FUTURELAB+

BIOMED

Behind the Scenes of Scientific Breakthroughs

Longevity Markers: How are you so old?

Laboratory Investigation

Developed in partnership with:

Bay Area Bioscience Education Community

In this Lesson Plan:

Print the **Teacher Section** →

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Cover Image
This is a conceptual illustration of genetic engineering.

BIOTECHNOLOGY / BEHIND THE SCENES OF SCIENTIFIC BREAKTHROUGHS

Lab: Longevity Markers: How are you so old?

ACTIVITY DURATION

Five class sessions
(45 minutes each)

DRIVING QUESTION

How can we detect unique genome features of humans who live the longest?

ESSENTIAL QUESTIONS

Are there genes that can tell us how long we might live?

What are different techniques and tools we can use to study DNA?

OVERVIEW

Understanding what determines someone's lifespan is a subject of fascination among many researchers. Though it is clear that environmental factors and lifestyle have the biggest impact on longevity, there is evidence that genetic factors contribute to about 25% of human lifespan variation (Passarino et al., 2016). However, which genes are linked to healthy aging and how their function leads to a long life is not well understood. Genome-Wide Association Studies (GWASs) are a tool that has allowed this research to move forward. In a GWAS, genetic data from thousands of individuals are analyzed to determine if there is a statistically significant association between a specific region of DNA and a particular trait.

In this lab, students will model the steps that may be conducted in a GWAS study of longevity. First, students have the opportunity to perform a DNA extraction on their own cheek cells (or from strawberries) and use gel electrophoresis to verify that they successfully isolated DNA. Next, they will "send their DNA off to be sequenced" and receive a string of nucleotides around the SNP rs2802288. Using BLAST, they will then be able to determine what gene this SNP is a part of. Finally, they will analyze genotype data from a mock GWAS of 100 individuals and determine if the data suggests there is an association between rs2802288 and longevity.

BACKGROUND INFORMATION

This lesson introduces students to important lab techniques that allow them to visualize DNA on various levels including: DNA extraction, gel electrophoresis (which requires proficiency with micropipetting), and BLAST to identify a nucleotide sequence. Prior to beginning this lesson, you may also wish to have students view their DNA under a microscope by staining cheek cells as another level of observation. It is useful for students to be well-grounded in their understanding of how variations in DNA affect the inheritance of traits. At this point in the unit, students may also have learned about cellular aging, the human genome project, and genetic sequencing as a concept, which would help provide further context.

Source: *Human Longevity*

Have you ever wondered…

Why do some people live longer than others?

Longevity is mostly determined by factors in the environment, but it is clear that there are certain genetic variations associated with long life. The more we understand the functions of these genes in the body, the more we can learn about age-related diseases, such as cancer and Alzheimer's. This may also lead to better diagnostic tests and treatments for diseases, as well as potentially lifespan-increasing therapies.

Sources:

The Quest for Genetic Determinants of Human Longevity: Challenges and Insights

Live Long and Proper: Genetic Factors Associated with Increased Longevity Identified

MAKE CONNECTIONS!

How does this connect to the larger unit storyline?

In the quest to understand the many factors that influence aging and lifespan, it is important to include a discussion of genetic factors. The study of longevity genes is a developing science that has many implications for how we treat and prevent age-related diseases and improve the quality of life for an aging population. This subject is also of great interest to researchers and individuals who are attempting to manipulate and increase human lifespan.

How does this connect to careers?

Geneticists focus on the discovery of genetic variants that influence human diseases and traits. In order to do so, they may collect and isolate DNA samples, and evaluate sequencing data to discover genetic alleles that influence a variety of phenotypes.

Bioinformaticians use technology and computer science to find solutions to problems in the area of biology. They may work on open technologies that reduce DNA sequencing costs or develop applications that allow for the processing of massive data sets. This can help researchers interpret variant data.

How does this relate to the product development life cycle?

When developing new techniques and treatments for aging-related illness or to increase lifespan, an important step is to understand what is unique about the genomes of humans that live the longest, which genetic markers are associated with them, and the mechanism by which they act.

Pedagogical Framing

Instructional materials are designed to meet national education and industry standards to focus on in-demand skills needed across the full product development life cycle—from molecule to medicine—which will also expose students and educators to the breadth of education and career pathways across biotechnology.

Through this collection, educators are equipped with strategies to engage students from diverse racial, ethnic, and cultural groups, providing them with quality, equitable, and liberating educational experiences that validate and affirm student identity.

Units are designed to be problem-based and focus on workforce skill development to empower students with the knowledge and tools to be the change in reducing health disparities in communities.

SOCIAL-EMOTIONAL LEARNING

Students work cooperatively with a partner and group of four throughout the lab, such as during the phenomenon and lab analysis, helping them co-construct knowledge and develop relationship skills. They also build social awareness during activities that require resource sharing and time management, such as during the DNA extraction and gel electrophoresis.

CULTURALLY AND LINGUISTICALLY RESPONSIVE INSTRUCTION

Using bioinformatics tools, such as BLAST and modeling a Genome-Wide Association Study, are advanced topics that bridge molecular biology and statistics. This rigorous content models high expectations for all students as well as illuminates the techniques behind personal genomics services with which they are already familiar. Additionally, students have the opportunity to extract DNA from their own cheek cells and visualize it using gel electrophoresis, allowing them to practice these lab skills in a personal context.

COMPUTATIONAL THINKING PRACTICES

Throughout this lab, students will engage in computational thinking strategies, such as finding patterns, analyzing data, and modeling with mathematics. As students engage with the phenomenon charts centered around centenarian population data, they will look for patterns that prime them to understand the concept of Genome-Wide Association Studies (GWAS). These studies aim to identify patterns between particular single nucleotide polymorphisms (SNPs) and particular phenotypes. Students will analyze data collected from their gel electrophoresis results, as well as using the BLAST database to determine if sample DNA contains a particular SNP. Lastly, they will model a GWAS by analyzing data and identifying patterns in a set of sample results.

OBJECTIVES

Students will be able to:

Extract DNA from their cheek cells, **visualize** the DNA using agarose gel electrophoresis, and **analyze** a sample DNA sequence with BLAST using protocols.

Describe how gel electrophoresis separates molecules based on size and charge using scientific text.

Describe factors that affect longevity, including particular genetic variations (SNPs) and the methods used to study these genes using scientific text.

Analyze data from a Genome-Wide Association Study (GWAS) to determine if there is an association between the SNP rs2802288 and longevity using a graph and data table.

Materials

Documents
Lab Preparation (for teacher)
Sample Permission Slip for Student DNA Extraction (for teacher to customize)
Building Lab Skills: Agarose Gel Electrophoresis (1 per student)
Background Reading: Genetic Markers of Longevity (1 per student)
Phenomenon Charts (1 per group)
GWAS Results (1 per group)
Vocabulary Tool (1 per student)
Student Protocol, Part 1: DNA Extraction (1 per pair)
Student Protocol, Alternative Part 1: Strawberry DNA Extraction (optional)
Student Protocol, Part 2: Agarose Gel Electrophoresis (1 per pair)
Student Protocol, Part 3: DNA Sequence Analysis with BLAST (1per pair)
Student Guide (1 per student)

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Materials

Reagents

Lab Part 1: DNA Extraction

- *0.9% saline solution (10 mL per student)*
- *5% Chelex (2 tubes per pair)*

Lab Part 2: Agarose Gel Electrophoresis

- *1X TAE running buffer (150–300 mL per group of four students—depending on which gel electrophoresis system you are using)*
- *0.8% agarose gel with DNA stain (1 per group of four)*
- *Students' DNA sample from Lab Part 1 (1 per student)*
- *Negative control (1 per group)*
- *10X loading dye (1 per group)*
- *1 kb+ ladder (1 per group)*

Equipment and Consumables

Lab Part 1: DNA Extraction

- *P1000 micropipettes (1 per pair*
- *P1000 tips (1 box per pair)*
- *P200 micropipettes (1 per pair)*
- *P200 tips (1 box per pair)*
- *Small disposable cups to portion out saline solution (1 per student)*
- *1.5 mL microtubes (4 per pair)*
- *Microtube rack (1 per pair)*
- *Centrifuge (1–2 per class)*
- *Heat block set at 99˚C (1 per class)*
- *Cap locks (4 per pair)*
- *Permanent marker (1 per pair)*
- *Dry waste beaker (1 per group of four)*
- *Sink or wet waste beaker (1 per group of four)*

Lab Part 2: Agarose Gel Electrophoresis

- *P20 micropipettes (1 per pair)*
- *P20 tips (1 box per pair)*
- *Microtube rack (1 per pair)*
- *Electrophoresis gel setup (1 per group of four)*
- *UV light source*
- *UV safety goggles (if needed for electrophoresis equipment)*

Day 1

LEARNING OUTCOMES

Students will be able to:

Demonstrate proper use of gel electrophoresis by loading practice dyes into agar-agar gels (inexpensive agarose substitute using protocols).

Describe how gel electrophoresis separates molecules based on size and charge using scientific text.

Procedure

Teacher Note > *If time allows for a pre-lab activity, students may complete a virtual gel electrophoresis in this DNA Extraction Simulation from Learn Genetics at the University of Utah. If materials and equipment are not available for an in-class gel electrophoresis lab, the instructor may adapt the pre-lab portions of the Case of the Crown Jewels from MdBioLab to allow students to cut DNA fragments and create a poster-sized gel electrophoresis to simulate and interpret the results of the process.*

Teacher Note > *Before implementing Part 1 of the lab, in which students extract DNA from their cheek cells, check your school district policy to determine if students are allowed to isolate their own cells and extract their own DNA. If so, we suggest modifying the Sample Permission Slip for Student DNA Extraction and requiring that students have it signed by a parent or guardian before participating in the lab. If not, we offer a Student Protocol, Alternative Part 1: Strawberry DNA Extraction.*

Prepare in advance materials for practice gels

1 Prepare one 12% agar-agar gel (e.g., "Golden Coin" brand) for each pair of students (can be one per two pairs).

a. Add 12 g of agar-agar powder to 100 mL of water (adjust numbers accordingly to make whatever volume you need):

- Mini-One electrophoresis equipment = ~15 mL per gel.
- Other electrophoresis equipment (e.g., Fotodyne) = ~25 mL per gel.

b. Microwave on a low-power setting (about 50% or on "defrost") until liquid is translucent. Check every 5 minutes until melted.

- CAUTION: agar-agar can be superheated and let off steam explosively. Microwaving at a low-power setting for longer reduces this possibility.
- After making sure there are no visible lumps, microwave at full power for 20 seconds to dissolve any remaining solute.

c. Pour into gel casting trays with combs.
(Use the side of the comb with the higher number of wells. If using large equipment, you have the option to use two combs in one gel to increase the number of wells).

d. Allow to cool until solid.

e. Carefully remove comb.

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Day 1

Continued

Procedure

2		Dilute and aliquot loading dye.
	a.	Dilute loading dye with water in a 1:10 ratio (e.g., 300 µL dye + 3 mL water).
	b.	Aliquot into 1.5 mL tubes with ~110 µL in each.
3		Set up the following materials per pair:
	a.	Agar-agar gel, an acting agarose substitute, one per two pairs.
	b.	Diluted loading dye (~110 µL), an acting DNA substitute.
	c.	Water, a 1XTAE buffer substitute, to fill the electrophoresis chambers.
	d.	P20 micropipette and tips.
	e.	Waste container.
	f.	Gel electrophoresis system without power supply or lid (can be one system per two pairs).

Whole Group (15 minutes)

1	Pass out copies of *Building Lab Skills: Agarose Gel Electrophoresis* and lab materials.
2	Warm up: Carefully touch the agar-agar gel without breaking it (poke/prod/pick up). Make three observations about what you notice. Encourage students to share their experiences with eating or cooking with agar (it is common in many Asian desserts).
3	Explain that today students will learn what that gel is used for in a biotechnology lab and practice loading samples into it.
4	Search for a video of "gel loading mistakes" and share with students. Ask them to record three things from the video they think will be especially important for when they load their gels.
5	Model the steps under *Building Lab Skills: Agarose Gel Electrophoresis, Hands-on Practice* (under a document camera if possible) while having students read and annotate the steps on their own copies.

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Day 1

Continued

Procedure

Small Group (25 minutes)	
1	Ask students to take turns loading a practice gel with their partners by following the *Building Lab Skills: Agarose Gel Electrophoresis, Hands-on Practice* steps. Each student should load half the number of wells. *(If two pairs of students are sharing one electrophoresis system, have one pair do the reading and questions before the hands-on practice)*.
2	Ask students to complete the reading and answer Questions #1–3. They should check their answers with the key.
3	Ask students to clean up by putting the gel in the trash and emptying the water down the drain.

Individual (5 minutes)	
1	Exit Ticket: What are some of the applications for running an agarose gel? What could be learned from it? What are some ways to avoid mistakes with loading samples into a gel?

Day 2

LEARNING OUTCOMES

Students will be able to:

Observe and ask questions about centenarian populations and longevity genes using charts.

Describe factors that affect longevity, including particular genetic variations (SNPs) and the methods used to study these genes using scientific text.

Procedure

Whole Group (20 minutes)

1. Warm up: How old is the oldest person you have ever known? Why do you think they have lived longer than other people?

2. Ask students to share with a partner and call on students randomly to share with the whole class.

3. Share the *Phenomenon Charts* with each group and ask students to record two observations and two questions on Question #1 of the *Student Guide, Part 1: Pre-Lab* about what they see. Possible sentence starters include: "I notice…Reminds me of…I wonder…Could it be…"

   Charts:

   1. By 2050, China is expected to have the largest centenarian population, followed by Japan, Italy, and India.

   2. Five genes were investigated in this study (ADIPOQ, FOXO1A, FOXO3A, SIRT1, and COQ7). Statistical analysis of results showed that FOXO3A was strongly associated with longevity with a P-value of < 0.05.

4. Have students share responses with their elbow partners or lab groups and add a new observation or question they hear from a peer to each phenomenon.

5. Prompt students to share their ideas and engage in a whole class discussion about each Phenomenon Chart. You may wish to compile class observations and questions on the board or document and keep visible throughout the lesson as a way to help students make connections as they progress through the lab. As students share their questions about Chart 2, there should be an opportunity to lead into a brief explanation of this lab and how it will model a Genome-Wide Association Study.

   a. Search for a video of 'genome-wide association studies beginners' and show the first four and a half minutes of the top result, which provides an explanation of GWAS.

   b. After the video, there is another opportunity to have a structured discussion and/or return to some of the class observations and questions and record newly acquired information.

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Day 2

Continued

Procedure

Small Group (20 minutes)

1 Ask students to read *Background Reading: Genetic Markers of Longevity* and answer the questions that follow. When all students have finished, review the answers as a class.

2 Ask students to write their own sentences for the words in the *Vocabulary Tool* and complete it for homework.

Individual (5 minutes)

Exit Ticket: In plain language, what is a 'genetic marker of longevity' and how are researchers identifying them?

Day 3

Procedure

LEARNING OUTCOMES

Students will be able to:

Extract DNA from their cheek cells using protocols.

Predict what gene the SNP rs2802288 is a part of and whether or not it is associated with longevity using their understanding of SNPs and genetic markers of longevity.

Teacher Note > *Before implementing Part 1 of the lab, in which students extract DNA from their cheek cells, check your school district policy to determine if students are allowed to isolate their own cells and extract their own DNA. If so, we suggest modifying the Sample Permission Slip for Student DNA Extraction and requiring that students have it signed by a parent or guardian before participating in the lab. If not, we offer an Alternative Student Protocol, Part 1: Strawberry DNA Extraction.*

Teacher Note > *Before class, prepare saline solution, set up heat block and centrifuge station(s) around the room, and set up lab stations using Lab Prep (approximately 45 minutes).*

Whole Group (10 minutes)

1. Introduce the lab to students by reading the lab overview in the *Student Guide, Part 1: Pre-Lab* as a class, making sure to annotate to increase student comprehension.

2. Instruct students to skip to Question #1 in the *Student Guide, Part 2: Lab and Data Collection* to introduce the overview flowchart that provides students with an overview of the major lab steps. Read each one together. Explain that identifying SNPs and determining if they are associated with a particular trait, in this case longevity, is a multi-step process that involves different teams of scientists working together. In this lab, students will get a glimpse of some of these critical steps.

   a. Give students a few minutes to predict the purpose of each of the four major lab steps considering the purpose of the investigation.

   b. Go over responses and fill in the table together using a document camera, explaining to students that information provided on the *Student Guide, Part 2: Lab and Data Collection Answer Key*.

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Day 3

Continued

Procedure

3		Inform students they will be performing step one today—extract DNA from their cheek cells. Point out where the heat block and centrifuge station(s) are and share the following safety precautions and guidelines:
	a.	Do not touch heated blocks (they may be extremely hot, even if off!).
	b.	Always make sure that the centrifuge is balanced with a counter weight before running.
	c.	Wash your hands before and after the lab.
	d.	Do not eat or drink during the lab.
	e.	Keep your lab station clean and clear of clutter.

Small Group (35 minutes)

1		Break students into lab pairs, pass out lab stations and ask them to complete the steps of *Student Protocol Part 1: DNA Extraction.*
2		During downtime, instruct students to discuss possible experimental outcomes with their lab teams and to record their predictions on Questions #2–3 in the *Student Guide, Part 1: Pre-Lab.*
3		Have students clean up and give you their tubes of isolated cheek cell DNA to store in the freezer until next class.

Day 4

LEARNING OUTCOMES

Students will be able to:

Visualize their cheek cell DNA using agarose gel electrophoresis using protocols.

Analyze a sample DNA sequence with BLAST to determine the gene that contains the SNP of interest (rs2802288) using protocols.

Procedure

Teacher Note > *Before class, remove student cheek cell DNA samples from the freezer and set up lab stations according to the Lab Prep document. Each group of four students (two lab pairs) will need one agarose gel with at least seven lanes. Suggest pre-pouring gels for students to reduce error but you may also have each group pour their own.*

Whole Group (5 minutes)	
1	Warm-Up: Describe what happens during agarose gel electrophoresis in one sentence or draw a picture.
2	Ask students to share with a partner and come up with one sentence together that combines both of their ideas.
3	Call on three students to share with the class. (This is a great opportunity to highlight student voices who rarely volunteer.)
4	Share the learning outcomes for the day.

Small Group (35 minutes)	
1	Pass out one copy per pair of *Student Protocol, Part 2: Agarose Gel Electrophoresis* and *Student Protocol, Part 3: DNA Sequence Analysis with BLAST.*
2	Ask students to retrieve their cheek cell DNA samples and go to their lab stations.
3	Share the following safety reminders with students and ask them to complete the steps of *Student Protocol, Part 2: Agarose Gel Electrophoresis.*
	a. Be careful with liquid agarose—it is extremely hot!
	b. If using non-minione electrophoresis equipment, turn off the power supply before opening the gel box.
	c. If using non-minione electrophoresis equipment, wear UV goggles when looking at the gel under UV light.

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Day 4

Continued

Procedure

4	While the gel is running, students can use their computer to follow the steps of *Student Protocol, Part 3: DNA Sequence Analysis with BLAST* and answer Question #3 in the *Student Guide, Part 2: Lab and Data Collection*. Share the DNA Sequence with students digitally so they can copy and paste it into the website.
5	After the gel has run, ask students to take a picture with their phones (or take a picture for them) and attach it to Question #2 in the *Student Guide, Part 2: Lab and Data Collection* and label the lanes.

Individual (5 minutes)

1	Exit Ticket: On the gel, did your cheek cell DNA look like a clear band or a blob/smear? Why do you think this is? (It should look like a smear due to the large quantity of DNA loaded on the gel of multiple sized fragments—the cheek cell DNA is the entire genome.)

Day 5

LEARNING OUTCOMES

Students will be able to:

Analyze data from a Genome-Wide Association Study (GWAS) to determine if there is an association between the SNP rs2802288 and longevity using a graph and data table.

Describe limitations of the Genome-Wide Association Study and a potential follow-up experiment.

Procedure

Whole Group (10 minutes)

1. Warm-Up: Ask students if they have ever heard of personal genomics such as 23andMe or Ancestry.com and prompt students to share anything they know about those services and the methods they use.
2. Ask students to share with a partner.
3. Call on three students to share with the class (This is a great opportunity to highlight student voices who rarely volunteer.)
4. Share with students that GWAS is the technology that personal genomics companies use to identify genetic variants associated with certain traits and diseases. Today, students will look at the results of a fictional GWAS to determine if there is an association between the SNP rs2802288 and longevity. Remind them that the study includes the rs2802288 genotypes of 100 individuals ages 21–112 (two possible alleles—A or G).

Small Group (20 minutes)

1. Pass out *GWAS: rs2802288 Results* and ask students to work with their partners to complete *Student Guide, Part 3: Data Analysis* and *Student Guide, Part 4: Extension.*
2. Option to pause at Question #4 in the *Student Guide, Part 3: Data Analysis* (observations of the graph) and have a whole class discussion about the GWAS results. Possible questions:
   - **a.** What patterns do you notice?
   - **b.** What is surprising?
   - **c.** What data is missing?
3. Ask students to prepare a two minute pitch for Question #2 in the *Student Guide, Part 4: Extension* to describe a follow-up experiment or study that they will share with three other lab pairs. Explain that they will be voting on the one they think will provide the most useful data in furthering our understanding of the genetics of longevity.

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Day 5

Continued

Procedure

Small Group (10 minutes)	
1	Break students into groups of four lab pairs.
2	Ask each pair to take turns sharing an idea for a follow-up experiment or study.
3	Ask the students in each group to vote on the experiment they think will provide the most useful data in furthering our understanding of the genetics of longevity.

Individual (5 minutes)	
1	Exit Ticket: Would you be interested in having some of your DNA sequenced and participating in a GWAS in the future? Why or why not?

National Standards

Next Generation Science Standards

LS3.A: Inheritance of Traits
Each chromosome consists of a single very long DNA molecule, and each gene on the chromosome is a particular segment of that DNA. The instructions for forming species' characteristics are carried in DNA. All cells in an organism have the same genetic content, but the genes used (expressed) by the cell may be regulated in different ways. Not all DNA codes for a protein; some segments of DNA are involved in regulatory or structural functions, and some have no as-yet known function.

LS3.B: Variation of Traits
Environmental factors also affect expression of traits, and hence affect the probability of occurrences of traits in a population. Thus the variation and distribution of traits observed depend on both genetic and environmental factors.

Science and Engineering Practices
Analyzing and Interpreting Data
Analyze data using tools, technologies, and/or models (e.g., computational, mathematical) in order to make valid and reliable scientific claims or determine an optimal design solution.

Crosscutting Concepts
Patterns
Recognizing patterns is an important part of interpreting data.

Math

MP.4 Model with mathematics.
Use a function to describe how one quantity of interest depends on another. Able to map the relationship between SNPs and phenotypes using a graph. Analyze those relationships mathematically to draw conclusions.

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National Standards

Continued

Career and Technical Education (CTE)	**A3.3** Employ standard techniques of DNA extraction, purification, restriction digests, bacterial cell culture, and agarose gel electrophoresis and document and evaluate results.
	A8.1 Follow written protocols and oral directions to perform a variety of laboratory and technical tasks.
	A8.6 Properly and safely use and monitor a variety of scientific equipment, including pH meters, microscopes, spectrophotometers, pipettes, micropipettes, and balances.
	A8.7 Determine which equipment is appropriate to use for a given task and the units of measurement used.

Lab

Preparation

KEY

When the preparation task should take place in relationship to the lab

The amount of time necessary to complete the preparation task

Quick Tips	
1	Before continuing, check the *Materials List* to make sure you have all the necessary equipment and reagents for the lab.
2	We recommend having students complete the *Student Protocol, Part 1: DNA Extraction* in pairs (with each student extracting his or her own DNA) and then team up with another pair to have a group of four students for the *Student Protocol, Part 2: Agarose Gel Electrophoresis.*
	a. Lab Part 1: Each pair is responsible for their own tube of extracted DNA.
	b. Lab Part 2: Each student will run their DNA sample in one of four wells that are available to use in each gel.
3	*Virtual Learning Options* for this lab, including digital-only resources, are provided.

Preparation, Lab Part 1: DNA Extraction

1	Before the lab	10 min
	Prepare 0.9% saline solution	
☐	Dissolve 4.5 g of non-iodized salt in 500 mL of water. (If you need to make a different volume, use 0.9 g of salt per 100 mL of water to calculate what you need)	
☐	Aliquot 10 mL of 0.9% saline solution into small disposable cups for each student.	

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Lab

Continued

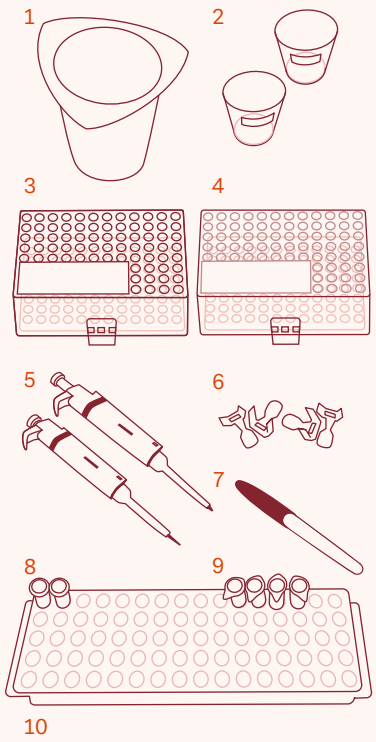
Preparation

2 | Before the lab | 30 min

Set up lab stations

☐ Give to each pair of students:

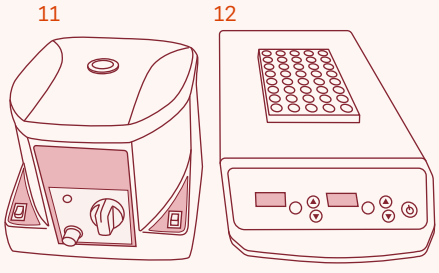
1	Waste bucket
2	2 cups with 10 mL 0.9% saline solution each
3	Blue tips (for P1000)
4	Yellow tips (for P200)
5	P200 and P1000
6	4 cap locks
7	Permanent marker
8	2 tubes with 5% Chelex
9	4 empty microtubes
10	Microtube rack



Note > *Cap locks prevent tubes from opening due to pressure and allow for easier handling. If you do not have cap locks available, you may cover all the tubes with a sheet of foil after all students have placed their tubes in the heat block.*

☐ Gather one or more per classroom:

11	Centrifuge (1–2 stations per class)
12	Heat Block (1–2 stations per class)



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Lab

Continued

Preparation

3	Before the lab	5 min
☐	Set-up the centrifuge(s): — You will need one to complete this lab with your class, but we recommend having more than one in the room to prevent bottleneck situations. — If you only have one, we recommend doing the DNA extraction part of the lab in lock step to allow for easier management of the centrifuge. — Never start the centrifuge with an uneven number of tubes—model for students how every tube must be counterbalanced with another tube, otherwise it can damage the equipment.	

4	Within one hour of the lab's start time	30 min
☐	Preheat the heat blocks: — Allow to preheat to 99˚C prior to the start or at the beginning of class. — We recommend leaving a note next to the heat block to caution students from touching the heated blocks because it may not be apparent that the unit is on. — If you do not have a heat block available, please set up a water bath at 99˚C—take similar precautions for safety.	

Lab

Continued

Preparation

Preparation, Lab Part 2: Agarose Gel Electrophoresis

1 Before the lab — 1 hour

☐ Prepare the positive control (calf thymus DNA). Add 20 µL of loading dye into the provided tube of Positive Control DNA and mix well.

☐ Prepare the Negative Control (water). Add 20 µL of loading dye into the provided tube of Negative Control and mix well.

☐ Aliquot reagents into 1.5 mL tubes

Sample	Volume/Group
Loading dye	25 µL
1 kb+ ladder	15 µL
Positive Control (calf thymus DNA)	15 µL
Negative Control (water)	15 µL

2 Before the lab — 1 hour

☐ Prepare 1X TAE and 0.8% agarose gels (one gel with at least seven wells per group of four). See *Preparing 0.8% Agarose Gels*.

☐ Suggest pouring extra gels in case students puncture theirs with the micropipette.

Options for pouring gels:

Students Pour During Lab	Teacher Pours Day *before* Lab	Teacher Pours Day *of* Lab
Give each group a 50 mL beaker with 20 mL[1] liquid agarose[2]. Gels typically take 15–20 minutes to solidify.	Gels can be stored in the refrigerator wrapped in plastic with 1–2 mL of 1X TAE buffer to keep moist.	You can reuse gels by remelting in the microwave.

[1] Amount of liquid agarose needed may vary between gel boxes

[2] Keep melted agarose in 65°C water bath to prevent solidifying

Lab

Continued

Preparation

3 Day of lab — 30 min

☐ Set up lab stations (one per group of four).

1	Gel electrophoresis system
2	Agarose gel
3	Yellow tips (for P20)
4	150–300 mL, 1X TAE buffer
5	Micropipette P20
6	Waste bucket
7	Loading dye
8	1 kb+ ladder
9	Negative control-water
10	Positive control calf thymus DNA
11	4 Student DNA samples
12	Microtube rack

4 After lab — 15 min

☐ Properly dispose of lab supplies:

- Any excess solutions can go down the drain.
- Used micropipette tips and microtubes can go in the trash.
- Agarose gels can also be discarded in the regular waste. GelGreen® DNA stain is not hazardous.
- Agarose gels can be reused. For best quality, do not reuse the agarose gel more than five times. To reuse gel, simply stuff the gel back into a bottle or a beaker. Make sure to keep different percentage gels in separate containers. Microwave gel until liquid to recast. You will need to re-add DNA stain if viewing DNA.

Continues next page >

Lab

Continued

Virtual Learning Options

1	Anytime	30 min

Digital Options

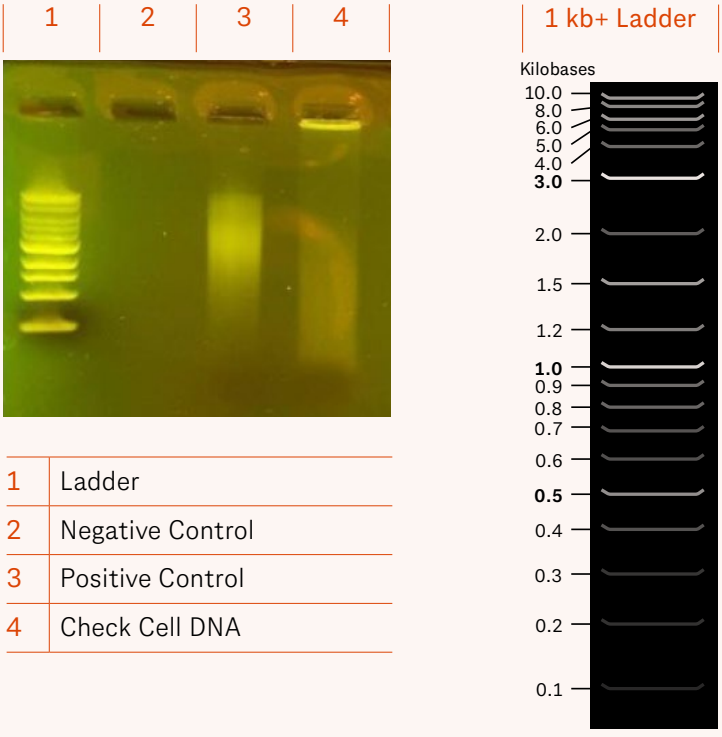
☐ Students click through and answer the questions embedded in this *DNA Extraction Simulation* from Learn Genetics at the University of Utah.

☐ Adapt the pre-lab portions of the *Case of the Crown Jewels* from MdBioLab to allow students to cut DNA fragments and create a poster-sized gel electrophoresis to simulate and interpret the results of the process.

☐ Students click through and answer the questions embedded in one of the following Gel Electrophoresis simulations:

— *DNALC*
— *University of Utah*
— *LabXchange*

☐ Give students the following example gel and have them complete the *Student Guide*:



1	Ladder
2	Negative Control
3	Positive Control
4	Check Cell DNA

Continues next page >

Lab

Continued

Virtual Learning Options

2	Anytime	30 min
	At Home	
☐	Students isolate strawberry DNA at home to mimic the process of extracting DNA from human cells. Follow steps in this *video* from the National Human Genome Research Institute.	

Skills

Preparing 0.8% Agarose Gels

Teacher Note > *Watch this video from the University of Leicester for an overview, however, be sure to follow the instructions below specific to this lab: Making an Agarose Gel—University of Leicester.*

Procedure	
1	Prepare 1X TAE by adding 20 mL 50X TAE to 980 mL of distilled water.
2	Add 2.4 g agarose powder to a 500 mL or larger glass bottle, flask, or beaker.
3	Add 1X TAE to the agarose powder to a final volume of 300 mL.

Note > *For volumes of agarose less than 300 mL, make sure to pour the amount of liquefied agarose you need to a clean beaker and use the corresponding amount of GelGreen® for the volume, e.g. for 100 mL of agarose, use 100 µL of 1000X GelGreen®.*

4		Microwave on a low power setting (like 50% or on "defrost") until liquid is translucent. Check every five minutes by removing the bottle with an oven mitt and swirling until melted.
	a.	CAUTION: Agarose can be superheated and let off steam explosively. Microwaving at a low power setting for longer reduces this possibility.
	b.	After making sure there are no visible lumps, microwave at full power for 20 seconds to dissolve any remaining solute.
5		Let the agarose cool slightly on the benchtop for 5–10 minutes (until you can touch the bottle without burning your hand and the agarose is still liquid or 50–60°C).

Continues next page >

Skills

Continued

Preparing 0.8% Agarose Gels

6	While the agarose is cooling, prepare ten gel trays with combs (this lab requires at least seven wells).
7	To the 300 mL of cooling agarose, add 300 µL of GelGreen® DNA stain (provided at a 1000X concentration). Swirl thoroughly to mix.
8	Immediately, pour approximately 15 mL (if using the miniPCR system) of agarose with GelGreen® and 25–30 mL (if using another system such as Fotodyne) to each of the prepared gel trays (work quickly to avoid agarose solidifying).

Note > *If the agarose has solidified after adding the GelGreen®, you can still microwave the gel to liquefy. The GelGreen® will lose optimal activity after microwaving, however.*

9	Do not move the tray until the gel has completely cooled and solidified.
10	Carefully pull the combs out to create the wells (pull straight up).

Agarose Gel Electrophoresis Questions

ANSWER KEY — **Do not share with students**

Directions
Answer the questions below after closely reading the building lab skills information and working through the hands-on practice.

1. Describe how and where DNA samples are loaded onto an agarose gel.

DNA samples are first mixed with loading dye and then transferred to small holes (called wells) in the gel with a micropipette.

2. Draw a picture that shows the direction that DNA molecules run through a gel when a current is applied.

3. Why do smaller molecules end up further from the start (where they were originally loaded into the wells) than larger molecules?

Smaller molecules can pass more easily through the agarose gel matrix so they move faster through the gel when a current is applied.

4. If you want to visualize small DNA fragments on a gel, is it better to use a lower or higher percentage of agarose? Why?

Higher percentages of agarose are better for visualizing smaller DNA fragments because it causes the molecules to move more slowly, resulting in a higher resolution image with crisper bands.

Genetic Markers of Longevity Questions

ANSWER KEY **Do not share with students**

Directions
Answer the questions below after closely reading the background material.

1. What is longevity and how much of it is determined by environmental vs. genetic factors?

Longevity refers to living longer; roughly 75% is determined by environmental factors and 25% by genetics.

2. Summarize the results of the 2008 study on long-lived Japanese men, making sure to include the terms SNP and rs2802292 in your answer.

In this study, there was found to be an association between the G-allele of FOXO3 rs2802292 and longevity. The long-lived individuals also showed stronger indicators of health than younger controls, including higher sensitivity to insulin and fewer instances of heart disease.

3. What is FOX03 and what does it do?

FOXO3 is a gene associated with longevity. It codes for a transcription factor that regulates genes involved in biological processes related to aging.

4. Describe the purpose of a genome-wide association study.

A genome-wide association study is a way to observe if there is any association between a particular SNP and a trait.

5. What would the regression line look like for a graph where there is NO association between a phenotype you are studying and a particular SNP? Explain why.

If there is NO association between the phenotype and SNP you would expect to see a flat linear regression line. This is because you would expect to see no significant differences between individuals with a certain phenotype and the number of alleles they have.

6. What would the regression line look like for a graph where there IS an association between a phenotype you are studying and a particular SNP? Explain why.

If there IS an association between the phenotype and SNP you would expect to see a sloped linear regression line—the more sloped the line is, the stronger the association. This is because you would expect to see a pattern between individuals with a certain phenotype and the number of alleles they have.

Student Guide, Part 1: Pre-Lab

ANSWER KEY **Do not share with students**

Directions
In this lab, you will play the role of a geneticist investigating how to detect unique genome features of humans who live the longest.

1. To begin, carefully examine each chart provided by your teacher and record two observations and two questions about what you see.

   Phenomenon:

	Chart 1	Chart 2
Observations *I notice...* *... reminds me of...*	Any observations are relevant—the purpose is to spark curiosity and elicit prior knowledge.	Any observations are relevant—the purpose is to spark curiosity and elicit prior knowledge.
Questions *I wonder...* *Could it be that...*	Any questions are relevant—the purpose is to spark curiosity and elicit prior knowledge.	Any questions are relevant—the purpose is to spark curiosity and elicit prior knowledge.

Continues on next page >

Student Guide, Part 1: Pre-Lab

ANSWER KEY **Do not share with students**

Continued

2. Predict what gene rs2802288 might be a part of if it is associated with longevity. Explain your reasoning. You may refer to *Background Reading: Genetic Markers of Longevity* and *Phenomenon: Chart 2*.

From the reading: FOXO3 is one of the best-studied longevity genes. It is located on chromosome 6 and codes for forkhead box protein O3. A SNP within the gene FOXO3 was first reported to be associated with human longevity in a study of Japanese men with a mean age of 97.9 years. In that 2008 study, the long-lived men were homozygous for the G-allele of FOXO3 rs2802292.

From the chart: ADIPOQ, FOXO1A, FOXO3A, CIRT1, COQ7 were all identified as candidate genes for human longevity. FOXO3A and ADIOQ have the lowest P-values, indicating the highest likelihood for association with longevity.

Continues next page >

Student Guide, Part 1: Pre-Lab

ANSWER KEY — **Do not share with students**

Continued

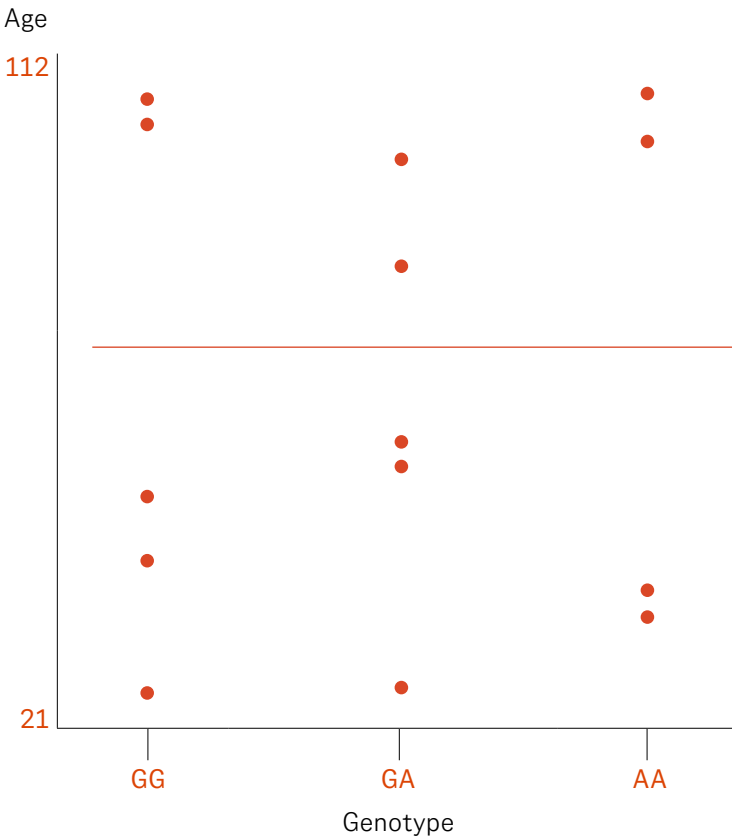
3. Use the information from *Background Reading: Genetic Markers of Longevity* to draw two graphs:

— Graph 1: What you would expect to see if there is NO association between rs2802288 and longevity.

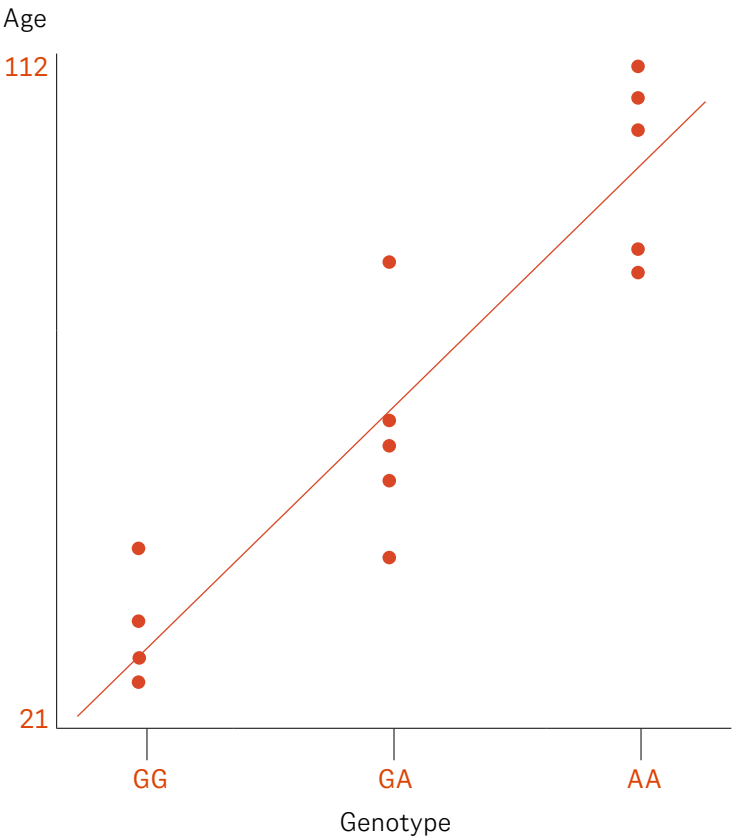
— Graph 2: What you would expect to see if there IS an association between one of the rs2802288 alleles (G or A) and longevity.

Graph 1 should have a horizontal regression line and Graph 2 should show some kind of slope (any direction and any magnitude). The AA and GG genotypes could be switched.

Graph 1



Graph 2



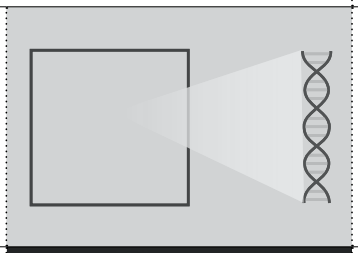
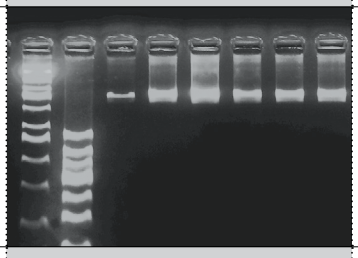
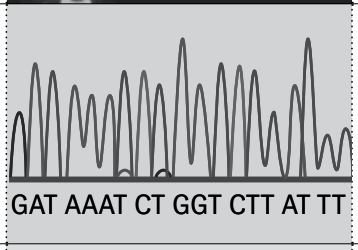
Student Guide, Part 2: Lab and Data Collection

ANSWER KEY

Directions

Answer the questions below to prepare for the lab and record your data after completing the lab.

1. The image below provides an overview of some of the main steps in a GWAS. Record the purpose of each step and whether or not you will be performing it.

	Lab Step		What is the purpose of this step?	Will you perform it?
a	Extract DNA from Cheek cells		Collect DNA samples from individuals to look for an association between a SNP and longevity (identify their genotype for rs2802288 and compare to their age)	Yes (with your own DNA)
b	Agarose Gel Electrophoresis		Confirm that DNA was successfully isolated.	Yes (with your own DNA)
c	DNA Sequencing		Determine the genotype for rs2802288 (AA, AG, or GG).	No
d	DNA Sequence Analysis		Identify the gene that rs2802288 is a part of. Plot the rs2802288 genotypes of individuals against their age.	Yes (with example DNA)

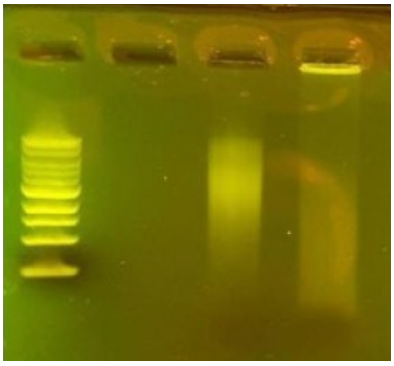
Continues on next page >

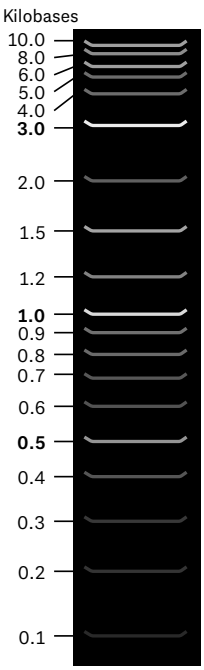
Student Guide, Part 2: Lab and Data Collection

ANSWER KEY

Continued

2. Draw or paste the picture of your gel below. Label each well and the DNA ladder (without writing directly on the picture). Record three observations about your gel.





1	Ladder
2	Negative Control
3	Positive Control
4	Check Cell DNA

My three observations:

Example observations:

- Lane 1 has lots of bright lines
- Lanes 3 and 4 have bright smears
- Lane 2 has no glowing sample
- Lane 4 has a bright line at the well at the top of the gel

3. Record the following information from the top match of the nucleotide BLAST you performed on your sequence:

Description	Homo sapiens forkhead box O3 (FOXO3), transcript variant 2, mRNA
Scientific Name	Homo sapiens
E Value	0.0
Percent Identity	100%

Differences between your sequence and that of the data base:

None

OPTIONAL: Scroll down to see if sequences from other organisms match with yours.

Student Guide, Part 3: Data Analysis

ANSWER KEY — **Do not share with students**

Directions

Analyze your results from the lab and the provided GWAS data by answering the questions below.

1. Did you successfully extract DNA from your cheek or strawberry cells? Describe what you see on your gel that supports your answer.

If yes, there should be a bright smear on the gel in the lane in which DNA was loaded into the gel. The DNA was stained with a substance that glows under UV light and migrated through the gel toward the positive electrode because it is negatively charged.

If no, the lane in which DNA was loaded into the gel will look dark. However, the positive control DNA and the DNA ladder should be visible, indicating the gel was successfully run and stained but the cheek cell DNA was not present.

2. According to the BLAST, what gene is SNP rs2802288 a part of? Use evidence from the E value and Percent Identity to support your answer. Does this support or refute your prediction?

FOXO3 (Percent Identity = 100% meaning all the nucleotides in the query sequence match FOXO3 and E value = 0.0 meaning there are no matches due to chance)

3. Review the *Background Reading: Genetic Markers of Longevity* or search the internet to find three pieces of information about this gene. Record them here.

- Located on chromosome 6
- Codes for forkhead box protein O3, a transcription factor
- Increases the production of genes that combat cellular aging, such as damage to DNA, proteins, and lipids, and loss of stem cell function.
- Increases the production of genes, including those that regulate DNA repair, tumor suppression, stem cell function, immune function, protein aggregation, and more.

Continues next page >

Student Guide, Part 3: Data Analysis

ANSWER KEY **Do not share with students**

Continued

4. After examining the GWAS: rs2802288 Results presented on this page and the next, answer questions 4a–4c to analyze the data in the graph and table.

   The graph and data table show the results from a fictional Genome-wide Association Study on the rs2802288 SNP. This study included samples from 100 individuals. Each individual's age is recorded as well as their genotype for rs2802288 (GG, GA, or AA).

4a. Identify at least three patterns or features of the data. *What do you notice about the graph or data table?*

— The regression line has a positive slope
— All individuals over 100 have at least one "A" allele
— Most individuals with "GG" genotype are around 60 years old
— Only one individual younger than 97 years old had an AA genotype

4b. Draw two conclusions from the patterns you identified. *What is the main takeaway from the data?*

— The slope indicates there is a pattern between the number of "A" alleles at rs280288 and older age
— Having an "A" allele of rs280288 may be associated with living longer

4c. Describe at least 3 limitations of the data and the implications of each. *What information is missing from the study? What features make it less reliable?*

Limitation	Implication
Low number of individuals in the study (only 100)	Since most GWAS's have thousands or hundreds of thousands of individuals, this small sample size in this study means the patterns identified are less reliable.
Unclear which samples were taken from dead vs. alive individuals	There is a difference between individuals who died at a young age vs. had their sample taken at a young age and continue to live to an old age. This could skew the data.
Unclear what the longevity history of the individuals is	In genetic studies, it is important to gather data about the individuals' relatives. If the individuals in this study who reached the oldest age also had genetic relatives that reached an old age, it would strengthen the association between the "A" allele and longevity.
Unclear what other environmental factors may be affecting age of death	Genetics is only one piece of aging. This study did not take into account things like smoking or other high risk factors that impact life span so it decreases the reliability of the association between the "A" allele and longevity.

Continues next page >

Student Guide, Part 3: Data Analysis

ANSWER KEY **Do not share with students**

Continued

5. Is there an association between rs2802288 and longevity? (Yes/No/Cannot determine)

   Explain your reasoning.

Examples:

Yes, there seems to be an association between the "A" allele of rs280288 and older age

- The regression line has a positive slope, indicating there is a pattern between a certain allele and the measured trait (age). If the line were horizontal, there would not be an association between an allele and the trait.
- All individuals over 100 have at least one "A" allele, indicating that this allele is more common in centenarians.

Cannot determine:

- The GWAS data indicates an association between the "A" allele of rs280288 but there are many limitations of the study that make it unreliable.
- For example, only 100 individuals were analyzed (which is too small a sample to identify reliable patterns) and it is unclear what other environmental factors may have impacted the individuals' longevity.

Student Guide, Part 4: Extension

ANSWER KEY — **Do not share with students**

Directions
Answer the questions below to learn more about the rs2802288 *SNP.*

1. Search for the SNP "rs2802288" on the internet and click on the first link. Scroll to the bottom of the page to find a horizontal bar graph that summarizes the percentage of different populations that have been found to have "AA," "AG," and "GG" alleles of the SNP.

   Record three observations and three questions about the graph:

	1	2	3
Observations *I notice…* *… reminds me of…*	All observations and questions are relevant.		
Questions *I wonder…* *Could it be that…*			

Continues on next page >

Student Guide, Part 4: Extension

ANSWER KEY **Do not share with students**

Continued

2. Describe a follow-up experiment or study. What would you want to find out next that would help you answer the questions you listed above?

— Sequence your own DNA through a genome sequencing company and see what your genotype is for rs280288 SNP

— Use the same set of DNA samples to look for an association between a different SNP and longevity (or a different trait)

— Collect DNA samples from individuals with the same ethnic background (ex. Puerto Ricans) and see if the GWAS results are repeated

— Translate the nucleotide sequences of the two different FOXO3 alleles into amino acid sequences to identify potential differences in the protein product

— Measure the expression of the two different FOXO3 alleles in the individuals (ie. measure the mRNA and/or protein production in different cells)

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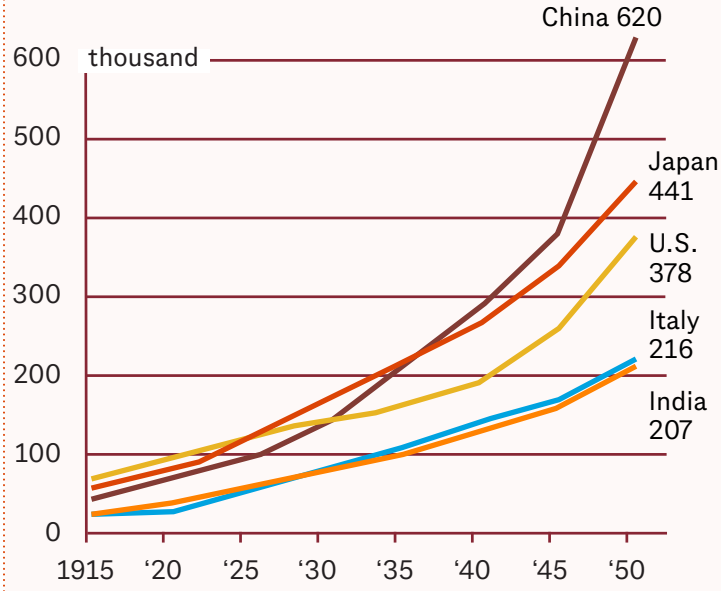
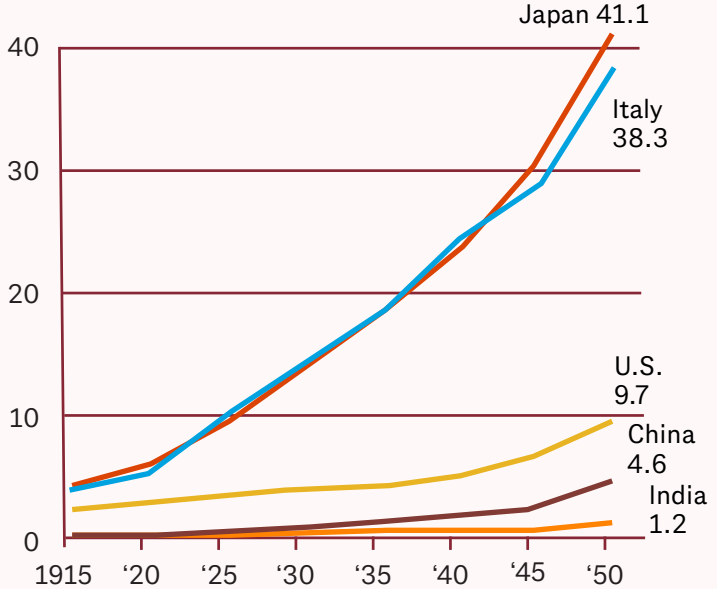
Phenomenon Chart 1

Number of Centenarians in China and Japan Expected to Surpass Number in U.S.

Number of persons ages 100 and older



Number of persons ages 100 and older per 10,000



Source:
World Population Ages 100 and Up to Grow Eightfold by 2050, UN Projects

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Phenomenon Chart 2

Candidate genes for human longvity (long life)

Gene	SNP ID	P-value
ADIPOQ	rs1063539	0.20
	rs182052	0.22
	rs266729	0.08
FOXO1A	rs2755209	0.48
	rs2721069	0.62
	rs2755213	0.77
FOXO3A	rs2764264	0.0002
	rs13217795	0.0006
	rs2802292	<0.0001
SIRT1	rs7069102	0.84
	rs10823112	0.44
	rs1885472	0.71
COQ7	rs8051232	0.90
	rs11074359	0.43
	rs7192898	0.73

Note: The lower P-value, the more likely there is an association between a SNP and longevity.

Source: *FOXO3A genotype Is Strongly Associated with Human Longevity*

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Sample Permission Slip for Student DNA Extraction

Directions
Use this example to generate your own permission slip.

Class

School

Contact Number or Email

Dear Parent(s) or Guardian(s),

Our class has the opportunity for students to participate in a class exercise in which an important technique in biotechnology will be used to visualize the students' DNA. The technique the students will be using is called agarose gel electrophoresis.It is a method used to separate molecules based on size and charge by putting a porous gel into an electric field.

Gel electrophoresis has a number of applications including uses in forensics, diagnostics, parentage testing, and evolutionary studies. However, in this class activity, we will be using it only to observe a student's DNA as a whole without analyzing anything specific about the students' DNA sequence.

If you agree to participate in this laboratory protocol, students will take a sample of DNA from their own cheek cells using a salty mouthwash. They will then use gel electrophoresis to verify that they successfully isolated DNA. **The results of this particular lab exercise are for teaching purposes only and will NOT be used for any diagnostic or identification purposes.** Your student's privacy will be protected. The student's name will not be linked to his or her DNA and the results of the lab exercise will remain anonymous. The resulting DNA will be destroyed at the end of this lesson.

Participation is voluntary. By signing this permission form, you are allowing your student to participate in this exciting learning experience. If you have any concerns or questions, please contact me at the mobile number or email to the left.

Sincerely,

Student Name *(please print)*

Student Signature

Parent/Guardian Signature

Date Signed

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Building Lab Skills: Agarose Gel Electrophoresis

Directions
After closely reading the background material, practice Agarose Gel Electophoresis with the Hands-on Guide.

One challenge with working with molecules such as DNA is that they are too small to see with the naked eye. To solve this problem, scientists use many different tools including a technique called *agarose gel electrophoresis*. Gel electrophoresis allows scientists to visualize otherwise invisible DNA molecules. They can use it to perform tasks such as confirm they have successfully extracted DNA from a sample, determine the size of a DNA molecule they are studying, and compare DNA fingerprints between different individuals.

What is an agarose gel?
Agarose is a substance derived from agar, which is extracted from seaweed (it is the same substance used in many dessert foods). It consists of long polymers that form a matrix of evenly sized and spaced openings. Samples of molecules (including DNA, RNA, or proteins) are mixed with a loading dye and transferred into small holes or "wells" in the gel with a micropipette. The loading dye adds color to the DNA sample and contains a dense substance such as glycerol. Because the glycerol makes the sample denser than the buffer solution in which the gel is immersed, the DNA sample will sink to the bottom of the well. Then, when an electrical current is passed through the gel, the molecules will move through this matrix. They will move at different speeds based on size and charge. In the image below, a sample of DNA mixed with blue loading dye is pipetted into a well.

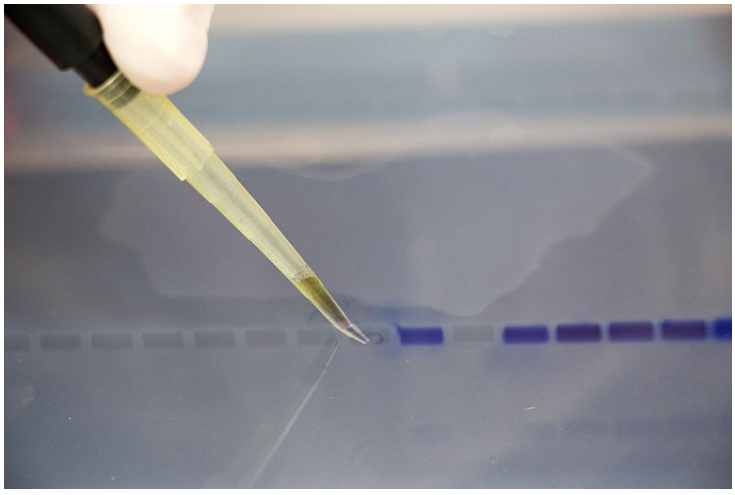
How does electrophoresis separate molecules based on size and charge?
When the current is running through the gel, one end of the gel becomes negatively charged (cathode) and the other end becomes positively charged (anode).

Molecules with a positive charge will move toward the (-) cathode because they are attracted to the negative charge. Molecules with a negative charge (such as DNA) will move toward the (+) anode because they are attracted to the positive charge.

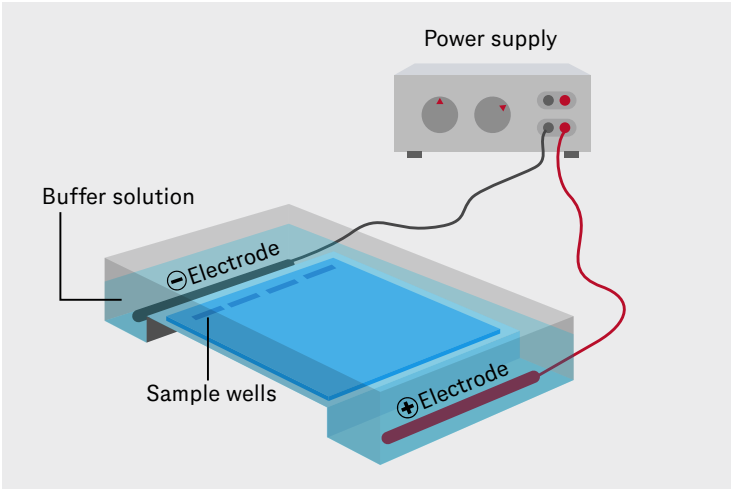
Large molecules will move slowly through the gel because it is more difficult for them to move through the gel matrix. This means they will travel a shorter distance from where they started when an electrical current is passed through the gel for a certain amount of time. Small molecules will move more quickly through the gel because it is easier for them to pass through the matrix. This means they will travel a farther distance from where they started. (larger = closer to start, smaller = farther away from start).

Continues next page >

Transferring DNA Samples into Agarose Gel Wells



Agarose Gel Electrophoresis



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Building Lab Skills: Agarose Gel Electrophoresis

Continued

To determine the particular size of DNA molecules on a gel, the samples are compared to a DNA ladder that is also run on the gel. A DNA ladder contains fragments of DNA of different known lengths measured in base pairs (bp) or kilobase pairs (kb).

Because small molecules move quickly through a gel, using a high percentage of agarose (like 2%) causes them to move slower, resulting in a higher resolution image with more crisply defined bands. Notice how the 100 bp–400 bp bands in the ladder above are thicker and blurrier than the larger fragments. This indicates that this ladder was run on a gel that contains a lower percentage of agarose (like 1%). Lower percentages of agarose are useful for visualizing larger DNA fragments because they can move more quickly through, resulting in better separation between bands. Notice the clear distinction between the 1000 bp and 900 bp bands in the ladder.

The brighter and thicker the band, the higher the concentration of DNA. Notice how the 500 bp, 1000 bp, and 1500 bp fragments in the ladder are brighter than the others. This means there is a higher concentration of these fragments present in the ladder than the other fragments.

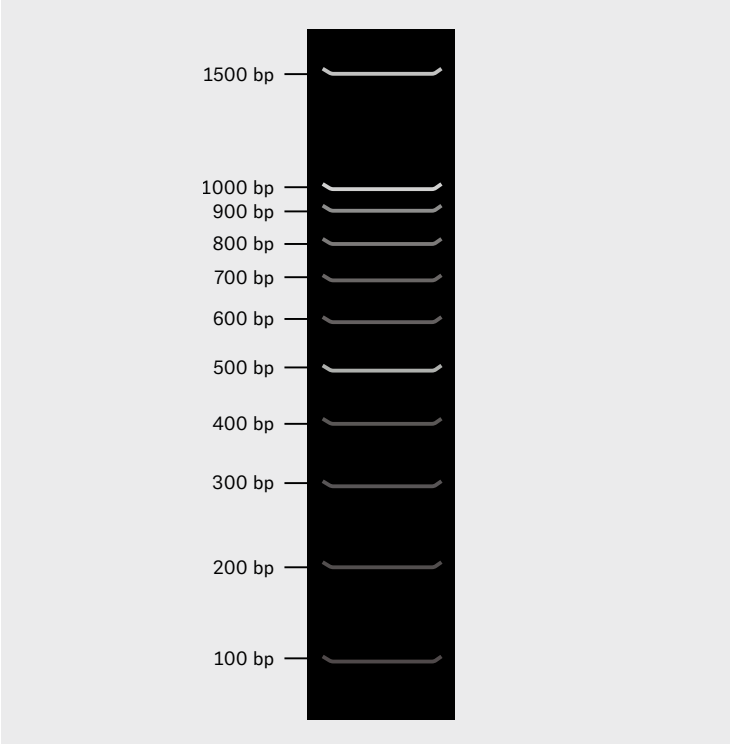
The image below shows a DNA ladder in the far left lane and three DNA samples that have been run through an agarose gel. The DNA bands are visible because they have been stained with a fluorescent dye that glows under UV light. The DNA molecules in the lane on the far right are the smallest out of the three samples because they have moved further down the gel than the other two samples.

Sources:

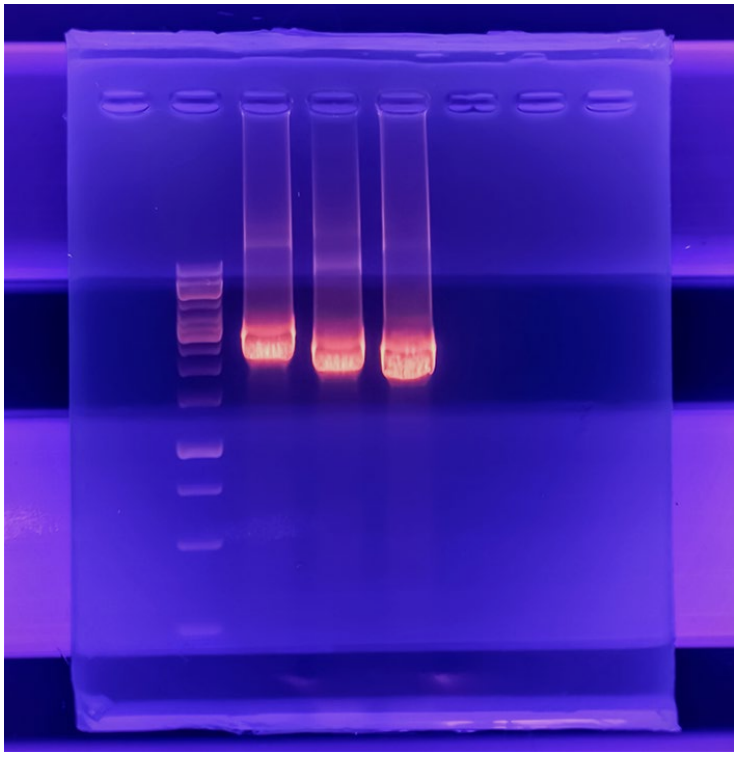
Agarose gel electrophoresis

What is gel electrophoresis

DNA Ladder



Three DNA Samples Run through an Agarose Gel



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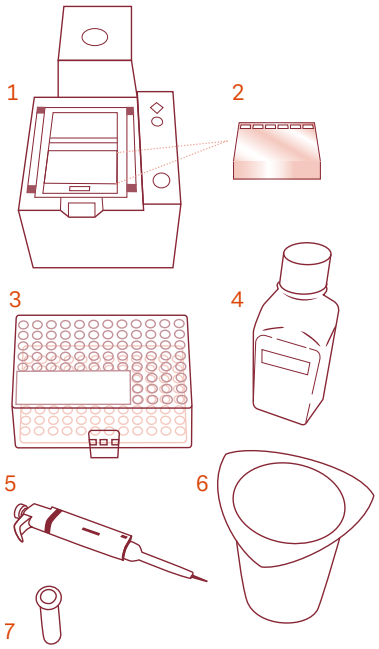
Building Lab Skills: Agarose Gel Electrophoresis

Continued

Hands-on Practice

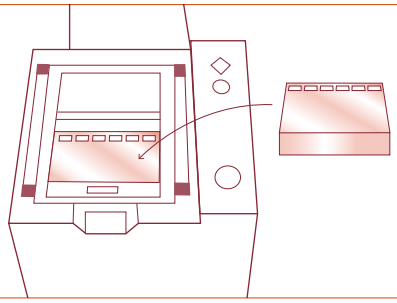
A Gather the following materials:

1	Gel electrophoresis system
2	Agar-agar gel (agarose gel substitute) in its tray
3	Yellow tips (for P20)
4	Water (1X TAE buffer substitute)
5	Micropipette P20
6	Waste bucket
7	Diluted loading dye (DNA sample substitute)



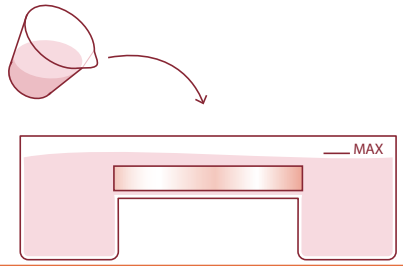
1 Place the practice gel into the electrophoresis system.

Make sure the wells are lined up with the (-) electrode.



2 Slowly add water to both chambers of the system until it just covers the gel entirely.

When running a real agarose gel you will use 1X TAE buffer instead of water.



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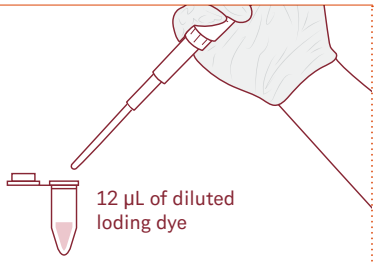
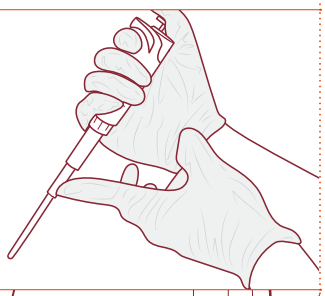
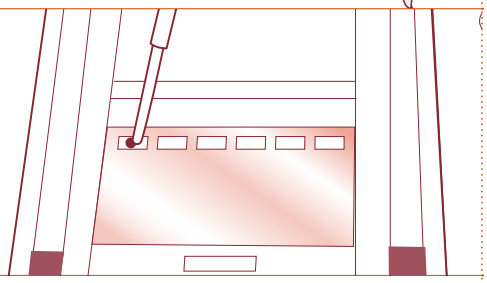
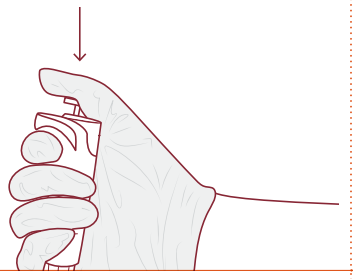
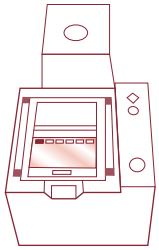
Building Lab Skills: Agarose Gel Electrophoresis

Continued

3	Use the P20 micropipette to pick up 12 µL of diluted loading dye and position yourself directly over the gel.	
4	Stabilize your micropipette by pressing the index finger of your opposite hand onto the base of the micropipette while pressing the micropipette back onto your index finger to create tension.	
5	Dip the tip of the micropipette to just under the surface of the water and slowly add the diluted loading dye into a well in the gel. *Do not puncture the gel with the pipette tip!*	
6	Keep your thumb DOWN on the micropipette plunger as you withdraw the micropipette from the gel to avoid accidentally sucking up the sample. Eject the tip.	
7	Stop here. Usually you would place the lid on the electrophoresis system, plug it into the power supply, and run a current through the system for ~15 minutes to allow the molecules in the sample to migrate through the gel. However, since we used water instead of 1X TAE buffer, no current can be carried through the gel.	
8	Repeat steps 3–6 until all the wells are full to practice loading samples into a gel.	

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Agarose Gel Electrophoresis Questions

Directions
Answer the questions below after closely reading the building lab skills information and working through the hands-on practice.

1. Describe how and where DNA samples are loaded onto an agarose gel.

2. Draw a picture that shows the direction that DNA molecules run through a gel when a current is applied.

3. Why do smaller molecules end up further from the start (where they were originally loaded into the wells) than larger molecules?

4. If you want to visualize small DNA fragments on a gel, is it better to use a lower or higher percentage of agarose? Why?

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Background Reading: Genetic Markers of Longevity

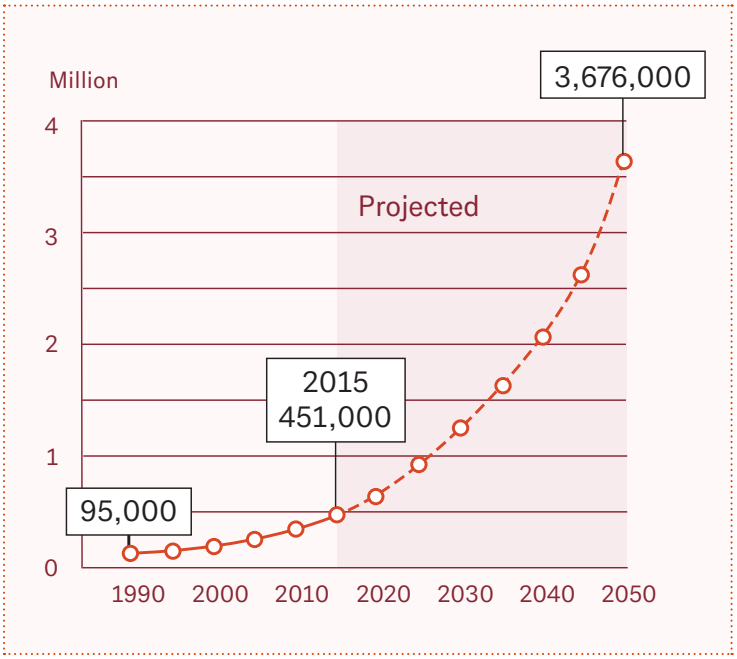
Longevity

Humans have always searched for ways to increase longevity (living a long life). Since the beginning of the 20th century, the life expectancy in the world has more than doubled. This is largely due to medical advances in industrialized countries (Roser et al., 2019). People who live to at least 100 years old, called centenarians, have also increased significantly in recent decades. In 1990 there were 2.9 centenarians for every 10,000 adults over 65 years old, 7.4 in 2015, and a projected 23.6 centenarians per 10,000 elderly adults by 2050. This means there are estimated to be 3.7 million centenarians in the world in 2050 compared to 95,000 in 1990 (Stepler 2016). However, life expectancy across countries is not equal. In 2019, the Central African Republic had the lowest life expectancy at 53 years, whereas the life expectancy in Japan was 83 years (Roser et al., 2019).

Longevity is a complex trait influenced by the interaction of genetic and environmental factors. These factors determine risks for certain diseases and the individual rate of aging. It is estimated that 75% of the factors that influence your lifespan come from your environment.

The World's Centenarian Population Projected to Grow Rapidly

Number of persons ages 100 and older



However, some of the reasons we do (or don't) live for a long time are genetic—about 25% of the variation in human lifespan is determined by genes (Passarino et al., 2016).

Single nucleotide polymorphisms (SNPs) and FOX03

The study of how longevity-associated genes contribute to a longer life is a developing field. While several variations of genes are associated with long life spans, it is important to remember that this only implies a correlation, not causation.

Single nucleotide polymorphisms (SNPs) are places in the human genome where a single base pair differs between people. They occur about every 300 nucleotides in the genome, making them the most common type of genetic variation (Nelson et al., 2004). Because the majority of the human genome consists of non-coding regions of DNA, most SNPs do not have a particular effect. However, some SNPs have been associated with diseases such as type 1 diabetes and multiple sclerosis (Castellanos-Rubio and Ghosh, 2019).

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Background Reading: Genetic Markers of Longevity

Continued

A SNP within the gene FOXO3 was first reported to be associated with human longevity in a Genome-wide Association Study in 2008. This study compared a group of Japanese-American men who ranged in age from 95–106 years old to a control group. The study showed that the Japanese-American men were homozygous for the G-allele of a SNP in FOXO3 called rs2802292 (Willcox et al., 2008). This association was later confirmed in studies of Italian, French, and German men and women. The long-lived individuals also showed stronger indicators of health than younger controls, including higher sensitivity to insulin and fewer instances of heart disease (Sanese et al., 2019).

Continues next page >

Example genotypes of six individuals for a SNP in Chromosome 2

Individual 1

Chr 2	C G A T A T T C C **T** A T C G A A T G T C
Chr 2	C G A T A T T C C **C** A T C G A A T G T C

Individual 2

Chr 2	C G A T A T T C C **C** A T C G A A T G T C
Chr 2	C G A T A T T C C **C** A T C G A A T G T C

Individual 3

Chr 2	C G A T A T T C C **T** A T C G A A T G T C
Chr 2	C G A T A T T C C **T** A T C G A A T G T C

Individual 4

Chr 2	C G A T A T T C C **T** A T C G A A T G T C
Chr 2	C G A T A T T C C **C** A T C G A A T G T C

Individual 5

Chr 2	C G A T A T T C C **C** A T C G A A T G T C
Chr 2	C G A T A T T C C **T** A T C G A A T G T C

Individual 6

Chr 2	C G A T A T T C C **C** A T C G A A T G T C
Chr 2	C G A T A T T C C **T** A T C G A A T G T C

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Background Reading: Genetic Markers of Longevity

Continued

FOXO3 rs2802292

GG = Longer than average lifespan, more likely to live past 100

TT = Less likely to live past 100

FOXO3 continues to be a focus of longevity genetic research. It encodes a type of protein called a forkhead box (FOX), which is a transcription factor. Transcription factors control gene expression, turning genes "on" or "off" in a cell. FOXO3 has been found to regulate many genes, including some that are related to healthy aging. The genes it regulates are involved in functions such as cellular stress response, programmed cell death, and metabolism (Sanese et al., 2019). These processes are all involved in cellular aging and therefore, human lifespan.

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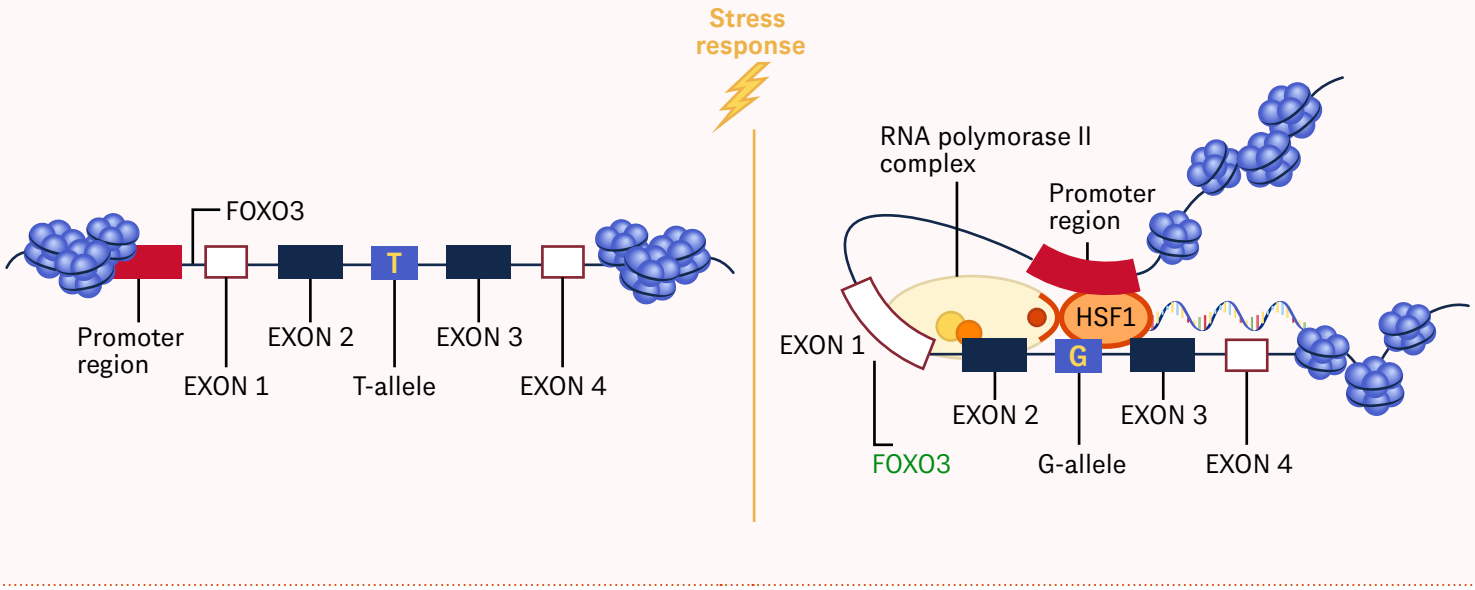
Proposed mechanism for how FOXO3 rs2802292 G-allele helps mediate cell stress response

rs2802292 T-allele

rs2802292 G-allele



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Background Reading: Genetic Markers of Longevity

Continued

Genomic Sequencing and Genome-Wide Association Study (GWAS)

In a Genome-wide Association Study (GWAS), thousands of individuals' genetic data are analyzed to determine if there is a statistically significant association between a specific region of DNA (often a SNP) and a particular trait (often a disease). Generally, the DNA of two groups of individuals is compared: those with the trait (for example, Crohn's disease) and a control group (those without the trait but from comparable backgrounds). If a particular genetic variant is found more frequently in the group with the trait, then that variant is associated with the trait.

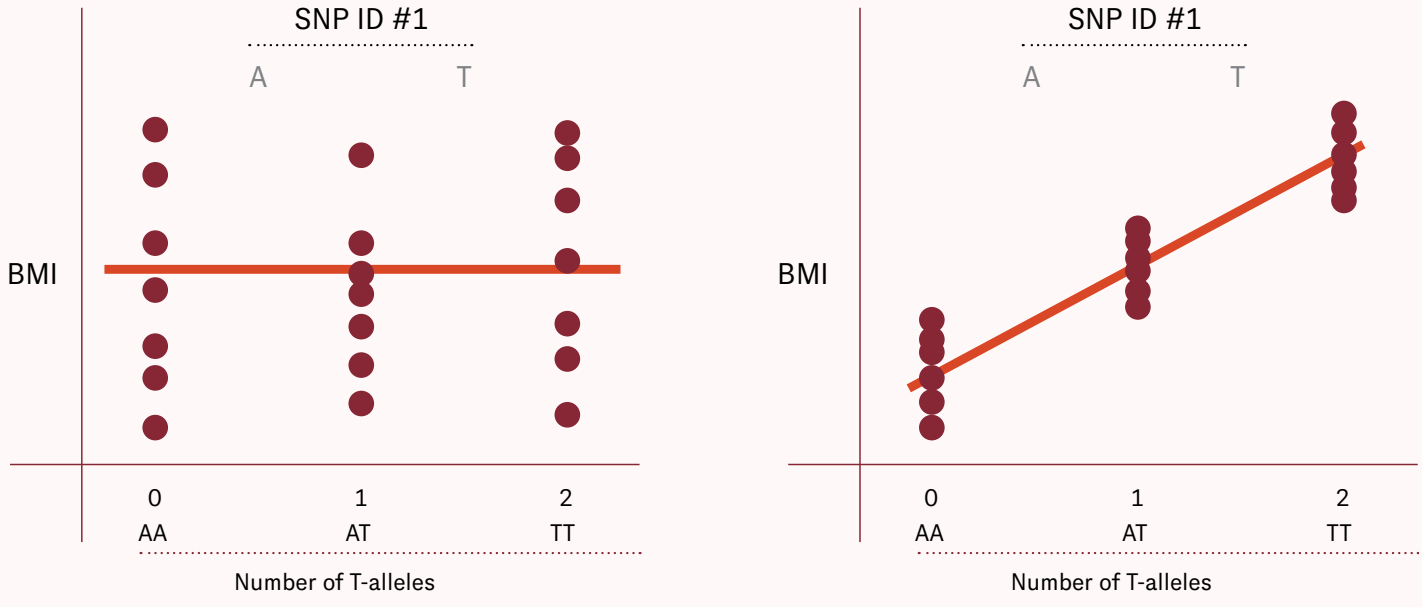
One way to visualize GWAS data is to plot the number of a given allele each individual has against the trait they have. (Allele is another word for a genetic variant or version of a gene.) Because everyone has two alleles for every gene (one from each parent), they can either have 0, 1, or 2 copies of an allele. In the example below, the scientists are looking for an association between SNP ID#1 and BMI (Body Mass Index).

There are two possible alleles for this SNP - "A" or "T". Each individual has a genotype of either "AA," (no T alleles) "AT," (one T allele) or "TT" (two T alleles).

If there is *no* association between either the "A" or "T" allele and BMI, you would expect to see *no* significant differences between individuals with a certain BMI and the number of "T" or "A" alleles they have. If you drew a regression line in the graph, it would be horizontal (graph on the left). However, if there is an association between either the "A" or "T" allele and BMI, you would expect to see a pattern between individuals with a certain BMI and the number of "T" or "A" alleles they have. If you drew a regression line in the graph, it would have a slope (graph on the right). The slope of the line represents the effect size. The greater the slope, the greater the effect size, and therefore the stronger the relationship between an allele and the trait.

In this example, having more "T" alleles is associated with having a higher BMI and having more "A" alleles is associated with having a lower BMI.

Example GWAS Results



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Genetic Markers of Longevity Questions

Directions
Answer the questions below after closely reading the background material.

1. What is longevity and how much of it is determined by environmental vs. genetic factors?

2. Summarize the results of the 2008 study on long-lived Japanese men, making sure to include the terms SNP and rs2802292 in your answer.

3. What is FOX03 and what does it do?

4. Describe the purpose of a genome-wide association study.

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Genetic Markers of Longevity Questions

Continued

5. What would the regression line look like for a graph where there is NO association between a phenotype you are studying and a particular SNP? Explain why.

6. What would the regression line look like for a graph where there IS an association between a phenotype you are studying and a particular SNP? Explain why.

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Background Reading: Genetic Markers of Longevity Sources

All Our Charts on Life Expectancy

Are You Genetically Predisposed to Live a Longer Life? (FOXO3)

Is Longevity Determined by Genetics?

FOXO3A Genotype is Strongly Associated with Human Longevity

Mouse Studies Show that Longevity Gene May Play a Role In Maintaining Stem Cells in the Brain

FOXO3 on the Road to Longevity: Lessons From SNPs and Chromatin Hubs

Sequence Alignment—an overview

Human Longevity

World's Centenarian Population Projected to Grow Eightfold by 2050

Large-Scale Validation of Single Nucleotide Polymorphisms in Gene Regions

Disease-Associated SNPs in Inflammation-Related lncRNAs

Genome-wide association studies

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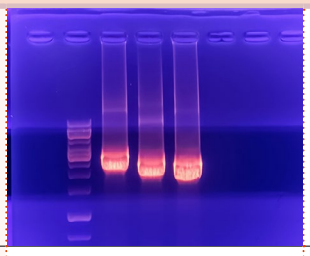
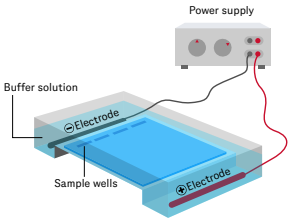
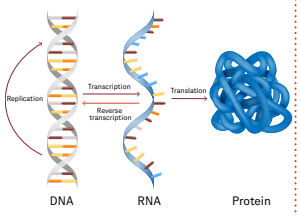
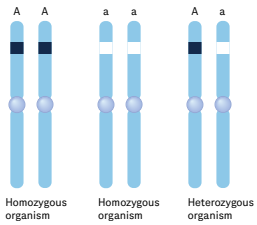
Vocabulary Tool

Directions
For each vocabulary word, write a new sentence that helps you practice using it.

Word	Image	Definition	Example Sentence	My Sentence
Agarose Gel		A jelly-like substance made from seaweed that can be used to separate molecules, such as DNA and proteins	Making an *agarose gel* is like making Jell-O: you pour the hot liquid agarose into a tray and then it solidifies as it cools.	
Gel Electrophoresis		A technique used to separate molecules based on their mass and charge	You can visualize DNA using *gel electrophoresis*.	
DNA Deoxyribonucleic Acid		A molecule found inside the cells of all living things. It contains the instructions for making the proteins that determine traits.	People look different from each other because of differences in their *DNA*.	
Gene		A segment of DNA that codes for a protein	As many as 16 different *genes* code for proteins that determine eye color in humans.	
Allele		A version of a gene	An individual inherits two *alleles* for each gene, one from each parent.	

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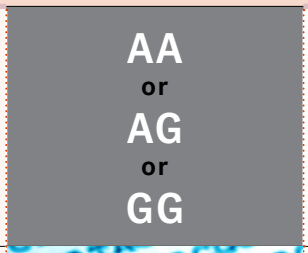
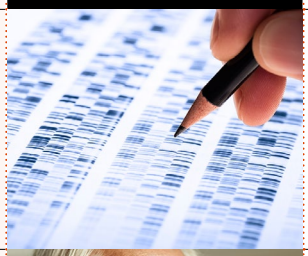
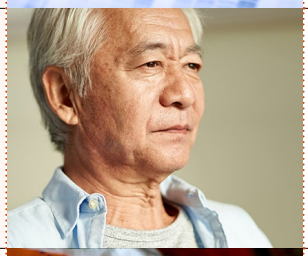
Vocabulary Tool

Continued

Word	Image	Definition	Example Sentence	My Sentence
Genotype		The set of genes an individual has. Often displayed as two letters (one representing the allele from each parent).	To find associations between genes and traits, peoples' *genotypes* are compared to a given trait.	
BLAST *Basic Local Alignment Search Tool*		An online tool that is used to find similarities between DNA, RNA, or protein sequences	To find out what gene a particular DNA sequence is from, you can search for it using *BLAST*.	
Single Nucleotide Polymorphism *SNP*		A genetic variation in the human genome of a single base pair	Certain *SNPs* have been associated with specific traits, such as longevity.	
Genome Wide Association Study *GWAS*		A method to identify an association between a genetic variation and a trait by comparing the DNA of thousands of individuals.	In a *GWAS*, the DNA from thousands of individuals is analyzed and compared to a trait, such as longevity.	
Longevity		Long life	Multiple environmental and genetic factors have been linked to a person's *longevity*.	
Centenarian		A person who is one hundred or more years old	Japan now likely has the largest proportion of centenarians in its population.	

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Student Protocol

Part 1: DNA Extraction

Directions

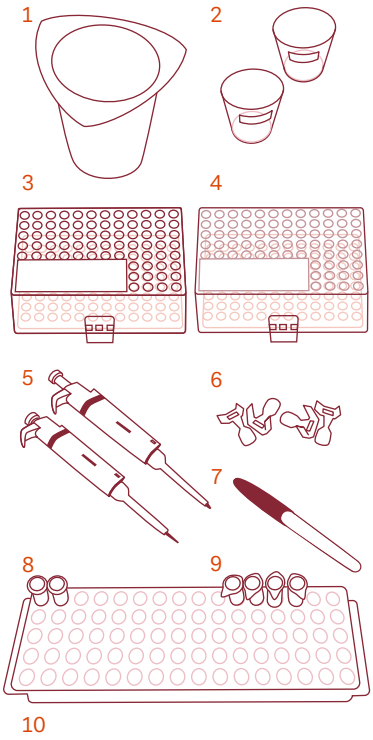
Follow the steps of the protocol to extract DNA from your cheek cells.

1 Prepare Lab Station

☐ Make sure your lab station is clean.

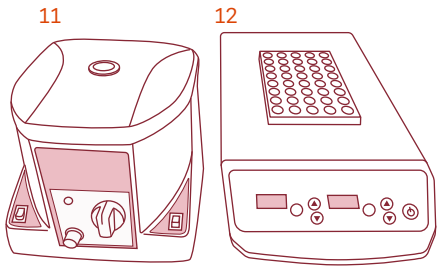
☐ Gather Materials needed per student pair:

1	Waste bucket
2	2 cups with 10 mL 0.9% saline solution each
3	Blue tips (for P1000)
4	Yellow tips (for P200)
5	P200 and P1000
6	4 cap locks
7	Permanent marker
8	2 tubes with 5% Chelex
9	4 empty microtubes
10	Microtube rack



☐ Locate these in your classroom:

11	Centrifuge (1–2 stations per class)
12	Heat Block (1–2 stations per class)



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Student Protocol

Part 1: DNA Extraction

Continued

2	**Prepare to Pellet Cells**
☐	Vigorously swish 10 mL of saline solution in your mouth for 45 seconds to dislodge cheek cells.
☐	Expel the saline into a cup and swirl to mix the cells—this is the cell suspension.
☐	Label a 1.5 mL microtube with your initials on the lid.
3	**Pellet Cells**
☐	Use the P1000 micropipette to transfer 1000 µL of your cell suspension into the tube you just labeled.
☐	In a centrifuge, spin your cell suspension tube for 2 min at 10,000 RPM to "pellet" your cells.
☐	After spinning, you should see a cell pellet with clear liquid on top of it at the bottom of the tube.
☐	Gently pour out the liquid above the cell pellet into the sink or waste container. There will be a little bit of liquid left remaining in the tube.
☐	Repeat these steps to pellet more cells.
4	**Rack the Tube**
☐	Close the lid firmly.
☐	Flick or "rack" the tube to resuspend (mix) the cell pellet in the remaining liquid.
5	**Transfer the Suspened Cells into a Tube with Chelex**
☐	Label a tube of 200 µL Chelex with your initials.
☐	Use the P200 micropipette to transfer all of your cell suspension from Step 4 to the tube containing the Chelex.
☐	Gently flick the tube to mix.

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Student Protocol

Part 1: DNA Extraction

Continued

6	Heat the Chelex and Cells Tube
☐	Prehead a heat block to 99˚C.
☐	Take the Chelex and cells tube to the 99˚C heat block.
☐	Slide a cap lock onto the lid of the tube before you place it into the heat block.
☐	Place the tube on the heat block and incubate for 10 minutes.
7	Rack the Tube
☐	Gently remove the cap lock.
☐	With the tube away from your face, open it to release the pressure.
☐	Close the lid firmly and “rack” or flick the tube to mix.
8	Spin your Chelex and Cells Tube in a Centrifuge
☐	In a centrifuge, spin your Chelex and cells at maximum speed for five minutes to “pellet” the chelex beads.
9	Transfer Liquid
☐	Get a clean 1.5 mL microtube and label it with your initials.
☐	Transfer 50µL of liquid from the top of the Chelex and cells tube to the new tube you just labeled (this is your DNA!).
☐	Do not pick up any of the chelex beads that are settled at the bottom of the tube.
10	Clean up by Placing Used Tubes, Tips, and Saline Wash Cups in the Trash.

Potential stopping point—store DNA in freezer until ready to continue.

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Student Protocol

Alternative Part 1: Strawberry DNA Extraction

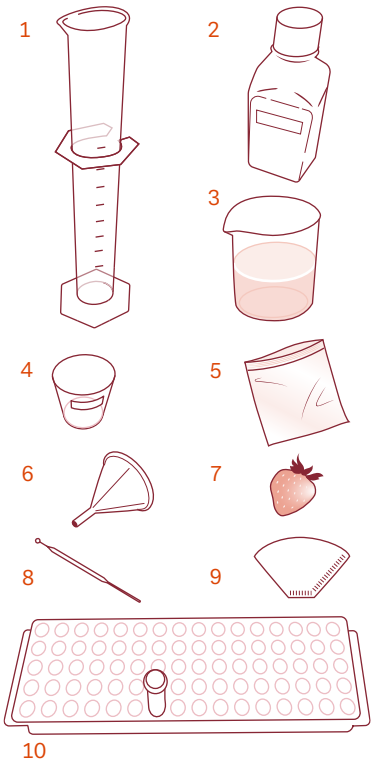
Directions

This is an alternative protocol for Part 1: DNA Extraction. If you are not extracting DNA from your cheek cells, follow the steps of this protocol to extract DNA from a strawberry.

1 Gather the Materials

☐ Materials needed per student:

1	25 mL graduated cylinder (or other measuring implement)
2	10 mL Extraction Buffer (six teaspoons of salt, 30 mL of dish soap, and 600 mL water)
3	4 mL 70–100% Isopropanol (cold)
4	1 small cup
5	1 plastic sandwich bag
6	1 small funnel
7	1 strawberry
8	1 coffee stirrer or any type of rod
9	1 coffee filter or piece of cheese cloth
10	1.5 mL microtube



2 Prepare the Strawberry

☐ Remove any green leaves from the strawberry.

☐ Put the strawberry in a plastic bag and gently squish the strawberry with your fingers for two minutes.

☐ Add 10 mL of Extraction Buffer to the bag, remove the air and close the bag (make sure it is sealed tight!).

☐ Squeeze, massage and squish gently, mixing for one minute.

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Student Protocol

Alternative Part 1: Strawberry DNA Extraction

Continued

3	Precipitate the DNA
☐	Pour the extract onto the filter in the funnel and let it drip into the small cup.
☐	Squeeze the filter to speed up the process.
☐	Slowly add 4 mL of alcohol down the side of the cup.
☐	Let the mixture sit about two minutes while observing the interface between alcohol and strawberry solution (where the two layers meet).
4	Spool the DNA
☐	Dip the rod into the tube at the interface between the alcohol and strawberry layers.
☐	Do a slow “stir the pot” motion to begin spooling the DNA. It will look like a clear, sticky glob.
5	Store the DNA
☐	Store the DNA in a microtube filled with water.

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Student Protocol

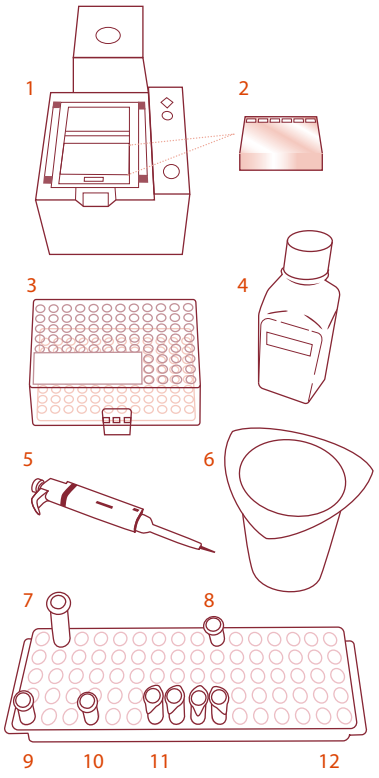
Part 2: Agarose Gel Electrophoresis

Directions

Follow the steps of the protocol to visualize your DNA samples on an agarose gel.

1	Prepare the Lab Station
☐	Make sure your lab station is clean.
☐	Gather materials needed (one per group of four).

1	Gel electrophoresis system
2	Agarose gel
3	Yellow tips (for P20)
4	150–300 mL, 1X TAE buffer
5	Micropipette P20
6	Waste bucket
7	Loading dye
8	1 kb+ ladder
9	Negative control-water
10	Positive control calf thymus DNA
11	4 Student DNA samples
12	Microtube rack



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Student Protocol

Part 2: Agarose Gel Electrophoresis

Continued

2	Prepare the DNA
☐	Add 5 µL of loading dye to your tube with 50 µL of DNA (from Part 1: DNA Extraction, Step 9).
☐	Gently flick the tube to mix.
☐	Optional: the sample also may be vortexed (if available) and spun down prior to loading on the gel.
3	Prepare the Agarose Gel
☐	Obtain a 0.8% agarose gel from your teacher.
☐	Place your gel into the gel box with the wells oriented towards the negative (black) electrode.
☐	Covered in enough 1X TAE buffer to just cover the gel entirely.
4	Load the Samples into the Wells
☐	Use the P20 micropipette to load 12 µL of each sample into a well.
☐	Load the samples from left to right with the wells at the top of the gel box.
☐	Load each of the following wells, taking care not to puncture the gel: — Well 1 (far left): 1 kb + ladder — Well 2: Negative control (water) — Well 3: Positive control (calf thymus DNA) — Wells 4–7: Cheek cell DNA samples

Continues on next page >

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Student Protocol

Part 2: Agarose Gel Electrophoresis

Continued

5	Run the Gel in the Electrophoresis System
☐	Plug your gel electrophoresis system into the power supply.
☐	Cover with the lid and run the gel at 150 volts for 5–15 minutes (the longer it runs, the more separation between bands you will see but the dimmer the DNA will appear). If you run the gel too long, the DNA will run off the gel and into the buffer where you can no longer see it.
☐	Check that the gel is running by looking for small bubbles streaming off the electrodes.
☐	Turn the power supply off and take a picture of the gel through the hood (Minione equipment) or carefully remove the gel from the tray and visualize it on a UV transilluminator using UV safety goggles.
6	Clean Up
☐	Place the gel and used tubes and tips in the trash.
☐	Gently rinse the gel electrophoresis system with water and air dry.

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Student Protocol

Part 3: DNA Sequence Analysis with BLAST

Directions

Now that you have isolated DNA and confirmed your success with agarose gel electrophoresis, the next step is to determine the sequence of nucleotides.

After submitting the DNA to a facility that sequences it (determines the order of nucleotides in your sample), you have received the following sequence back. It consists of 365 nucleotides surrounding SNP rs2802288*.*

Note: This is a role-playing exercise. You will not have your DNA sample sequenced.

DNA Sequence

G G G G G G A A G A G C G G A A A A G C C C C C C G G C G G C
G G G C T G T C T C C A T G G A C A A T A G C A A C A A G T A T A
C C A A G A G C C G T G G C C G C G C A G C C A A G A A G A A G
G A G C C C T G C A G A C A G C C C C C G A A T C A G C T G A C G
A C A G T C C C T C C C A G C T C T C C A A G T G G C C T G G C A
G C C C C A C G T C A C G C A G C A G T G A T G A G C T G G A T G
C G T G G A C G G A C T T C C G T T C A C G C A C C A A T T C T A
A C G C C A G C A C A G T C A G T G G C C G C C T G T C G C C C A
T C A T G G C A A G C A C A G A G T T G G A T G A A G T C C A G G
A C G A T G A T G C G C C T C T C T C G C C C A T G C T C T A C A G
C A G C T C A G C C A G C C T G T C A C C T T C A G T A A G C A A
G C C

BLAST

Follow the steps on the next pages to use a tool called BLAST (Basic Local Alignment Search Tool) to learn more about the sequence. BLAST is a free program hosted by the NIH (National Institutes of Health) that is used to find similarities between nucleotide or protein sequences. It compares the sequence of interest to sequence databases and calculates the statistical significance of matches. It can be used to infer evolutionary relationships between sequences as well as identify members of gene families.

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Student Protocol

Part 3: DNA Sequence Analysis with BLAST

Continued

1	Go to the BLAST website
☐	*BLAST: Basic Local Alignment Search Tool.*
2	Find the Nucleotide BLAST button
☐	Click on the button called “Nucleotide BLAST” under “Web BLAST”.
3	Enter your DNA Sequence
☐	Highlight and copy the DNA sequence from the previous page.
☐	Paste it in the box that says “Enter Query Sequence.”
☐	Enter a name for the job title (ex. Longevity SNP).
4	Click the BAST button
☐	Click on the button called “BLAST” in the bottom left corner.
☐	Wait for the processing. It will take a minute to compare the submitted sequence to the sequence database.

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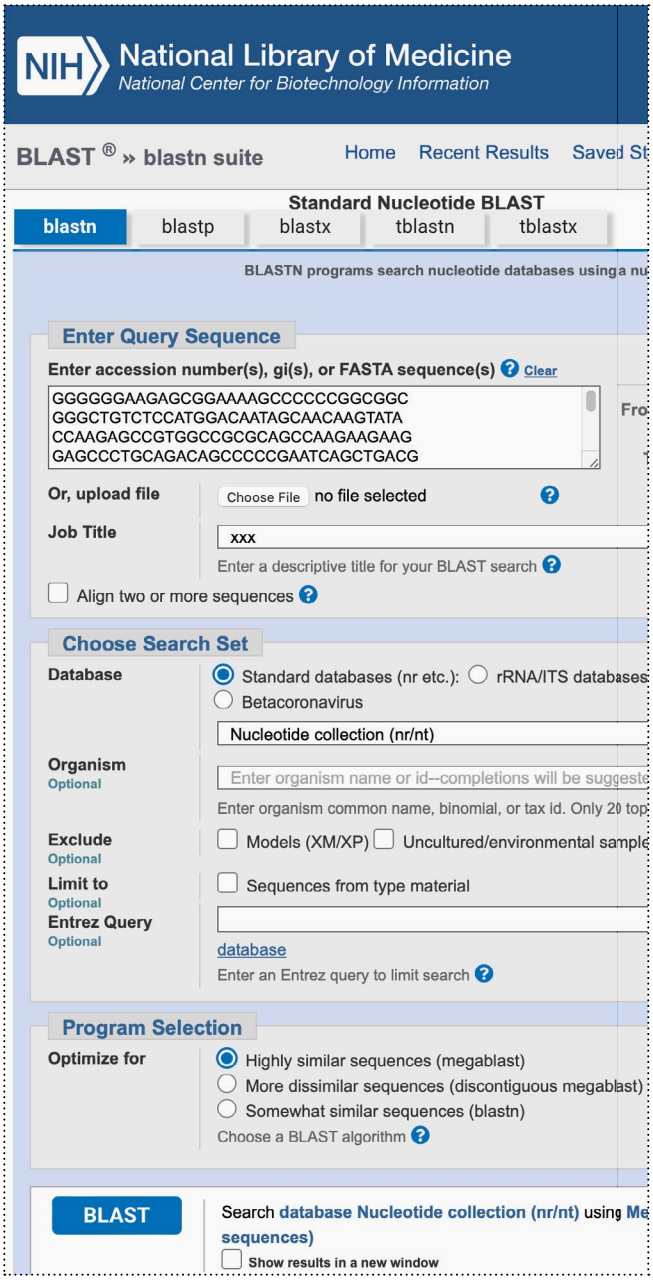

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Student Protocol

Part 3: DNA Sequence Analysis with BLAST

Continued

5	Find *Sequences producing significant alignments*
☐	Scroll down until you see "Sequences producing significant alignments.
☐	Directly below, find the blue tabs to the right: Description, Scientific Name, E value, Per Indent
☐	Here are the definitions:

Description	name of the sequence that matches most closely to your sequence
Scientific Name	organism that contains the matching sequence
E value	expected matches that are due to chance (the lower the number, the more significant the match is
Per Indent	percentage of your sequence that matches that sequence

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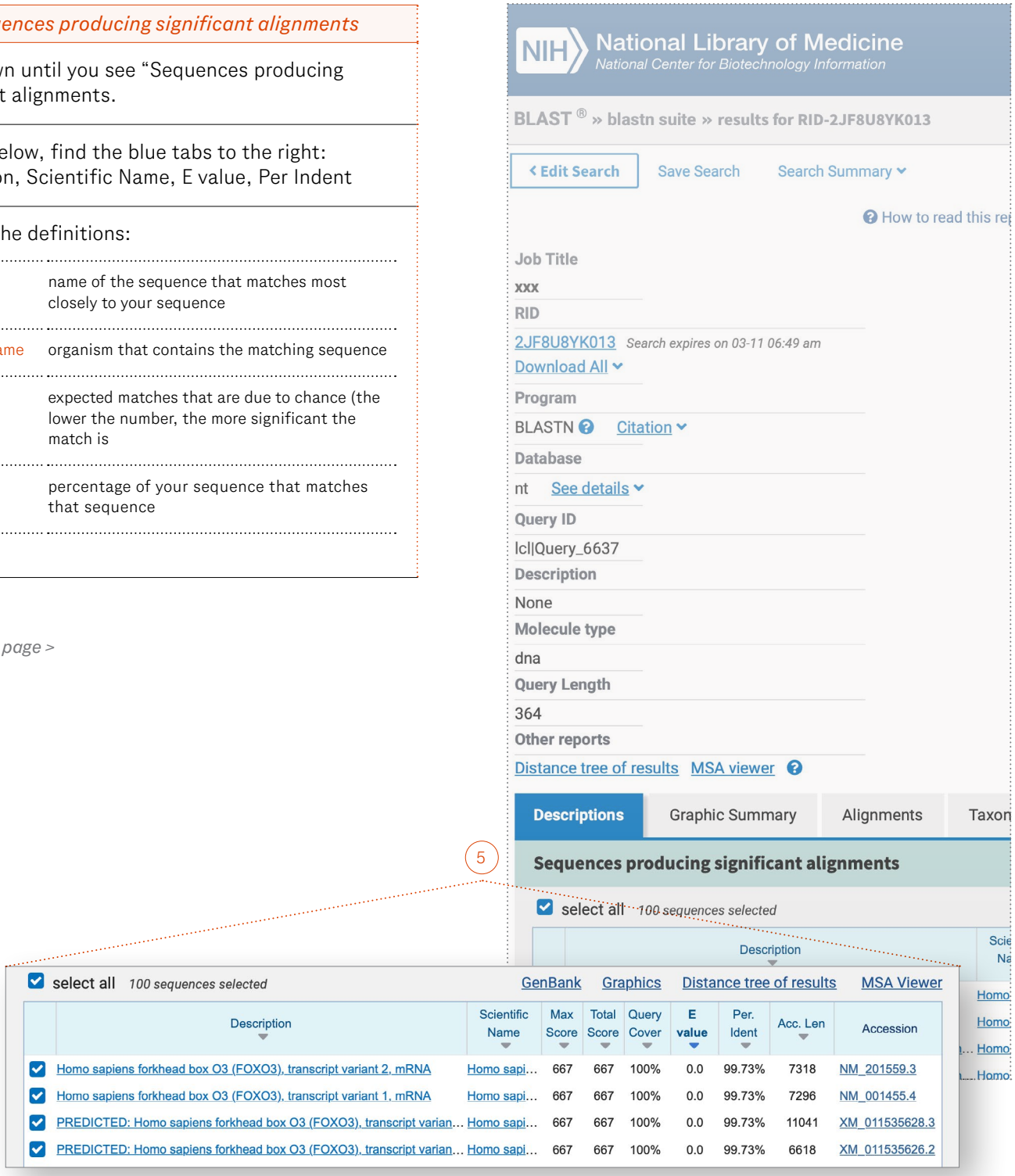

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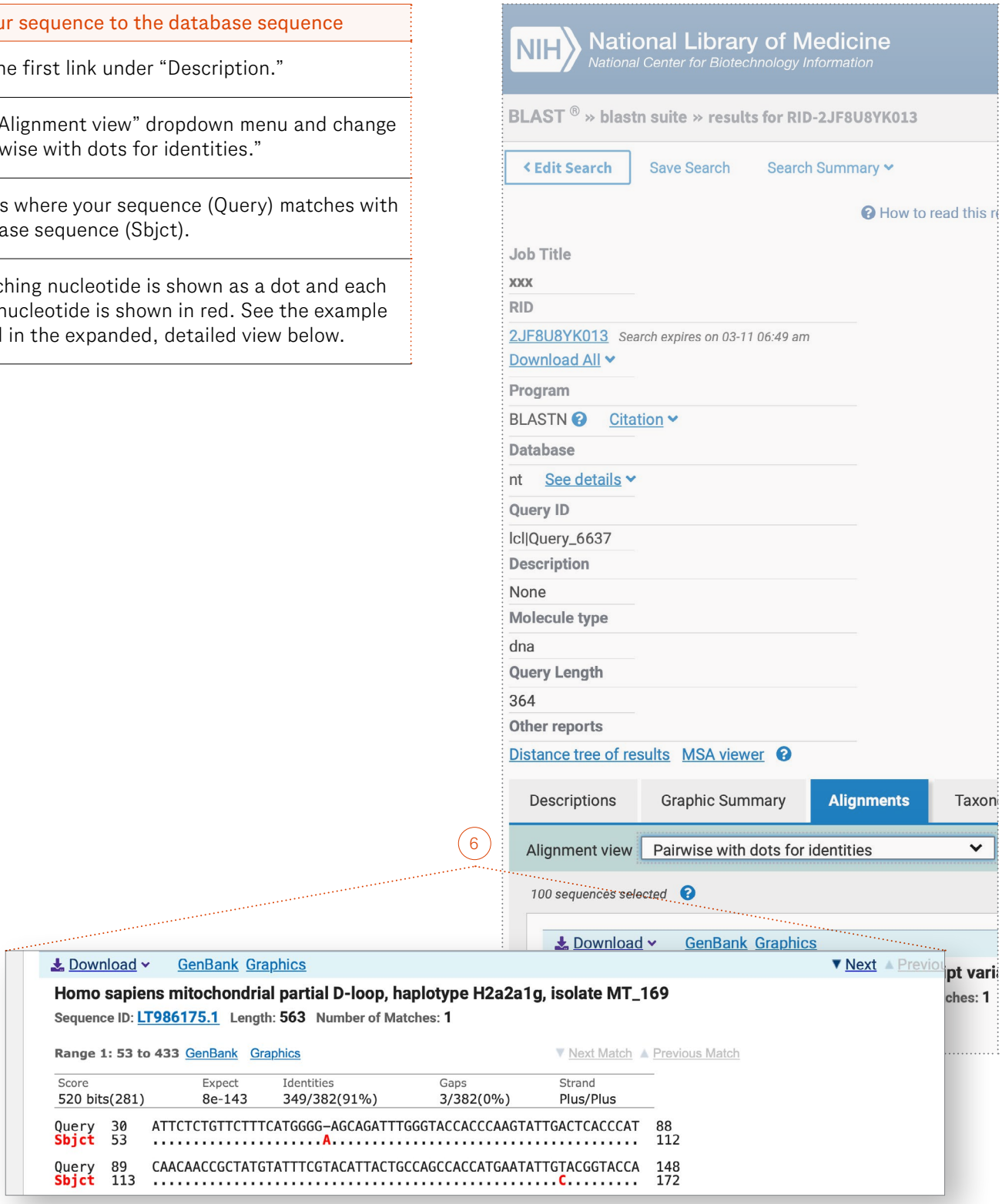
Student Protocol

Part 3: DNA Sequence Analysis with BLAST

Continued

6	Match your sequence to the database sequence
☐	Click on the first link under “Description.”
☐	Find the “Alignment view” dropdown menu and change it to “Pairwise with dots for identities.”
☐	This shows where your sequence (Query) matches with the database sequence (Sbjct).
☐	Each matching nucleotide is shown as a dot and each different nucleotide is shown in red. See the example illustrated in the expanded, detailed view below.



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Student Guide, Part 1: Pre-Lab

Directions
In this lab, you will play the role of a geneticist investigating how to detect unique genome features of humans who live the longest.

Overview
In a Genome-Wide Association Study (GWAS), the DNA sequences of thousands of individuals are compared to look for associations between certain genetic variations and a certain trait. In this lab, you will simulate the steps of a fictional GWAS to determine if there is an association between a certain SNP (single nucleotide polymorphism) and longevity. The SNP you will focus on is called rs2802288 and was previously identified in a population of South American centenarians (people over 100 years old).

There are two possible alleles of rs280228—"G" or "A" (at this location in the human genome, there is either a guanine or adenine). In this study, you will determine what gene rs2802288 is a part of and if there is an association between one of the alleles and longevity. To do this, you will compare the rs2802288 alleles of 100 people from a variety of ages spanning 21–112 (the samples are from a mix of living and dead individuals).

Driving Question

How can we identify if a particular SNP is associated with longevity?

Continues on next page >

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Student Guide, Part 1: Pre-Lab

Continued

1. To begin, carefully examine each chart provided by your teacher and record two observations and two questions about what you see.

   Phenomenon:

	Chart 1	Chart 2
Observations *I notice...* *... reminds me of...*		
Questions *I wonder...* *Could it be that...*		

Continues on next page >

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Student Guide, Part 1: Pre-Lab

Continued

2. Predict what gene rs2802288 might be a part of if it is associated with longevity. Explain your reasoning. You may refer to *Background Reading: Genetic Markers of Longevity* and *Phenomenon: Chart 2*.

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Student Guide, Part 1: Pre-Lab

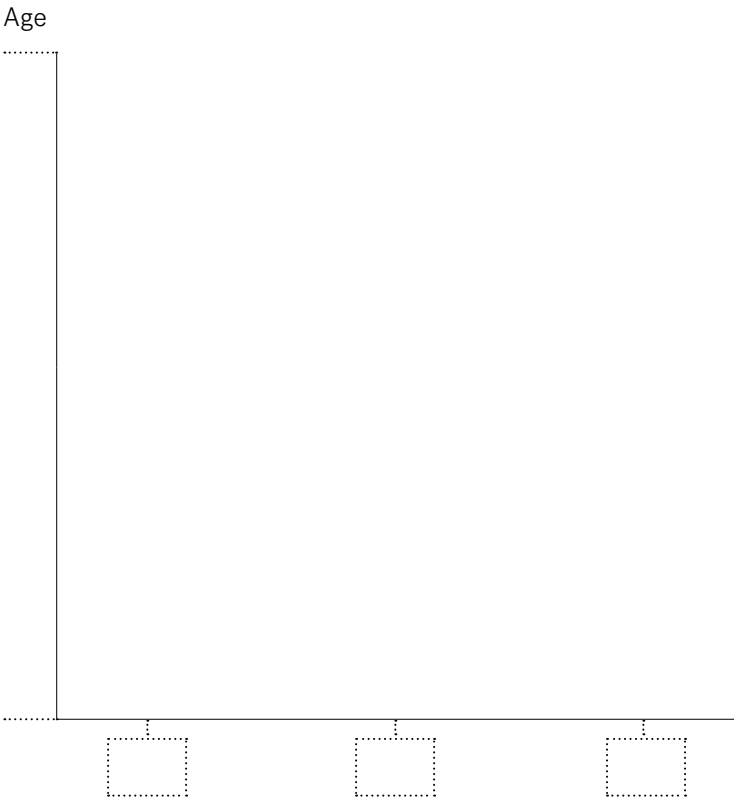
Continued

3. Use the information from *Background Reading: Genetic Markers of Longevity* to draw two graphs:

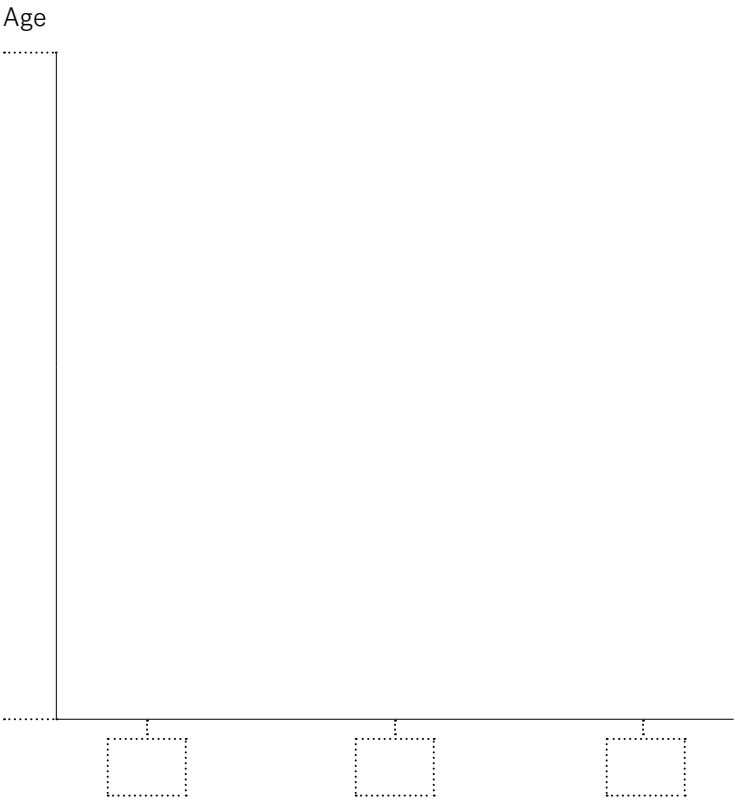
— Graph 1: What you would expect to see if there is NO association between rs2802288 and longevity.

— Graph 2: What you would expect to see if there IS an association between one of the rs2802288 alleles (G or A) and longevity.

Graph 1



Graph 2

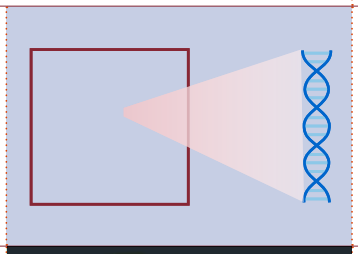
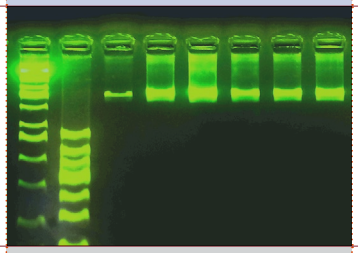
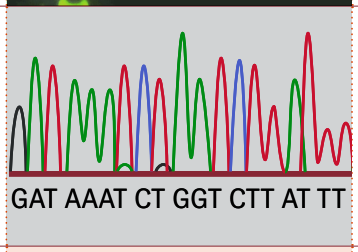
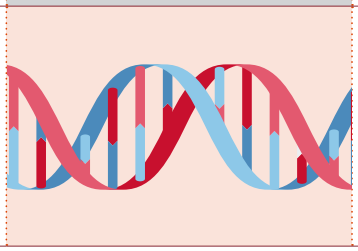


FUTURELAB+

Student Guide, Part 2: Lab and Data Collection

Directions
Answer the questions below to prepare for the lab and record your data after completing the lab.

1. The image below provides an overview of some of the main steps in a GWAS. Record the purpose of each step and whether or not you will be performing it.

	Lab Step		What is the purpose of this step?	Will you perform it?
a	Extract DNA from Cheek cells			
b	Agarose Gel Electrophoresis			
c	DNA Sequencing			
d	DNA Sequence Analysis			

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Student Guide, Part 2: Lab and Data Collection

Continued

2. Draw or paste the picture of your gel below. Label each well and the DNA ladder (without writing directly on the picture). Record three observations about your gel.

3. Record the following information from the top match of the nucleotide BLAST you performed on your sequence:

Description

Scientific Name

E Value

Percent Identity

My three observations:

Differences between your sequence and that of the data base:

OPTIONAL: Scroll down to see if sequences from other organisms match with yours.

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Student Guide, Part 3: Data Analysis

Directions

Analyze your results from the lab and the provided GWAS data by answering the questions below.

1. Did you successfully extract DNA from your cheek or strawberry cells? Describe what you see on your gel that supports your answer.

2. According to the BLAST, what gene is SNP rs2802288 a part of? Use evidence from the E value and Percent Identity to support your answer. Does this support or refute your prediction?

3. Review the *Background Reading: Genetic Markers of Longevity* or search the internet to find three pieces of information about this gene. Record them here.

Continues next page >

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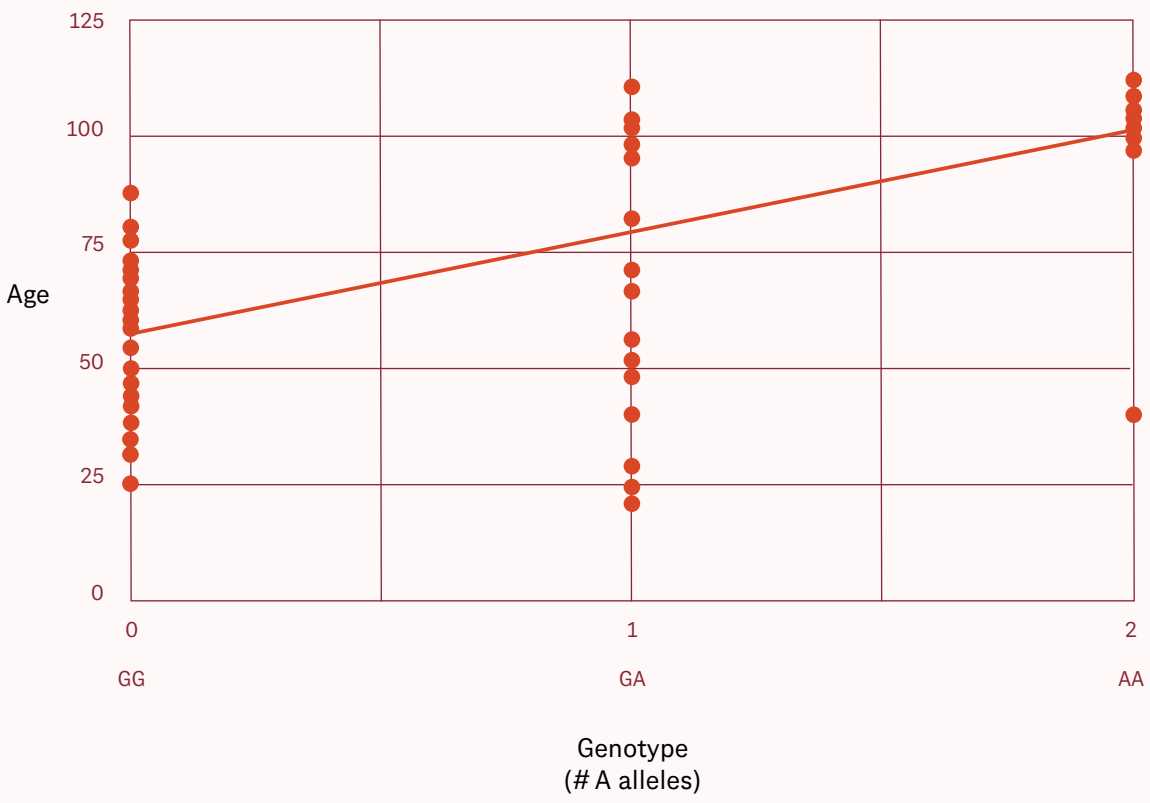
Student Guide, Part 3: Data Analysis

Continued

4. After examining the GWAS: rs2802288 Results presented on this page and the next, answer questions 4a–4c to analyze the data in the graph and table.

   The graph and data table show the results from a fictional Genome-wide Association Study on the rs2802288 SNP. This study included samples from 100 individuals. Each individual's age is recorded as well as their genotype for rs2802288 (GG, GA, or AA).

GWAS: rs2802288 Results



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Student Guide, Part 3: Data Analysis

Continued

GWAS: rs2802288 Results

These results are fictional and have not been collected from an actual study.

Age	Genotype	Age	Genotype	Age	Genotype	Age	Genotype
21	1	55	0	96	1	100	2
24	1	56	1	97	2	101	2
25	0	57	1	97	2	101	1
29	1	59	0	97	1	102	2
32	0	60	0	97	1	102	2
34	0	61	0	98	1	102	1
35	0	63	0	98	1	102	1
38	0	64	0	98	1	102	2
39	0	66	0	98	2	102	2
39	0	66	0	98	1	103	2
40	1	66	0	98	1	104	1
40	2	67	0	99	2	104	1
42	0	67	1	99	2	105	2
44	0	69	0	99	2	105	2
45	0	71	1	99	2	106	2
47	0	71	0	99	2	107	2
49	1	72	0	99	2	107	2
50	0	73	0	99	2	108	2
51	0	74	0	100	2	108	2
51	1	78	0	100	1	109	2
51	0	80	0	100	2	110	1
51	0	81	0	100	2	110	1
52	1	82	1	100	1	111	1
54	0	88	0	100	2	112	2
55	0	95	1	100	1		

Genotype Key
0 = GG
1 = GA
2 = AA

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Student Guide, Part 3: Data Analysis

Continued

4a. Identify at least three patterns or features of the data. *What do you notice about the graph or data table?*

4b. Draw two conclusions from the patterns you identified. *What is the main takeaway from the data?*

4c. Describe at least three limitations of the data and the implications of each. *What information is missing from the study? What features make it less reliable?*

Limitation	Implication

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Student Guide, Part 3: Data Analysis

Continued

5. Is there an association between rs2802288 and longevity? (Yes/No/Cannot determine)

   Explain your reasoning.

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Student Guide, Part 4: Extension

Directions
Answer the questions below to learn more about the rs2802288 SNP.

1. Search for the SNP "rs2802288" on the internet and click on the first link. Scroll to the bottom of the page to find a horizontal bar graph that summarizes the percentage of different populations that have been found to have "AA," "AG," and "GG" alleles of the SNP.

   Record three observations and three questions about the graph:

	1	2	3
Observations *I notice...* *... reminds me of...*			
Questions *I wonder...* *Could it be that...*			

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Student Guide, Part 4: Extension

Continued

2. Describe a follow-up experiment or study. What would you want to find out next that would help you answer the questions you listed above?

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Data Analysis Rubric

Score	4	3	2	1
Content and Clarity	Identifies at least three important features or patterns in the data and clearly connects them to relevant science concepts.	Identifies at least three features or patterns in the data and connects them to relevant science concepts.	Identifies two important features or patterns in the data and provides some connection to science concepts.	Identifies one important graph feature or does not provide a connection to science concepts.
Limitations	Identifies at least three relevant limitations of the data and clearly describes the implications.	Identifies three limitations of the data with some description of implications.	Identifies two limitations of the data.	Identifies one limitation of the data.
Final Score				