutation highlighted, insert it into your word processing document, and type a descriptive title for your image.

Example Title: Visualization of location of a mutation in the SOD1 reference protein. [A variety of descriptive titles are acceptable.]

(+1 point for the image and +1 point for inclusion of a descriptive title that refers to the reference protein structure. Note: students may not zoom in this much with their image.)

28. Capture the image of the mutated protein structure, insert it into your document, and type a descriptive title for your screen image.

Example Title: Visualization of location of a mutation in the mutated SOD1 protein. [A variety of descriptive titles are acceptable.]

(+1 point for the image and +1 point for inclusion of a descriptive title that refers to the mutated protein structure.)

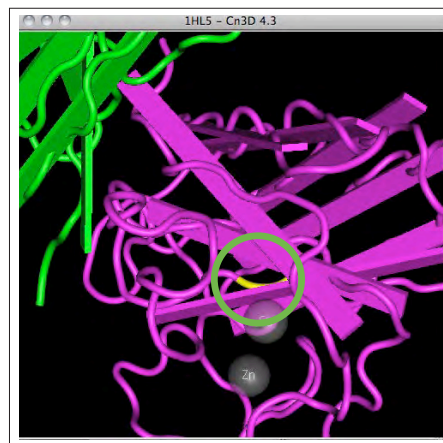


Figure 2: Structure 1HL5.
Credit: Adapted from Strange et al., 2003.

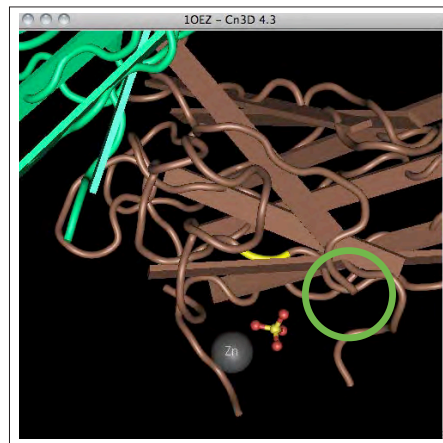


Figure 3: Structure 1OEZ.
Credit: Adapted from Elam et al., 2003.

29. Answer the following questions under your screen image in your word processing document.

a. In general, how can mutations impact the function of a protein?

In general, changes/mutations can alter the protein's shape or chemical characteristics and thus impact the protein's function.
(+1 point for noting that mutations change a protein's shape and/or chemical characteristics and +1 point for noting that this change impacts protein function.)

b. How might the specific mutation that you looked at impact the protein?

The shape of the protein is drastically altered, with a loop swinging out of the main protein core in the mutant.
(+2 points for a clear explanation of the change in protein shape; +1 point for simply noting that the change in shape impacts protein function.)

c. Answer the following in a few complete sentences: How can tools such as Cn3D help scientists study and treat genetic diseases?

Tools such as Cn3D can help scientists visualize the location of mutations on important proteins associated with disease. This knowledge can help them understand how the protein's function might be impacted, and the role the protein might play in the development of disease.
(+3 points for inclusion of at least three of the underlined phrases, with an emphasis on demonstration of student understanding that being able to visualize protein shape aids understanding of protein function.)

[Note: understanding the structure of a protein associated with a disease-causing mutation might also aid in the development of treatments.]

Challenge: Look up the full names of the amino acids involved in the mutation using the "Amino Acid Abbreviations and Chemistry Resource" showing one-letter abbreviations. How are they chemically different? How might this difference impact the protein's function? **[Note:** This exercise is considered extra credit.]

H=Histidine, R=Arginine

Both are positively charged, but histidine has an [aromatic] ring, while arginine does not. The histidine has been rendered as a ball and stick (see Lesson Three for a description of how to do this). Students would not be expected to do this during the assessment, but they may remember how to do it.

(+1 point for noting that histidine is larger than arginine; +2 points for explaining that the size difference among the two amino acids can alter the protein's shape and thus impacts the protein's function.)

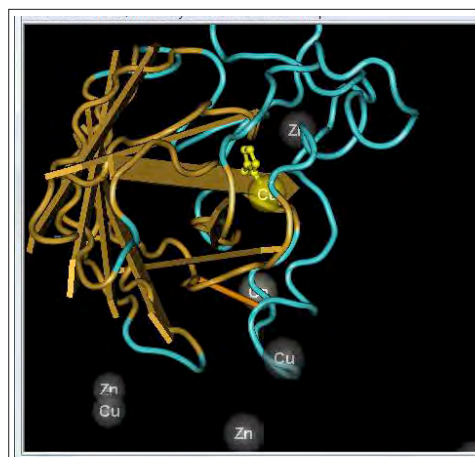


Figure 4: Structure 1OEZ.
Credit: Adapted from Elam et al., 2003.

PART III: Genetic Testing

(15 points possible)

30. How clinically valid is the genetic test? (Low, Medium, or High) Explain why you chose that level.

Clinical validity – middle/low [both acceptable]. (+1 point) Most ALS (90%) is not hereditary, and of the ALS that is inherited, most types (80%) are not due to changes in SOD1. There are no tests currently for those types not due to changes in SOD1. A positive genetic test also does not give information about when symptoms might arise (low penetrance). (+1 point for noting that the test is not reliable or clinically valid, and +1 point for explaining why, i.e., that there are multiple genes involved.)

31. Is there an effective treatment for this condition, whether medical or behavioral? Explain your answer.

Yes, effective treatment is available. (+1 point.) However, currently, there are no preventive treatments, only life-prolonging ones. (+1 point.)

32. If you were a genetic counselor, and this condition was found in your patient's family, would you recommend that your patient be tested for this condition? Why or why not?

Recommendations for testing if the condition was found in patient's family: no, clinical validity low and even if there is a family history and a negative SOD1 test, there is a chance that the individual might still have a different form of ALS. Also, there are no preventive treatments. However, if an individual strongly desired the test to have as much information as possible to plan for the future, a recommendation could be considered. (+1 point for recommending 'no,' +1 point for each reason: test is not valid (+1) and no effective preventive treatment available (+1).)

33. If you were a genetic counselor, and this condition was not found in your patient's family, would you recommend that your patient be tested for this condition? Why or why not?

Recommendations for testing if the condition was NOT found in patient's family: No, even if it is a sporadic case of ALS, the SOD1 test may not be informative for reasons outlined above. (+1 point for recommending 'no,' +1 point for each reason: test is not valid (+1) and no effective preventive treatment available (+1).)

34. What are the characteristics of a good genetic test, in your opinion? To what extent do direct-to-consumer genetic tests that consumers can purchase on their own meet your criteria?

A good genetic test should be clinically valid (the test accurately predicts a certain clinical outcome, such as getting a particular disease or symptom), and should have an effective treatment, whether through lifestyle modification or clinical invention (surgery, drug treatment, etc.). Many of the tests offered as direct-to-consumer (DTC) genetic tests do not meet these criteria. Some DTC tests have only preliminary scientific research to support their clinical validity, and many have no effective treatment, or treatment options are unclear. In addition, the lack of genetic counseling associated with many DTC tests makes it difficult to advise consumers about these potential shortcomings.

(+2 points for noting good tests are clinically valid (+1) and have effective treatment (+1); +2 points for explaining that many DTC tests do not meet these criteria because: research findings are preliminary (+1), there is no effective treatment (+1), and no genetic counseling is offered to advise patients about the risks.)



Appendix

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BRCA1 and BRCA2 Background Information

BRCA1, BRCA2 and the Risk of Cancer

The names *BRCA1* and *BRCA2* stand for **breast cancer** susceptibility gene **1** and **breast cancer** susceptibility gene **2**, respectively. The *BRCA1* (sometimes pronounced BRA-kah 1) and *BRCA2* (sometimes pronounced BRA-kah 2) proteins play vital roles in genomic stability and can act as tumor suppressors in both men and women. A tumor suppressor is a gene that normally prevents cancer. Mutations in these genes can lead to cancer when the normal function is lost. Together, mutations in these genes account for 5-10% of all breast cancer cases and approximately 45% of all familial [inherited] breast cancer.

By convention, the names of genes are usually italicized, while the names of their encoded proteins are not. Therefore, the gene is written as *BRCA1*, while the protein is simply “BRCA1.” Mutations in *BRCA1* or *BRCA2* can result in amino acid changes or changes in the mRNA reading frame that lead to shorter proteins. Some of these changes dramatically increase the risks of breast and ovarian cancer. The lifetime risk of breast cancer for the average woman is 12%, and the lifetime risk of ovarian cancer is 2%. Many factors such as excess weight, lack of exercise, having a first period at a young age, and not having children can increase the risk of breast cancer in all women. Increased weight and lack of exercise are associated with increased estrogen, which can promote cancer. Menstruation is associated with physiological changes in the breast that are conducive to the development of cancer, and beginning menstruation at a young age allows more total time for cancer to develop; some of these changes are mitigated by pregnancy and breastfeeding. 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. All carriers are also at increased risk for other types of cancer including pancreatic, laryngeal, and stomach cancers, as well as melanoma.

The risk of breast and ovarian cancer associated with *BRCA1* and *BRCA2* alleles containing cancer-causing mutations is inherited in an autosomal dominant fashion, because only a single defective copy must be passed from parent to offspring for the offspring to inherit the cancer risk. However, both copies of the gene must be mutated for cancer to develop, making this BRCA-associated cancer autosomal recessive. See the next *Appendix* section, “*BRCA1*: Is it Dominant or Recessive?” for more information. 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 that 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* or *BRCA2* genes. This phenomenon is known as “incomplete penetrance.” In some cases, the functional copy of the gene is lost when a tumor develops, while the germline-encoded mutant copy is retained. Inheritance of two mutated copies of *BRCA2* is associated with another kind of genetic disease called “Fanconi anemia.” Inheritance of two mutated copies of *BRCA1* is lethal to an embryo.

Cellular Functions of BRCA1 and BRCA2

Both *BRCA1* and *BRCA2* play crucial roles in genomic stability – making sure that DNA remains intact. In particular, they participate in a biochemical pathway for repairing breaks in double-stranded DNA. They also have a number of other functions. They act as transcriptional coactivators and ubiquitin-protein ligases, and they bind to zinc and tubulin. Many of these seemingly unrelated abilities may contribute to their role in repairing DNA.

If DNA is damaged, the cell must repair the DNA before proceeding through the cell cycle. BRCA1 and BRCA2 both interact with other proteins that respond to DNA damage and participate in homologous recombination. Cells respond to damaged DNA by making proteins to repair the damage and by stopping cell division. BRCA1 might put the brakes on cell division by binding to other proteins that act as tumor suppressors, DNA damage sensors, or signaling proteins. BRCA1 also binds to RNA polymerase II and stops transcription by interacting with a protein that chemically modifies histones. Transcription often involves modifying histones so that the chromosomes decondense or “loosen up” and allow RNA polymerase to access the target genes. The BRCA2 protein contains several copies of a 70 amino acid sequence called the BRC motif, which functions by binding to a DNA repair protein called RAD51.

BRCA1

BRCA1 is encoded by 24 exons and is located on the long arm of chromosome 17. Alternative splicing occurs frequently with *BRCA1*-encoded mRNA and many forms of alternatively spliced mRNAs have been isolated. Some alternatively-spliced forms are associated with disease-causing mutations, including frameshifts and premature truncations. A related pseudogene, which is also located on chromosome 17, has been identified. (Pseudogenes are genes that do not encode functional proteins. This is usually because they contain stop codons that prevent the translation of a functional protein.)

The BRCA1 protein contains three types of domains: a RING finger domain near the beginning of the protein (the N-terminus), Nuclear Localization Signals in the middle of the protein, and two BRCT domains at the end of the protein (see **Figure 1**). BRCT stands for “BRCA1 C-Terminal.” The “RING” in RING finger stands for “Really Interesting New Gene.” These protein domains are characterized by an amino acid sequence motif containing cysteines and histidines (Cys3HisCys4) that binds to DNA, RNA, and protein or lipid substrates through interactions with zinc cations. The BRCA1 RING finger is thought to facilitate binding of the protein to DNA. The Nuclear Localization Signals are kind of like an address on a piece of mail. They tell the cell to send the BRCA1 protein to the nucleus. BRCT domains consist of repeated sequences of 90-100 amino acids each that bind to other proteins, including other molecules of BRCA1. The BRCT domains are found in multiple proteins that participate in cell cycle regulation and DNA repair, including DNA ligase III. BRCT domains adopt a characteristic parallel four-stranded beta sheet, with two to three alpha helices packed against one face and a single alpha helix packed against the opposite face of the sheet. In BRCA1, the two BRCT repeats interact in a head-to-tail manner and facilitate the interaction of BRCA1 with protein partners such as p53.

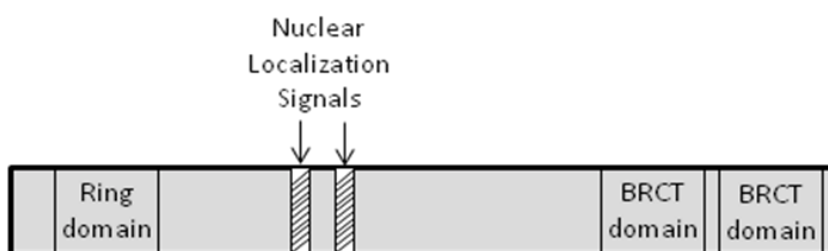


Figure 1: BRCA1 Protein Domains.

BRCA2

BRCA2 is encoded by 27 exons and is located on the long arm of chromosome 13. Common *BRCA2* mutations associated with cancer include small insertions and deletions, which can result in frameshifts that create defective proteins. The N-terminal region (exon 3) of *BRCA2* has been shown to function in transcription of genes involved in DNA repair, the cell cycle, and programmed cell death (apoptosis) via RNA polymerase II. As with BRCA1, Nuclear Localization Signals in *BRCA2* target the protein to the nucleus, where it interacts with RAD51 via a series of BRC repeat domains (see **Figure 2**).

Gene and Protein Structures



Figure 2: BRCA2 Protein Domains.

Options for Those Who Test Positive for Cancer-Associated Mutations

There are three main options for those who test positive for cancer-associated mutations in either *BRCA1* or *BRCA2*.

The first option is surgery. A prophylactic oophorectomy – removal of both ovaries before cancer can strike – can reduce the risk of breast cancer by 50% due to the subsequent reduction in estrogen production, and reduces the risk of ovarian cancer by 95%. Because minute amounts of tissue may remain, the risk of cancer is not completely eliminated. Female carriers of cancer-associated mutations in *BRCA1* or *BRCA2* are encouraged to have an oophorectomy by age 40. A prophylactic mastectomy – removal of both breasts before cancer can strike – reduces the risk of breast cancer by 90%. As with the oophorectomy, there is a possibility that some residual tissue or cancer cells might be left behind in the chest wall. As a consequence, the risk of breast cancer can never be completely eliminated.

The second option is chemoprevention (treatment with drugs). Because estrogen can promote breast cancer, drugs that block estrogen like tamoxifen or raloxifene can help reduce the risk of cancer. They also cause temporary, reversible menopause, with all its side effects, including hot flashes and disrupted ovulation.

The third option is to test frequently so that breast cancer can be found at an early stage. This includes increasing the frequency of mammograms to twice yearly; increased breast exams, breast MRIs, and blood tests; and at least yearly Pap smears, pelvic exams, and vaginal ultrasounds to screen for ovarian cancer. Unfortunately, screening for ovarian cancer is not very effective, and mortality rates for this form of cancer are quite high: the average 5-year survival rate is only 46%.

For more information about *BRCA1*, *BRCA2* and genetic testing, see the “National Cancer Institute Fact Sheet: *BRCA1* and *BRCA2*: Cancer Risk and Genetic Testing” at <http://www.cancer.gov/cancertopics/factsheet/risk/brca> and see the *Sources* below.

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BRCA1: Is it Dominant or Recessive?

Both BRCA1 and BRCA2 proteins are involved in DNA synthesis and repair of DNA breaks, helping to maintain the integrity of the genome. But is the inheritance of cancer risk associated with mutations in the *BRCA1* or *BRCA2* genes dominant or recessive?

A genetic trait is considered **dominant** if it is expressed in a person who has only one copy of that allele.

A genetic trait is considered **recessive** if it is expressed only when two copies of the allele are present.

Unfortunately, the classical Mendelian terms of “dominant” and “recessive” don’t apply well in the case of *BRCA1* and *BRCA2*.

Many *BRCA1* and *BRCA2* resources say that cancer-predisposing alleles of the *BRCA1* and *BRCA2* genes are **dominant**. If an individual inherits a single copy of a mutated *BRCA1* or *BRCA2* gene, that individual has inherited an **increased risk of cancer**.

However, *BRCA1* and *BRCA2* function as tumor suppressors, making them functionally **recessive**. Both copies must be mutated in a cell and made non-functional for cancer to develop. However, inheritance of two mutated copies of *BRCA1* or *BRCA2* is lethal to an embryo. In a pedigree, *BRCA1* and *BRCA2* **appear to be autosomal dominant**, because only one mutated allele is inherited. The second copy is mutated later in life.

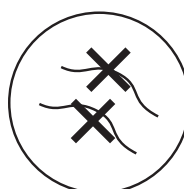
This is often understood in terms of Knudson’s Hypothesis or the “Two-Hit Hypothesis.”

Let’s consider that case of *BRCA1*.

A “normal cell” (i.e., non-cancerous) contains two functional copies of the *BRCA1* gene. Neither allele contains mutations associated with cancer. Functional BRCA1 proteins are made from both alleles, and help repair DNA damage as it occurs.

An individual who has inherited a germline *BRCA1* mutation in one allele (i.e., one “hit”) has a much higher lifetime risk of developing cancer than an individual born with two functional copies of the gene. Every cell in the body contains this mutated allele. Because the non-mutated *BRCA1* allele still encodes a functional protein, the cell is able to repair DNA damage and remains non-cancerous.

If DNA damage or an error in DNA replication causes the second copy of the *BRCA1* gene to become mutated in any given cell (i.e., a second “hit”), that cell can no longer make any functional BRCA1 protein. Researchers believe that a defective or missing BRCA1 protein is unable to help repair damaged DNA or fix mutations that occur in other genes. As these defects accumulate, they can allow cells to grow and divide uncontrollably and form a tumor.



Objective

Clear up any confusion about whether *BRCA1* and *BRCA2* mutations are dominant or recessive.

In genetic terms, a **dominant trait** is one that is phenotypically expressed in heterozygotes.

In genetic terms, a **recessive trait** is one that is phenotypically expressed only in homozygotes.

It is possible that a mutation in one copy of the *BRCA1* (or *BRCA2*) gene makes it more likely that an individual will eventually develop a mutation in the second copy of the gene.

Over 1600 different mutations have been identified in *BRCA1* and over 1800 have been found in *BRCA2*. Many families have their own type of mutation that stays within the family. 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 is. About a third of mutations identified in either the *BRCA1* or *BRCA2* gene so far are of uncertain clinical significance.

Additional Resources:

The Bio-ITEST Program has developed a two-part animation to highlight the normal function of *BRCA1* in the cell, and how mutations in the *BRCA1* gene can lead to cancer. The animation can be found under the Resources tab on the Bio-ITEST Genetic Testing web page at: <http://www.nwabr.org/curriculum/introductory-bioinformatics-genetic-testing>.

National Cancer Institute Fact Sheet, "*BRCA1* and *BRCA2*: Cancer Risk and Genetic Testing." <http://www.cancer.gov/cancertopics/factsheet/risk/brca>.

Genetics Home Reference: *BRCA1*. <http://ghr.nlm.nih.gov/gene=brca1>.

The Breast Cancer Information Core: An Open Access Online Breast Cancer Mutation Database through the National Human Genome Research Institute: <http://research.nhgri.nih.gov/bic/>.



GINA

The Genetic Information Nondiscrimination Act of 2008 Information for Researchers and Health Care Professionals April 6, 2009

The information presented in this fact sheet is intended for general informational purposes only. While this fact sheet does not cover all of the specifics of GINA, it does provide an explanation of the statute to assist those involved in clinical research to understand the law and its prohibitions related to discrimination in health coverage and employment based on genetic information. The information should not be considered legal advice. In addition, some of the provisions discussed involve issues for which the rules have not yet been finalized, and this information is subject to revision based on publication of regulations.

What is GINA?

The Genetic Information Nondiscrimination Act of 2008 (P.L. 110-233, 122 Stat. 881)¹, also referred to as GINA, is a new Federal law that prohibits discrimination in health coverage and employment based on genetic information. The President signed the act into law on May 21, 2008. The section of the law relating to health coverage (Title I) generally will take effect between May 22, 2009, and May 21, 2010.² The sections relating to employment (Title II) will take effect on November 21, 2009. GINA requires regulations pertaining to both titles to be completed by May 2009.

¹ http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=110_cong_public_laws&docid=f:publ233.110.pdf.

² The effective date of the insurance provisions is not the same in all cases because for group health plans, Title I will take effect at the start of the “plan year” beginning one year after GINA’s enactment. Because some health plans do not designate their “plan years” to correspond to a calendar year, there will be variation among plans as to when Title I takes effect for the plans. However, for individual health insurers, GINA will take effect May 22, 2009.

How does the Federal law affect state laws?

GINA provides a baseline level of protection against genetic discrimination for all Americans. Many states already have laws that protect against genetic discrimination in health insurance and employment situations. However, the degree of protection they provide varies widely, and while most provisions are less protective than GINA, some are more protective. All entities that are subject to GINA must, at a minimum, comply with all applicable GINA requirements, and may also need to comply with more protective State laws.

What will GINA do?

GINA generally will prohibit discrimination in health coverage and employment on the basis of genetic information. GINA, together with already existing nondiscrimination provisions of the Health Insurance Portability and Accountability Act, generally prohibits health insurers or health plan administrators from requesting or requiring genetic information of an individual or the individual’s family members, or using it for decisions regarding coverage, rates, or preexisting conditions. The law also prohibits most employers from using genetic information for hiring, firing, or promotion decisions, and for any decisions regarding terms of employment.

The statute defines 'genetic information' as information about:

- an individual's genetic tests (including genetic tests done as part of a research study);
- genetic tests of the individual's family members (defined as dependents and up to and including 4th degree relatives);
- genetic tests of any fetus of an individual or family member who is a pregnant woman, and genetic tests of any embryo legally held by an individual or family member utilizing assisted reproductive technology;
- the manifestation of a disease or disorder in family members (family history);
- any request for, or receipt of, genetic services or participation in clinical research that includes genetic services (genetic testing, counseling, or education) by an individual or family member.

Genetic information does not include information about the sex or age of any individual.

The statute defines 'genetic test' as an analysis of human DNA, RNA, chromosomes, proteins, or metabolites that detects genotypes, mutations, or chromosomal changes. The results of routine tests that do not measure DNA, RNA, or chromosomal changes, such as complete blood counts, cholesterol tests, and liver-function tests, are not protected under GINA. Also, under GINA, genetic tests do not include analyses of proteins or metabolites that are directly related to a manifested disease, disorder, or pathological condition that could reasonably be detected by a health care professional with appropriate training and expertise in the field of medicine involved.

How will the law be enforced and what are the penalties for violation of the law?

The law will be enforced by various Federal agencies. The Department of Labor, the Department of the Treasury, and the Department of Health and Human Services are responsible for Title I of GINA, and the Equal Employment Opportunity Commission (EEOC) is responsible for Title II of GINA. Remedies for violations include corrective action and monetary penalties. Under Title II of GINA, individuals may also have the right to pursue private litigation.

What won't GINA do?

- GINA's health coverage non-discrimination protections do not extend to life insurance, disability insurance and long-term care insurance.
- GINA does not mandate coverage for any particular test or treatment.
- GINA's employment provisions generally do not apply to employers with fewer than 15 employees.
- For health coverage provided by a health insurer to individuals, GINA does not prohibit the health insurer from determining eligibility or premium rates for an individual based on the manifestation of a disease or disorder in that individual. For employment-based coverage provided by group health plans, GINA permits the overall premium rate for an employer to be increased because of the manifestation of a disease or disorder of an individual enrolled in the plan, but the manifested disease

or disorder of one individual cannot be used as genetic information about other group members to further increase the premium.

- GINA does not prohibit health insurers or health plan administrators from obtaining and using genetic test results in making health insurance payment determinations.

What is the status of regulations to implement GINA?

The law requires regulations by May 2009. The Department of Health and Human Services (Centers for Medicare & Medicaid Services (CMS) and the Office for Civil Rights), the Department of Labor, the Department of the Treasury (the Internal Revenue Service), and the EEOC are currently working on the regulations. The Department of Labor, the Department of the Treasury, and CMS put forth a Request for Information about issues relevant to some of the health coverage provisions in Title I on October 10, 2008, which closed on December 9, 2008.³

³ <http://www.dol.gov/federalregister/PdfDisplay.aspx?DocId=21604>.

Is GINA retroactive?

GINA will not be retroactive, i.e., it cannot apply to acts or omissions that occurred prior to GINA's effective dates. However, once GINA takes effect, it will prohibit certain uses of genetic information in connection with health coverage and employment, no matter when the information was collected. For example, a health insurer that has been collecting or using genetic information for underwriting would need to change its business practices once GINA takes effect. Likewise, certain employers requiring genetic tests or family history information from employees or prospective employees will no longer be able to do so after GINA takes effect and will be prohibited from discriminating based on any genetic information that they had already collected.

Does GINA have specific research provisions?

Yes. GINA's prohibitions apply to 'genetic information' which is defined as including receipt of genetic services (genetic tests, genetic counseling, or genetic education) by an individual or family member participating in clinical research. There is, however, a research exception.

GINA provides a specific "research exception" to allow health insurers or group health plans engaged in research to request (but not require) that an individual undergo a genetic test. This exception permits the request to be made but imposes the following requirements:

1. the request must be made pursuant to research that complies with HHS regulations at 45 CFR part 46, or equivalent Federal regulations, and any applicable state or local laws for the protection of human subjects in research;
2. there must be clear indication that participation is voluntary and that non-compliance has no effect on enrollment or premiums or contribution amounts;
3. no genetic information collected or acquired as part of the research may be used for underwriting purposes;

4. the health insurer or group health plan must notify the Federal government in writing that it is conducting activities pursuant to this research exception and provide a description of the activities conducted; and
5. the health insurer or group health plan must comply with any future conditions that the Federal government may require for activities conducted under this research exception.

What information about GINA should be communicated as part of the informed consent process to individuals participating in a research study or those considering study participation?

Although GINA has not yet taken effect, there may currently be situations where it is appropriate for researchers to discuss the provisions of the law with individuals participating in a research study or those considering study participation. For more information, see the following guidance document prepared by the Office for Human Research Protections: <http://www.hhs.gov/ohrp/humansubjects/guidance/gina.html> (URL), <http://www.hhs.gov/ohrp/humansubjects/guidance/gina.pdf> (PDF).

Courtesy: National Human Genome Research Institute. <http://www.genome.gov>.

This document is available from the National Human Genome Research Institute at: <http://www.genome.gov/Pages/PolicyEthics/GeneticDiscrimination/GINAInfoDoc.pdf>.



Ethics Background

Principles: Respect, Maximize
Benefits/Minimize Harms, and Justice

Summary

The focus of this perspective is on the four **principles** supported by or compromised by the question or issue at hand.

Philosophers Tom Beauchamp and Jim Childress identify four principles that form a commonly held set of pillars for moral life:

Respect for Persons/Autonomy	Value the worth and dignity of each individual. Acknowledge a person's right to make choices, to hold views, and to take actions based on personal values and beliefs.
Maximize Benefits	Provide benefits to persons and contribute to their welfare. Refers to an action done for the benefit of others. Also known as beneficence .
Minimize Harms	Obligation not to inflict harm intentionally; in medical ethics, the physician's guiding maxim is "First, do no harm." Also known as nonmaleficence .
Justice	Treat others equitably, distribute benefits/burdens fairly.

Contributions

- Draws on principles or pillars that are a part of American life—familiar to most people, although not by their philosophical terms.
- Compatible with both outcome-based and duty-based theories (respect for persons and justice are duty-based, while minimizing harms and maximizing benefits are outcome-based).
- Provides useful and fairly specific action guidelines.
- Offers an approach that is appropriate for general bioethics and clinical ethics.
- Requires weighing and balance—flexible, responsive to particular situations.

Challenges

- Lacks a unifying moral theory that ties the principles together to provide guidelines.
- Principles can conflict and the theory provides no decision-making procedure to resolve these conflicts.
- Difficult to weigh and balance various principles.
- Autonomy in some cultures refers to individual autonomy, while in others it refers to group/family/community autonomy.

Additional Information

Additional information about ethical theories and perspectives can be found in *An Ethics Primer: Lesson Ideas and Ethics Background* by Jeanne Ting Chowning and Paula Fraser, produced through the Northwest Association for Biomedical Research. The complete Ethics Primer is available free for download from <http://www.NWABR.org>.



Creating Discussion Ground Rules

Introduction

The study of ethics involves consideration of conflicting moral choices and dilemmas about which reasonable people may disagree. Since a wide range of positions is likely to be found among students in most classrooms, it is especially important to foster a safe classroom atmosphere by creating some discussion ground rules. These ground rules are often referred to as “norms.” An agreed-upon set of ground rules should be in place before beginning the *Using Bioinformatics* curriculum.

Procedure

Ask the students, “What can we do to make this a safe and comfortable group for discussing issues that might be controversial or difficult? What ground rules should we set up?” Allow students some quiet reflection time, and then gather ideas from the group in a brainstorming session. One method is to ask that students generate a list of ground rules in small groups and then ask each group to share one rule until all have been listed. Clarify and consolidate the ground rules as necessary.

Post norms where they can be seen by all and revisit them often. If a discussion gets overly contentious at any time, it is helpful to stop and refer to the ground rules as a class to assess whether they have been upheld.

Some possible student ground rules/norms could include:

- A bioethics discussion is not a competition or a debate with a winner and a loser.
- Everyone will respect the different viewpoints expressed.
- If conflicts arise during discussion, they must be resolved in a manner that retains everyone’s dignity.
- Everyone has an equal voice.
- Interruptions are not allowed and no one person is allowed to dominate the discussion.
- All are responsible for following and enforcing the rules.
- Critique ideas, not people.
- Assume good intent.

Objective

Students will be able to:

- Create and agree to classroom discussion norms



Amino Acid Abbreviations and Chemistry Resources

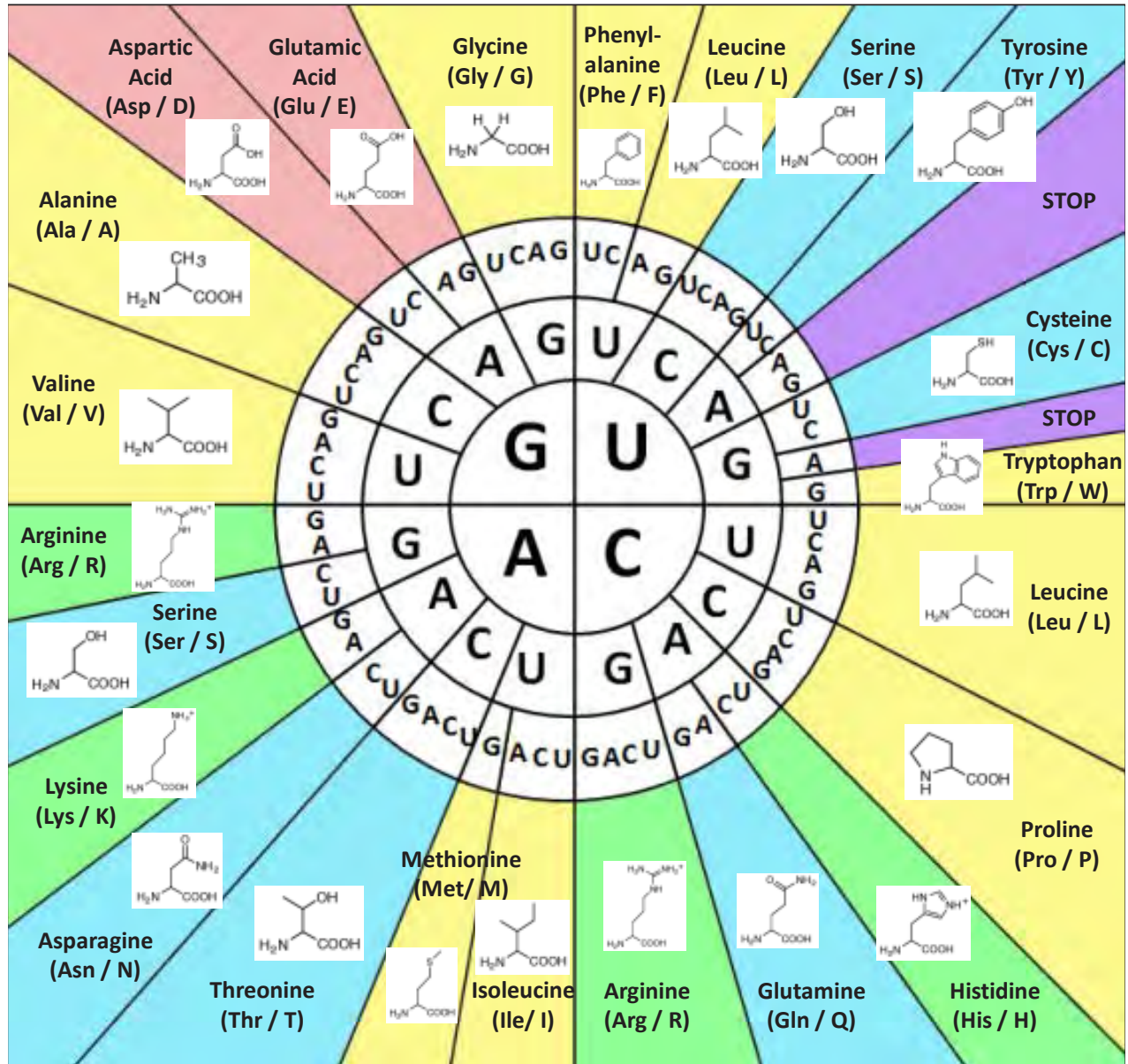
Single-letter Amino Acid Abbreviations

A – Alanine
 C – Cysteine
 D – Aspartic Acid
 E – Glutamic Acid
 F – Phenylalanine
 G – Glycine
 H – Histidine
 I – Isoleucine
 K – Lysine
 L – Leucine
 M – Methionine
 N – Asparagine
 P – Proline
 Q – Glutamine
 R – Arginine
 S – Serine
 T – Threonine
 V – Valine
 W – Tryptophan
 Y – Tyrosine

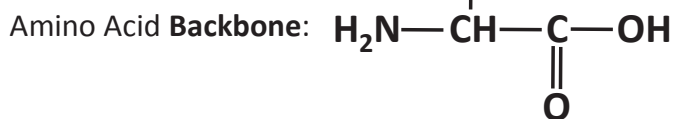
Amino Acid Abbreviations and Categorization by Chemistry

	Uncharged	Positive	Negative
Hydrophilic	Asparagine (Asn – N) Cysteine (Cys – C) Glutamine (Gln – Q) Serine (Ser – S) Threonine (Thr – T)	Arginine (Arg – R) Histidine (His – H) Lysine (Lys – K)	Aspartic Acid (Asp – D) Glutamic Acid (Glu – E)
Hydrophobic	Alanine (Ala – A) Glycine (Gly – G) Isoleucine (Iso – I) Leucine (Leu – L) Methionine (Met – M) Phenylalanine (Phe – F) Proline (Pro – P) Tryptophan (Trp – W) Tyrosine (Tyr – Y) Valine (Val – V)		

Codons and Amino Acid Chemistry



Amino Acid **Side Chain**
(R-Group):



Side Chain (R-Group) Chemistry:

- Hydrophobic / Nonpolar
- Hydrophilic/ Polar
- Acidic / Negative
- Basic / Positive

Behind the Scenes with the NCBI Databases and the *Entrez* Search Engine

We have already discussed the similarity between the NCBI databases and iTunes®. Now, we're going to go a little bit farther and consider what happens when data are submitted to NCBI and when we use Entrez to do a database search.

When researchers submit data to the NCBI, they do so by filling in a form from the NCBI website. The sections in the form where information gets entered are called "fields." Different data types have different kinds of fields. For example, the nucleotide database (GenBank) has fields for the gene name, organism, sequence length, and other information related to DNA or RNA sequences. The taxonomy database entry form includes fields for information about the common name, the scientific name, and the rank. Field names are used to help organize and find information.

Entrez is the software system that searches NCBI databases. When you type terms into the NCBI search box, Entrez takes those terms and searches all the fields, in all the database records, to see if those terms can be found. Sometimes this can lead to some puzzling results. For example, searching the nucleotide database with the word "lion" returns several records that come from *Sus scrofa*. *Sus scrofa* is the scientific name for "pig." While some lions might act like pigs, their DNA sequences should be different.

To solve this mystery, we can select the link to one of the *Sus scrofa* records and look at the results. If we search the record for the word "lion," we see that the journal is published from an address at "Lion Mountain 1" street.

What if we were searching for something from lions but instead found thousands of records from pigs? What could we do to improve our results?

We can get ideas by looking at the way Entrez did the search. Selecting the "Details" tab from our search results shows us that Entrez searched the "Organism" field with the scientific name for lion (*Panthera leo*) and Entrez searched all the fields with the word "lion."



Figure 1: Select the "Details" tab.

Consequently, our results included all the records where *Panthera leo* could be found in the organism field plus all the records that included "lion" anywhere in the record. We can use this information to help guide our quest for more specific results.

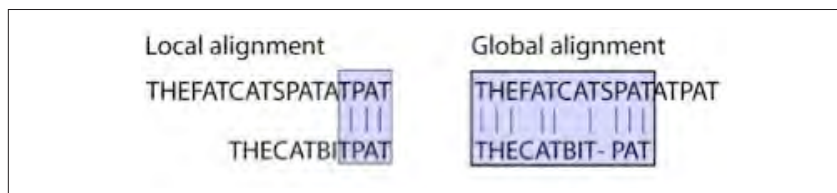
Discussion: What do you think would happen if you used "*Panthera leo*" [Organism] as a query instead of "lion?"

You can do the experiment and find out.

Understanding BLAST

BLAST stands for Basic Local Alignment Search Tool. An alignment is a way of lining sequences up in rows so they're easier to compare. A local alignment is one where short regions of the sequence are aligned preferentially over long regions.

Figure 1: Local vs. global alignments.



Although the name “BLAST” sounds like one program, BLAST is really a family of programs that are widely used by biologists all over the world to compare sequences from DNA, RNA, and proteins. Nucleotide blast (blastn) is used to compare nucleotide sequences. Protein blast (blastp) is used to compare protein sequences. Other kinds of blast programs add a step where nucleotide sequences are translated to protein sequences before searching. Blastx, for example, translates a nucleotide query in all six reading frames and compares the predicted amino acid sequences to a protein database. Tblast compares a protein query to a translated nucleotide database; and tblastx translates both a nucleotide query sequence and the nucleotide database sequences before doing a comparison.

How does BLAST work?

BLAST begins the process of comparing sequences and aligning matching regions by breaking sequences into shorter strings of text, called “words.” A typical “word” might be 11 bases or amino acids long with each base or amino acid represented by a single letter in the word. BLAST creates words for both the query sequence (the one we’re testing) and all the sequences in a database. Then, every word from the query sequence is compared to every word in the database until words are found that match perfectly.

Once BLAST has found a word from the query that matches a database word, the program evaluates the letters at the end of each word to determine whether the matching region can be extended. This process continues until the end of the sequence is reached or the sequences no longer match.

BLAST scores and statistics

When the BLAST programs were first written in 1990, their major function was to determine whether two sequences were similar enough to make it likely they evolved from a common ancestor. Since the original goal for BLAST was to find matching sequences and measure the significance of the match, BLAST provides many statistics for each search and assigns different scores that can be used to evaluate the results.

BLAST scores from protein comparisons are based on evolution. If a mutation occurs in a nucleic acid sequence that changes an amino acid, the altered protein experiences natural selection. If the change has a beneficial or neutral effect, the change can persist and be inherited. If an amino acid change is harmful, negative selection will make it less likely to persist in a population. In general, amino acid replacements are tolerated better when the new amino acid is either chemically similar or located in a less important part of a protein.

When researchers wrote the scoring system for BLAST, they looked at all the changes that took place between amino acid sequences from the same protein in different organisms and used that data to calculate probability values for each possible amino acid replacement. A BLAST score for a pair of two protein sequences is calculated by looking at each position, finding the likelihood for each position that one amino acid will be replaced by another, and adding those values together. For example, say we had one protein sequence like “ELVIS” and another like “ELVES.” We would look at the BLAST scoring table to find the probability of E replacing E is 5, the value for L replacing L is 4, for V replacing V is 4, for E replacing I is -3, and for S replacing S is 4. We add these together: $5 + 4 + 4 + -3 + 4$ and get a BLAST score of 14. For nucleotide sequences, BLAST calculates a score based on identity. BLAST assigns two points for each position where a pair of nucleotides matches and subtracts points for each position where they do not. Once BLAST has calculated a score, the program applies corrections based on the size of the database and the length of the sequence to arrive at a value called the “E” or “Expect” value. The E value corresponds to the number of sequences that one would expect to find, with an equivalent number of matching residues, in a database of certain size, containing random sequences. If a BLAST result has an E value of 5, it means we would expect to find five sequences in a random set. If a BLAST result has an E value so low that BLAST rounds it off to zero, we would not expect to find a match that good in a random set.

Other applications where BLAST is used

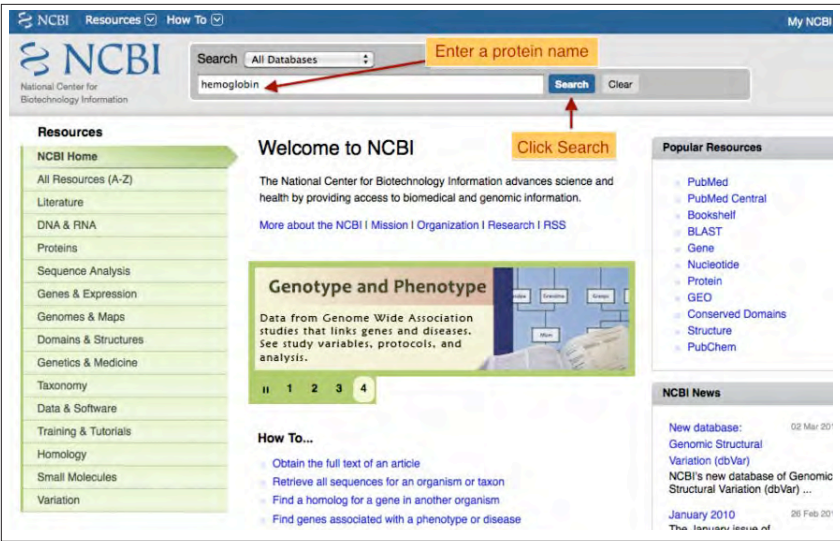
Although BLAST was written with the goal of finding homologous sequences, scientists use BLAST for many other tasks. BLAST can be used to determine where sequences with matching regions are positioned relative to one another, to view the relationship between mRNA and genomic DNA, to design and test PCR primers, to distinguish between different species, and to identify genetic variation and mutation sites. The NCBI even uses BLAST as a step in producing phylogenetic trees. Over the years, BLAST has become one of the most commonly used programs in biology.



Finding Structures in the NCBI Structure Database

- 1. Go to the NCBI website (http://www.ncbi.nih.gov).
- 2. Enter the name of a protein or gene in the text box and click the “Search” button (see **Figure 1**).

Figure 1: Enter the protein or gene name and click “Search.” Credit: NCBI MMDB.



Searches that begin at the NCBI home page scan the contents of all the NCBI databases and provide the results on a page like the one below. The number of matching records appears next to the name for each database.

- 3. Click “Structure” to obtain the search results from the structure database. (See **Figure 2**).

Figure 2: Choose the “Structure” database. Credit: NCBI MMDB.



The search results consist of a list of records from the database. Each record has a unique number (an accession number) that can be used to access the record.

If you find too many search results, you may narrow the search by using the word “AND” in combination with other search terms. For example, if you wish to find structures for human proteins, you may wish to search with the terms “hemoglobin AND homo.” (See **Figure 3**).

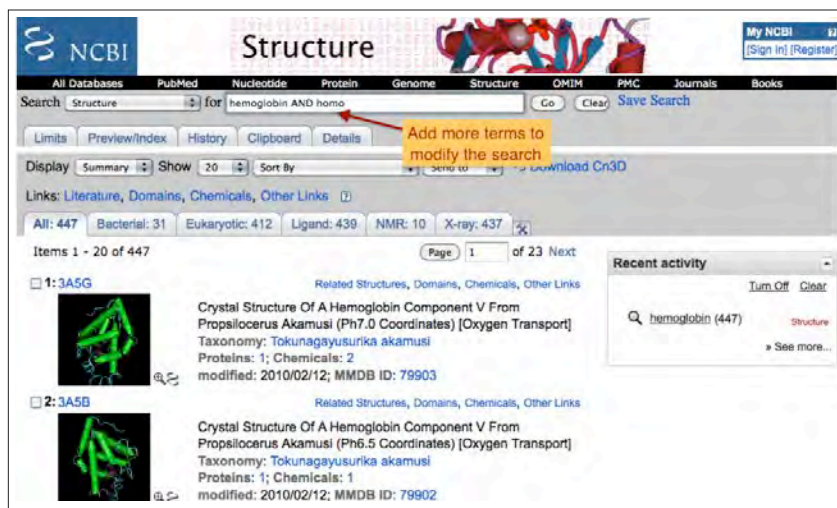


Figure 3: Narrow your search by using a combination of search terms. Credit: NCBI MMDB.

- Click the accession number (see **Figure 4**) for a record to view the complete information and access the download link for an individual structure.

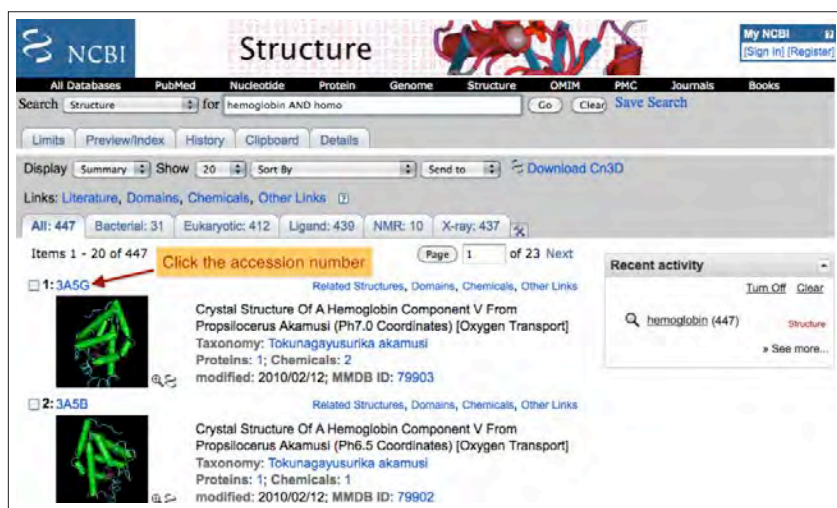
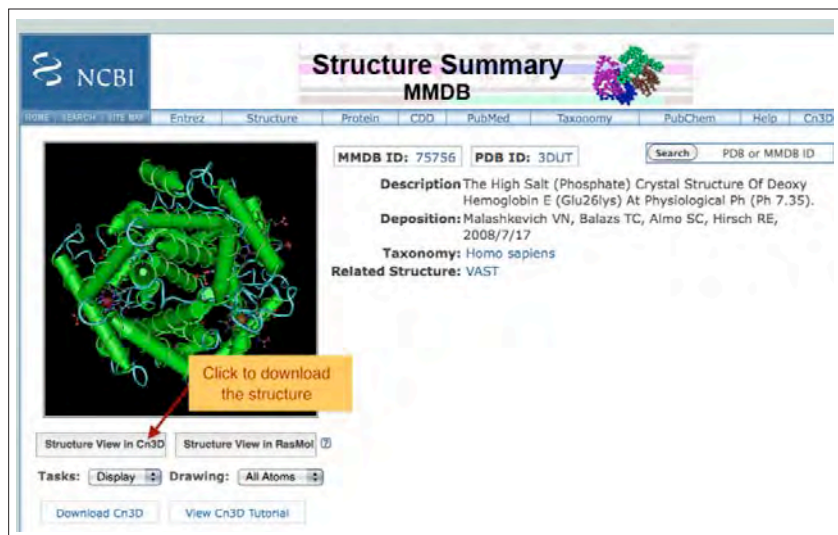


Figure 4: Click the accession number. Credit: NCBI MMDB.

- Click the “Structure View” in Cn3D box to download the structure to your computer. (See **Figure 5**).

Figure 5: Click “Structure View” to download the structure. Credit: NCBI MMDB.



- Save the file on your computer and open it in Cn3D.