AMGEN® Biotech Experience

Scientific Discovery for the Classroom

Lab-based

Exploring Precision Medicine

Student Science Notes

www.amgenbiotechexperience.com

AMGEN® Foundation

TABLE OF CONTENTS

Science Note 1.1

WHAT IS PRECISION MEDICINE?

Welcome to the Amgen Biotech Experience (ABE) *Exploring Precision Medicine* module! You probably are already familiar with how your genes affect your visible traits, such as your hair color and eye color. And you may have examined the heritability of disease. In this module, you will have a chance to explore how the understanding of genetics can help to personalize the medical treatments we receive. Throughout this module, you will examine medical cases, explore how your DNA may make it so things taste differently to you than to other people and why, investigate how we know what genes control what traits, and carry out a lab in which you extract your own DNA to see what it tells you about your ability to taste a particular flavor.

Science Note 1.2

WHAT CONTROLS TRAITS?

What are traits? What determines our traits? In this activity, you'll think about the factors that control our traits and discuss your ideas with your teammates.

QUESTIONS

1. Write a working definition of the term ***trait***:

   You will revise this definition throughout the module.

2. Read through the following list of traits:

Eye color	Height	Scar on abdomen
Hair length	Foot size	Enjoyment of hip hop music
Skin color	Religiosity	Ability to ride a bicycle
Affinity for cats	Ability to speak Spanish and Arabic	Hair color

3. Use the table below to sort these traits into categories based on how you believe they are controlled: by genetics only, by the environment only, or by both genetics and the environment.

Controlled by genetics only	Controlled by the environment only	Controlled by both

4. Read over the traits in all three columns. Write a rule (or group of rules) for determining what influences a trait.

5. Can you think of traits people might have that are related to their health?
   a. Make a list of these traits in the first column, below.
   b. Decide whether each trait is controlled by genetics only, by the environment only, or by both, and put a check in the appropriate column.
   c. Be prepared to discuss how you decided which column to check off for each trait.

HEALTH-RELATED TRAITS

Trait related to health	Controlled by genetics only	Controlled by the environment only	Controlled by both

Science Note 1.3

A MEDICAL DILEMMA

The fact that no two medical patients are alike makes the practice of medicine challenging. People's unique bodies mean that each surgery is different, and the dosing of medication is dependent on an individual's genetic makeup and lifestyle. When caring for a patient, a medical professional must quickly identify any problems, determine an appropriate treatment or combination of treatments, properly administer medication or therapy, continually monitor the patient's progress, and adjust the course of treatment accordingly—and each step must take the patient's medical history into account and also respect their individual wishes.

A crucial aspect of providing treatment is providing the right medication in the right dosage, but this is not as simple as it might seem. Patients can have vastly different responses to the same medication; gender, body size, metabolism, allergies, and genetics are just a few of the many factors that can change a patient's response to a particular treatment. Some people may need larger or more frequent doses, and other patients might require medication far less frequently and in smaller amounts. The enormous variety of medications currently on the market complicates matters still more. Sometimes even very subtle differences between multiple forms of a single drug can determine whether a patient will respond better to one version versus another.

In this activity, you'll read about a patient who isn't reacting as expected to a medication, and you'll consider how you might respond to this situation if you were a doctor.

Pain After Surgery

Sarah Smith is in the hospital for a minor outpatient surgical procedure. Dr. Washington performs the operation quickly and easily and is able to neatly close the incision. As the wound heals, the stitches will fall out, so Dr. Washington does not anticipate a need for a follow-up appointment. Sarah is discharged with instructions to keep the incision site clean, and she is given a prescription for a 7-day supply of the pain medication hydrocodone, if she needs it.

NOTE: Hydrocodone is also known by the brand names Vicodin, Lortab, Paracetamol, among others.

After 72 hours, however, Sarah calls Dr. Washington to complain that her pain relievers don't seem to be working well. She describes her pain as an 8 on a scale of 1 to 10 (10 being the worst). The doctor asks if she has taken her hydrocodone, and Sarah says that she has taken it every few hours but it isn't helping. She says she is exhausted after going several nights without sleep and asks if Dr. Washington can recommend a different, stronger medication.

Dr. Washington knows that pain could indicate that there have been complications from the surgery. She schedules a follow-up appointment with Sarah for that afternoon. After a thorough examination, Dr. Washington determines that the pain is not being caused by complications from the procedure. Sarah, however, insists that she has pain at the incision site as well as general discomfort in her muscles and joints. She appears quite distressed; her face is pale and sweaty, and she vomits into the exam room wastebasket partway through the appointment.

An Ethical Dilemma

Dr. Washington is now faced with a dilemma. Some degree of discomfort is to be expected following surgery, though Sarah's level of pain is unusual given the minor nature of the procedure. Something seems to be complicating what should be an easy recovery. Dr. Washington genuinely wants to help Sarah, and relieving her patient's pain to the extent possible is an important aspect of providing medical care.

However, hydrocodone is an opioid: a powerful pain reliever that can have a range of unpleasant side effects, such as constipation and drowsiness. Dr. Washington also knows that it is very easy for patients to become addicted to opioids and that giving Sarah a higher dose or a stronger medication significantly increases her risk of dependency. Many patients who become addicted to an opiate prescribed by a physician often eventually begin using heroin, whose chemical structure is nearly identical to hydrocodone. Their city has been hit hard by the opioid overdose crisis, and Dr. Washington is concerned that Sarah may be attempting to obtain a prescription for more opioids, either for personal use or to sell after she leaves the hospital.

A few days after Sarah left the hospital, Dr. Washington attended a medical conference, where she discussed Sarah's case with a number of other physicians. The other doctors expressed concern about the growing crisis of opioid misuse and the difficulty of determining if a patient is in pain, if they are engaging in drug-seeking behavior, or both.

A complicating factor is that opioids, including hydrocodone, are commonly prescribed to treat pain, but they don't affect every person in the same way. Several doctors at the conference had noted differences in their patients' responses to these medications; some patients experienced almost instant relief from opioids, and others seemed to experience no pain relief at all. Some patients developed a powerful addiction to these medications, and others had been able to take the prescribed doses without any further issues. The physicians all agreed that balancing the risks of addiction with the need to make their patients more comfortable remains an ongoing ethical dilemma and that more research is needed.

QUESTIONS

1. In Dr. Washington's position, what would you do? Why?

2. What questions do you think Dr. Washington should ask Sarah?

3. It seems that Sarah is in more pain than Dr. Washington expected. Do you think that you feel pain differently than other people? Do some people have lower pain thresholds than others? Why do you think that could be?

4. Historically, the vast majority of medical research has been conducted on patients who are wealthy, male, and white. Based on Sarah's experience in the case study, why do you think this might be problematic in terms of evaluating the effectiveness of a particular drug?

5. In what ways might the people on whom medical research is normally conducted be different from Sarah, and how might that affect what the doctor might know about Sarah and how she should be treated?

FOR HOMEWORK

Read the article "Do We Have the Right Guinea Pigs?" from *Politico Magazine* to learn more about how medications have traditionally been tested and why that approach may not be the best way.

Science Note 2.1

RATE THE BITTERNESS OF THESE FOODS

Lots of things are controlled at least partially by your genes, including your eye color, your hair color, and your height. But what about things that you can't see, such as the way you react to pain medication or whether you are color-blind? How do your genes factor into these unseen traits?

And what about your sense of taste? You probably like some foods and dislike others. You may really love some things that taste bitter (such as coffee, olives, or bitter melon) or absolutely hate them. In this activity, you will explore your sense of taste and think about how your genes might influence this trait—starting with an inventory of how a variety of different foods taste to you.

PROCEDURE

As your teacher shows each slide depicting a food, rate the bitterness of that food on a 1-to-5 scale, with 1 being "Very bitter" and 5 being "Not at all bitter." When rating each food, assume that it will be served raw and without condiments of any kind. After you've rated the bitterness of each food, decide which taste word you would use to describe it: salty, sweet, sour, bitter, or umami.

Food	Very bitter 1	Bitter 2	Neutral 3	Slightly bitter 4	Not at all bitter 5	How would you describe this taste—salty, sweet, sour, bitter, or umami?

Science Note 2.2

CAN YOU TASTE IT?

In 1931 in a laboratory at DuPont chemical company, chemist Arthur Fox accidentally spilled a powdered chemical called phenylthiocarbamide (PTC). As the powder swirled in the air, a nearby scientist complained that the dust tasted bitter. However, Fox didn't taste anything. Fox was curious as to why he couldn't taste the PTC and his colleague could, so he began to run tests on others. Fox asked his friends and family members to taste the chemical and tell him how it tasted. He found that some people didn't taste it at all, some had a mild sensation of bitterness, and others found the taste unbearably bitter.

Today, scientists know that there is a connection between your genes and your ability to taste bitter foods. There are several versions of the gene responsible for bitter-tasting ability. The variant you personally carry within your chromosome number 7 is your ***genotype***. The trait that gene codes for, in this case your bitter-tasting ability, is your ***phenotype***. While you won't know for certain which version of the gene you carry after completing this activity, you can easily determine your tasting phenotype by doing a simple taste test.

Can you taste PTC? Let's find out!

MATERIALS

FOR EACH STUDENT

- Control taste paper
- PTC taste paper
- 1 cup of water (should be drinkable water; you will use it to rinse your mouth)

FOR THE CLASS

- Waste container

PROCEDURE

1. Place a piece of control taste paper on your tongue. Remember the taste of it, so you can compare it to the PTC taste paper.
2. Remove the control paper from your mouth and dispose of it in the waste container. Rinse your mouth with water.
3. Place a PTC taste paper on your tongue. If you sense a bitter taste, then you are a PTC-taster (strong or weak).
    - o If the paper tastes just awful, you are a strong-taster.
    - o If you perceive only a slight bitter taste, you are a weak-taster.
    - o If it tastes like the control paper, you are a non-taster.

4. Remove the PTC taste paper from your mouth and dispose of it in the waste container. Rinse your mouth, if needed.
5. Record if you are a strong-taster, weak-taster, or non-taster in the table below.
6. Check with your teammates, and record their statuses in the table as well.

Name	Strong-taster	Weak-taster	Non-Taster
Remainder of class (number)			
Total			
Percentage			

7. As your teacher calculates the class totals, record them in your table. Calculate the percentage of students with each phenotype.
8. Make a bar chart showing the number of students with each phenotype below.

OUR CLASS'S TASTER STATUS BAR CHART

Science Note 2.3

REVIEW AND PLAN

Earlier you considered the connection between our genes and how we respond to medications. Do all medications work for each and every one of us in exactly the same way? The answer is no: A number of factors can influence how a patient responds to medications, just as a number of factors also play into whether a person enjoys bitter foods.

To better meet people's individual needs, scientists are exploring the idea of ***precision medicine***—an approach to the prevention and treatment of disease that takes into account variability in genes, environment, and lifestyle. Scientists have recognized that different medicines affect different people in different ways, based in part on their genetics.

MATERIALS

- Computer, Chromebook, or tablet with internet access

REVIEW

Let's review some important genetics concepts:

1. Watch *What Is a Gene?* (4:56) from Stated Clearly, and then answer the following questions:
   a. What is a gene?

   b. What is the relationship between genes and traits?

2. Read the article "Mamas, Don't Let Your Babies Grow Up to Be Broccoli-Haters" by Lisa Bramen, *Smithsonian Magazine*, and then answer these questions, briefly.

   a. How might a mother's diet impact the flavors her baby is exposed to?

   b. What role, if any, do you think environment plays in a person's food preferences?

PLAN

In the upcoming lab, you will extract your DNA from your cheek cells. You will prepare a very small portion of your DNA to use later in the module. Read through these questions and answer them here or in your science notebook.

3. In the first part of the lab, you'll take cells from your cheek and boil them. This will cause the cellular and nuclear membranes to break down. Once these membranes are broken down, components that are normally in the cytoplasm of the cell will mix with the DNA. These components include enzymes called *DNases* that break down DNA. DNases require metallic ions called *cofactors* that assist them in performing their activities. These metallic ions are also found inside cells. In this lab, you will combine your DNA with Chelex, which is a chelating (binding) agent often used for DNA extraction. The Chelex resin will bind these metal cofactors away from the DNases.

   Why do you think it is important to bind away these ions at this point?

4. Read the article "What Is PCR?" from Science Learning Hub and watch the first video in the article. Answer the following questions:

   a. What is in the solution of a PCR reaction?

   b. Why does the temperature increase to above 90°C?

   c. What happens during the annealing phase?

   d. What happens during the extension phase?

   e. What does a primer do?

   f. In your own words, explain how you end up with copies of the target sequence of DNA when the initial copies go from a primer site to the end of a strand.

Science Note 3.1

USING CHEEK CELLS TO COLLECT DNA

Your task is to extract DNA from the nuclei of your cheek cells. The procedure described below and shown in the flow chart on **Science Note 3.2** will allow you to remove the other cellular components and isolate the DNA from these cells.

The process you will perform is similar to the one that doctors use to collect, isolate, amplify, and examine a patient's DNA to determine which medication might be most effective for them.

MATERIALS

FOR EACH STUDENT:

- 1 pair of gloves
- 1 flat toothpick
- 100 µL of Chelex beads in a green 0.2-mL or 0.5-mL PCR tube
- 1 clean clear 0.2-mL or 0.5-mL PCR tube labeled with your initials and the letter "T"
  This is the Chelex extraction of cheek cells, which will serve as the template for your PCR reaction.

FOR THE TEAM:

- 1 P-10 or P-20 micropipette and tips
- 1 fine-point permanent, waterproof marker
- 1 microcentrifuge tube rack
- 1 cup of ice
- 1 computer, Chromebook, or tablet with thermocycler software installed, as needed

FOR THE CLASS:

- Thermocycler (PCR machine) or heat block
- Microcentrifuge
- Waste container

PROCEDURE

Step	Notes
1. Put on gloves.	
2. Turn on your PCR machine or heat block, and set it to 99°C.	*This step may be done as a class with a single large thermocycler or with individual machines.*
3. Using a flat toothpick, gently swab the inside of your mouth for at least 30 seconds.	
4. Swirl the toothpick in the tube of the Chelex beads for at least 30 seconds to dislodge the cells.	*Swirling thoroughly will allow you to dislodge as many cheek cells as possible.*
5. Cap the tube tightly. Label the tube with your initials.	

Step	Notes
6. Place the used toothpick in the waste container.	
7. Incubate the Chelex bead tube at 99°C for 10 minutes, using a PCR machine or heat block.	
8. Spin at a minimum of 4,800 g for 1 minute to pellet the cells and the Chelex beads.	*Make sure that you balance the microcentrifuge with other students' Chelex bead tubes or a balance tube of the same mass.*
9. Label a clean 0.2-mL or 0.5-mL PCR tube with your initials and the letter "T."	*"T" stands for "template." This tube is the* ***template tube****, which will be used in the PCR.*
10. Set your P-10/P-20 pipette to 20 µL and add a clean tip.	
11. Carefully transfer 20 µL of the ***supernatant*** (liquid above the beads) from the Chelex bead tube to your template tube.	*Be very careful when removing the Chelex bead tube from the centrifuge and moving the tube to your station to not disrupt the Chelex bead bed.* ***Do not*** *transfer any Chelex beads to this new tube or it will interfere with the PCR and further analysis.*
12. Discard the Chelex bead tube in a designated waste container for proper disposal (autoclave).	*This must be disposed of properly because the solution contains human body fluids and/or tissues.*
13. Optional stop point: Store your template tube at –20°C to be used in the next lab.	

Science Note 3.2

USING CHEEK CELLS TO COLLECT DNA FLOW CHART

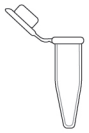
MATERIALS

For each student

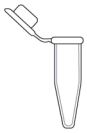
Gloves

Flat toothpick

0.2-mL or 0.5 mL tube with Chelex® beads



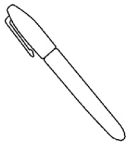
0.2-or 0.5 mL mL PCR tube



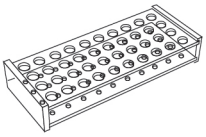
For the team

P-10 or P-20 micropipette and tips

Permanent, waterproof marker

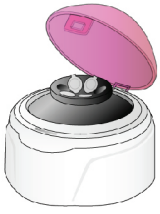


Microcentrifuge tube rack

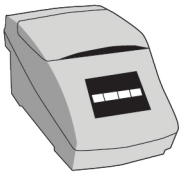


Your team needs access to

Microcentrifuge

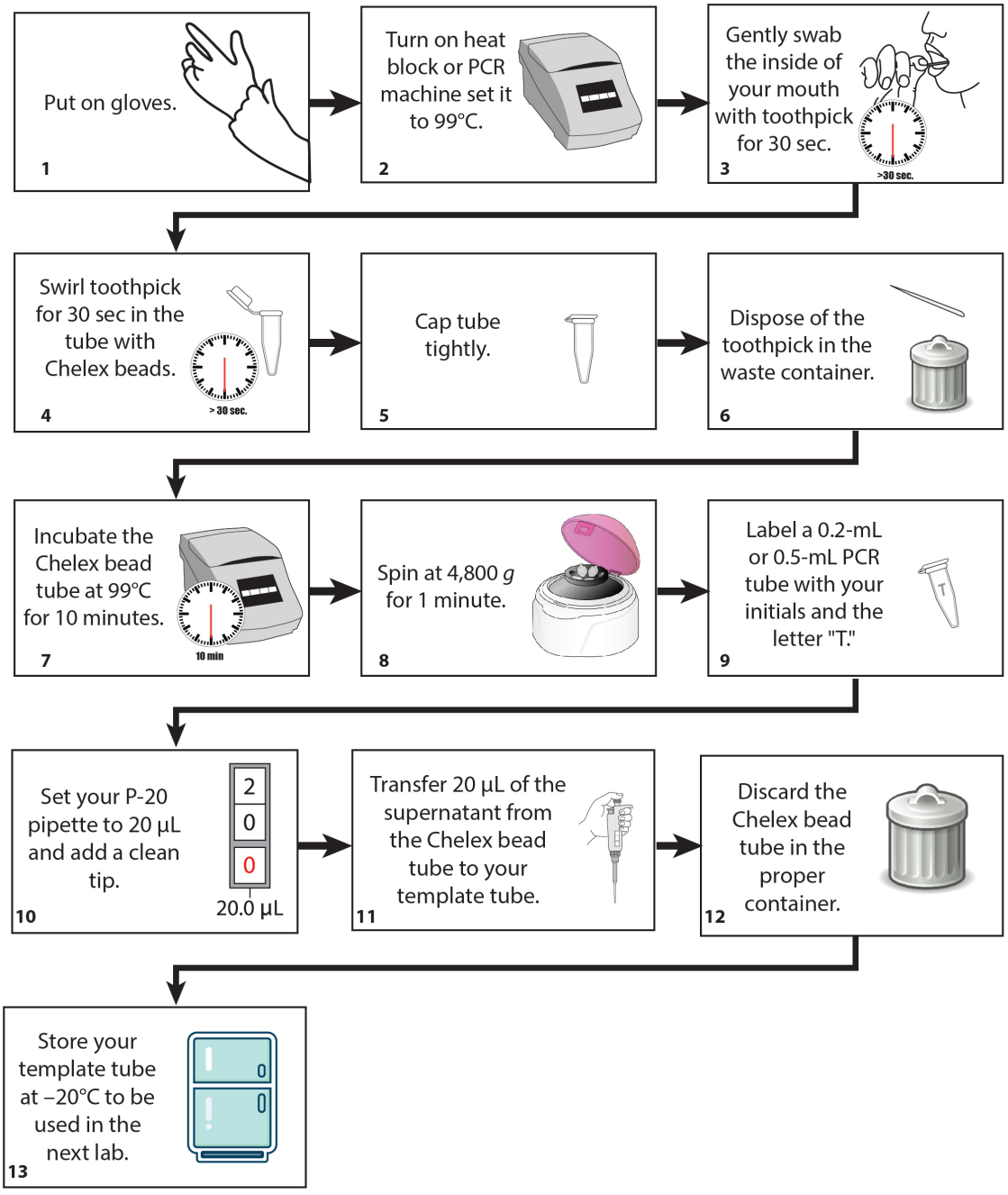


PCR machine or heat block



Waste container

PROCEDURE



Science Note 3.3

PREPARING FOR THE NEXT LAB

Your next task is to amplify a specific section of your DNA. You will use a procedure known as *polymerase chain reaction* (PCR), which is carefully controlled by software. With your team, you'll create a program for your thermocycler that will use PCR to copy the desired DNA sequence.

MATERIALS

- Computer, Chromebook, or tablet
- Software that controls your thermocycler

PROCEDURE

1. Using the software that controls your thermocycler, name the program that you are creating "Copy the DNA."
2. Set up the thermocycler program as shown below:

Step	Temperature	Time
Initial Denaturation	94°C	300 seconds
Denaturation	94°C	30 seconds
Annealing	64°C	30 seconds
Extension	72°C	30 seconds
Number of Cycles	35	
Final Extension	72°C	60 seconds

Science Note 3.4

COPY THAT DNA!

Your task is to use PCR to amplify (copy) a small fragment of DNA from your cheek cells (called the ***template DNA***—the sample DNA that contains the target sequence). Remember, the gene associated with the ability to taste PTC is called TAS2R38, and it is located on chromosome 7. Rather than amplify your entire genome, which has over 3 billion base pairs, you will amplify only this small portion of your DNA—fewer than 300 base pairs. When you complete the lab, your sample will contain over a million copies of that small fragment of DNA!

In a medical context, a patient's DNA would be amplified in a very similar way to ensure that there is sufficient DNA for testing and sequencing. Technicians might amplify a patient's entire genome or only a small portion of particular interest. The patient's DNA sample would then be analyzed for the presence or absence of a specific gene.

MATERIALS

FOR EACH STUDENT:

- 1 pair of gloves
- 1 clear 0.2-mL or 0.5-mL template (T) PCR tube (from **Science Note 3.1**)
- 1 clear 0.2-mL or 0.5-mL PCR tube containing TAS2R38 Primer Master Mix (MM) (must be kept on ice)

FOR EACH TEAM:

- 1 microcentrifuge tube rack
- 1 fine-point permanent, waterproof marker
- 1 P-10 or P-20 micropipette
- Micropipette tips

FOR THE CLASS:

- Microcentrifuge
- Thermocycler
- Computer, Chromebook, or tablet (if necessary)

PROCEDURE

Step	Notes
1. Put on gloves.	
2. Label the top of the TAS2R38 Primer Master Mix (PTC MM) with your initials or team name with a fine-point permanent, waterproof marker.	*PTC MM contains all the enzymes, primers, raw ingredients, cofactors, and buffers to create an optimal condition to amplify your DNA fragment.*
3. Carefully transfer 2.5 µL from your template tube (T) into your labeled PTC MM tube.	*This is now your PCR reaction tube.*

Step	Notes
4. Ensure that the tube is capped tightly. Gently invert the tube (tip the tube over and back) three times to mix the template and PTC MM.	*This tube now contains everything you need to run a successful PCR: template DNA, primers, and PCR MM. Store your sample on ice until you are ready to begin the reaction.*
5. Centrifuge the tubes for ~5 seconds. Place the tubes in the thermocycler.	
6. Run the "Copy the DNA" program.	*You created this program when you did **Science Note 3.3.** Depending on your thermocycler, you may be sharing with other teams.*

You may need to stop here and allow your teacher to complete the remaining steps of the Procedure.

7. Once the Copy That DNA program is finished, allow your teacher to remove your tubes from the PCR machine.	*The thermocycler interior and/or tubes might be hot!*
8. Give your teacher your PCR reaction (PTC MM) tube to store at 4°C. You will use the sample later in the module.	

Science Note 3.5

MAPPING OUR GENES

In the previous session, you extracted and amplified a segment of DNA from the TAS2R38 gene. But, how did people figure out where the TAS2R38 gene is located in our DNA? Researchers have spent years deciphering the order and location of the nucleotides in the human genome. While the advent of DNA sequencing technology allows researchers to determine the precise order of nucleotides within a piece of DNA, scientists must use other methods to determine the role of specific genes. To work out the importance of a particular genetic region, scientists use a variety of methods, for example:

- **Comparing chromosomes between organisms.** Chromosomes have two arms: the longer "q" arm and the shorter "p" arm. If scientists observe that a malformation in one arm is associated with a particular trait, they can draw conclusions about the location of the gene for that trait.
- **Deliberately knocking out (removing) certain portions of the DNA molecule** in organisms such as mice or fruit flies and then observing the results.
- **Comparing the genomes of individuals with and without specific traits.** Scientists have deduced that genes that are close together are inherited together more frequently than genes that are far apart. They use this information to develop a complex algorithm to predict the distance between genes.

Your task is to map a number of fictitious genes onto a fictitious chromosome. Using "results" obtained through a variety of experiments and other research methods, you will determine exactly where each gene is located on the chromosome.

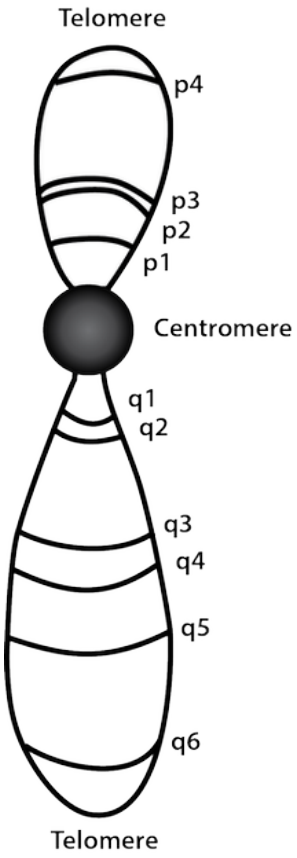
PROCEDURE

1. With your team, read through this list of fictitious genes, which you will map onto a chromosome.

FICTITIOUS GENES

Extremely smelly feet	Allergy to exams
Sneezing when angry	Fear of carrots
Purple nostril hair	Abnormally deep belly button
Fast-growing fingernails	Obsession with astronomy
Sweaty eyelids	Speaking in rhymes

2. Examine the chromosome shown at right. Notice the different location and sizes of each band. For the purpose of this exercise, assume that each band contains a single gene and that the thickness of the band denotes the length of the gene. (In reality, the scale would be quite different!)
3. Read through the experimental results below, and use these results to determine the location of each gene.



Experimental Results

Below are the results of scientists' experiments on a group of 10 subjects.

After running all 10 sequences through gel electrophoresis, scientists found:

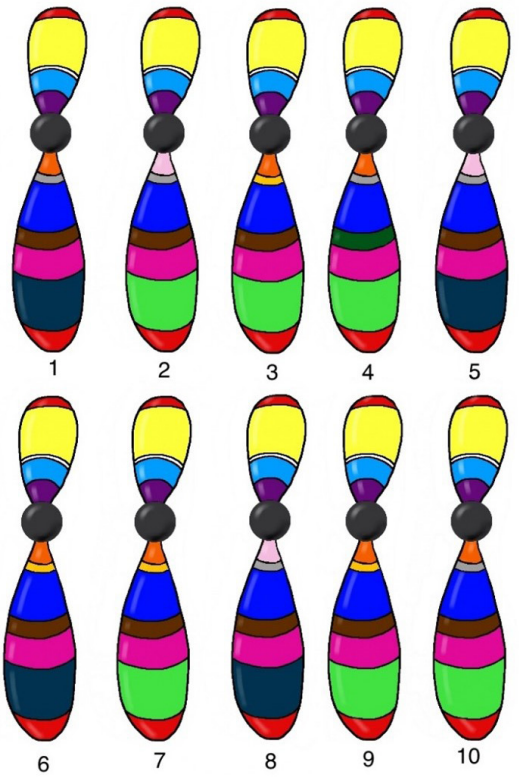
- The *allergy to exams* gene progressed the farthest through the gel.
- The *speaking in rhymes* gene progressed a much shorter distance than the *abnormally deep belly button* gene.

HINT: When performing gel electrophoresis, the smallest fragments progress furthest. Take the size of the various colored regions in the image below into account.

The inheritance computer algorithm found the following correlations:

- 99.9% of individuals who have sweaty eyelids also sneeze when they become angry.
- 0.0001% of individuals who are obsessed with astronomy also have purple nostril hair.

HINT: Segments that are located close together on a chromosome are more likely to be inherited together than segments located far apart. Items that are inherited together all the time are likely to be next to each other, or very close to it.



Knockout experiments on loci q2 and q3 resulted in:

- All test subjects' fingernails growing at normal speed
- All test subjects still having extremely smelly feet

Studying chromosomes with partially malformed Q arms showed:

- A chance of eliminating the tendency to sneeze uncontrollably when angry
- No chance of eliminating the tendency to speak in rhymes

Scientists have determined the following information about several genes' loci:

- The *purple nostril hair* gene is farther away from the center of the chromosome than the *allergy to exams* gene.
- The *obsession with astronomy* gene is located immediately next to a telomere.
- The *speaking in rhymes* gene is located immediately next to the centromere.

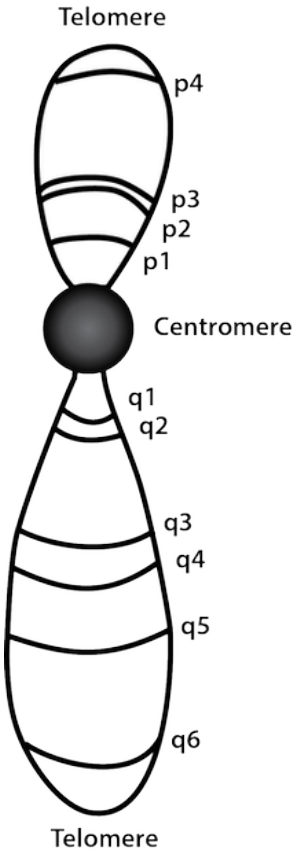
While studying the genome of the 10 subjects whose chromosomes are pictured at right, the scientists gathered several key pieces of information:

- Only subjects 2, 5, and 8 are terribly afraid of carrots.
- Only subjects 3, 6, 7, and 9 have abnormally deep belly buttons.
- All subjects have very sweaty eyelids.

4. Write each gene in the appropriate space in the chart below.

GENE LOCATION

Location	Gene
Telomere	Protects the chromosome during cell division
p4	
p3	
p2	
p1	
Centromere	Spindle fiber attachment point during cell division
q1	
q2	
q3	
q4	
q5	
q6	
Telomere	Protects the chromosome during cell division



Science Note 4.1

EXPLORING DNA SEQUENCES

The human genome contains approximately 3 billion base pairs in our 23 pairs of chromosomes. Our DNA contains the codes for approximately 20,000 different traits. The billions of nucleotides in our genomes are very similar across all human beings. In fact, somewhere between 99% and 99.9% of your DNA reads exactly the same as your friend's, your relative's, and a perfect stranger's.

Some genes are very long—far too long to be easily analyzed by hand. Researchers often use software to examine genomic sequences, quickly compare them, and identify areas where they are similar and where they are different.

In **Science Note 3.5**, you learned about how the locations of genes are determined. In this activity, you will learn about DNA sequencing, which was used to figure out the order and location of the nucleotides that make up the entire human genome in the Human Genome Project. You will then explore the data that DNA sequencing generates and compare several different people's TAS2R38 DNA sequences using software to try to determine how these differences correlate with the ability (or inability) to taste bitterness. But first, it is important to learn a little bit about what you will be seeing when you use the sequencing software.

PROCEDURE

1. A ***chromatogram*** is a visual representation of a DNA sample that is produced by a sequencing machine.
2. In the chromatogram, each color represents a specific nucleotide. Each color peak in the chromatogram indicates the likelihood of the corresponding nucleotide appearing at that position; taller peaks indicate that it is more likely that a particular nucleotide is in that position. The computer analysis also assigns the appropriate nucleotide to that position. Should the computer program be unable to assign a nucleotide base with confidence, an "N" (for nucleotide) will appear as a placeholder.

Key to the Chromatogram Colors	
Black = Guanine (G)	Red = Thymine (T)
Blue = Cytosine (C)	Green = Adenine (A)

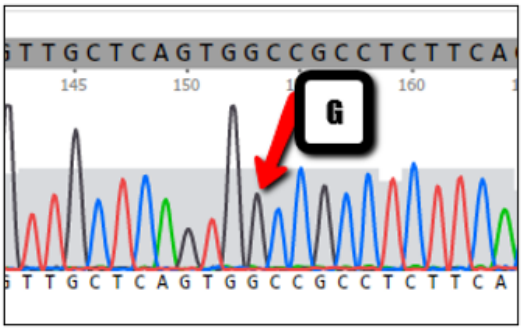
3. Chromatograms look like the image below—four different colored strands ("traces") representing the four different nucleotides. The gray blocks behind the traces indicate the ***confidence interval***—a measure of the probability that the computer-assigned nucleotide actually is the nucleotide in the DNA sequence. Basically, the taller the gray area, the more confident the computer is in assigning the nucleotide.

EXAMPLE CHROMATOGRAM

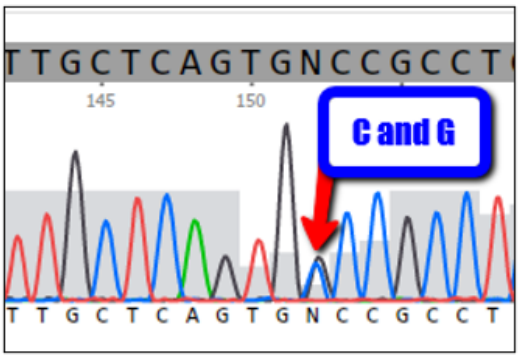
4. In a "good" chromatogram, the peaks are spaced evenly (for example, the peaks after #70 highlighted in green). Peak heights may vary, which is normal. Usually, the first 20–40 nucleotides of a sequence are not very good (as shown below in yellow), and you can see many "N" placeholders at positions where the computer cannot assign a nucleotide base with confidence (messy traces, low confidence).

5. If a person is heterozygous for a particular nucleotide, you should see two peaks at that location. The images below represent sequences that are (1) homozygous G, (2) heterozygous G and C, and (3) homozygous C.

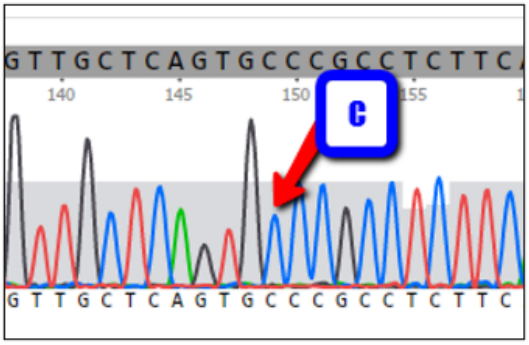
1



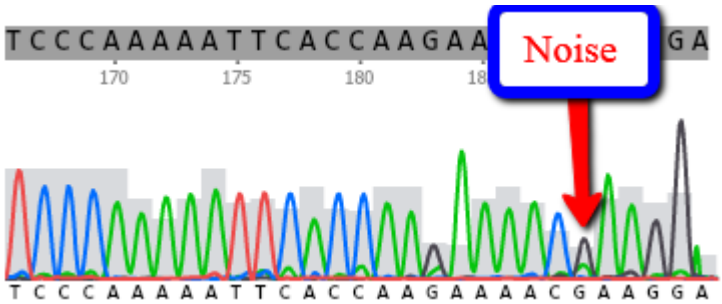
2



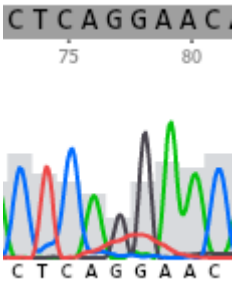
3



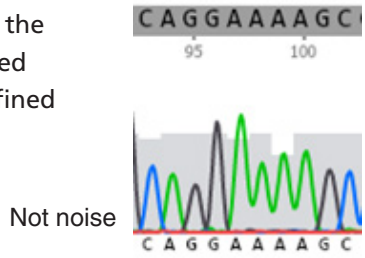
6. There is always some background "noise" from the other trace strands. The computer program can usually determine what is "noise" from a sample that is heterozygous. For the purpose of this chromatogram analysis, peaks that are less than half the height of the main peak at a nucleotide position can be considered "noise."



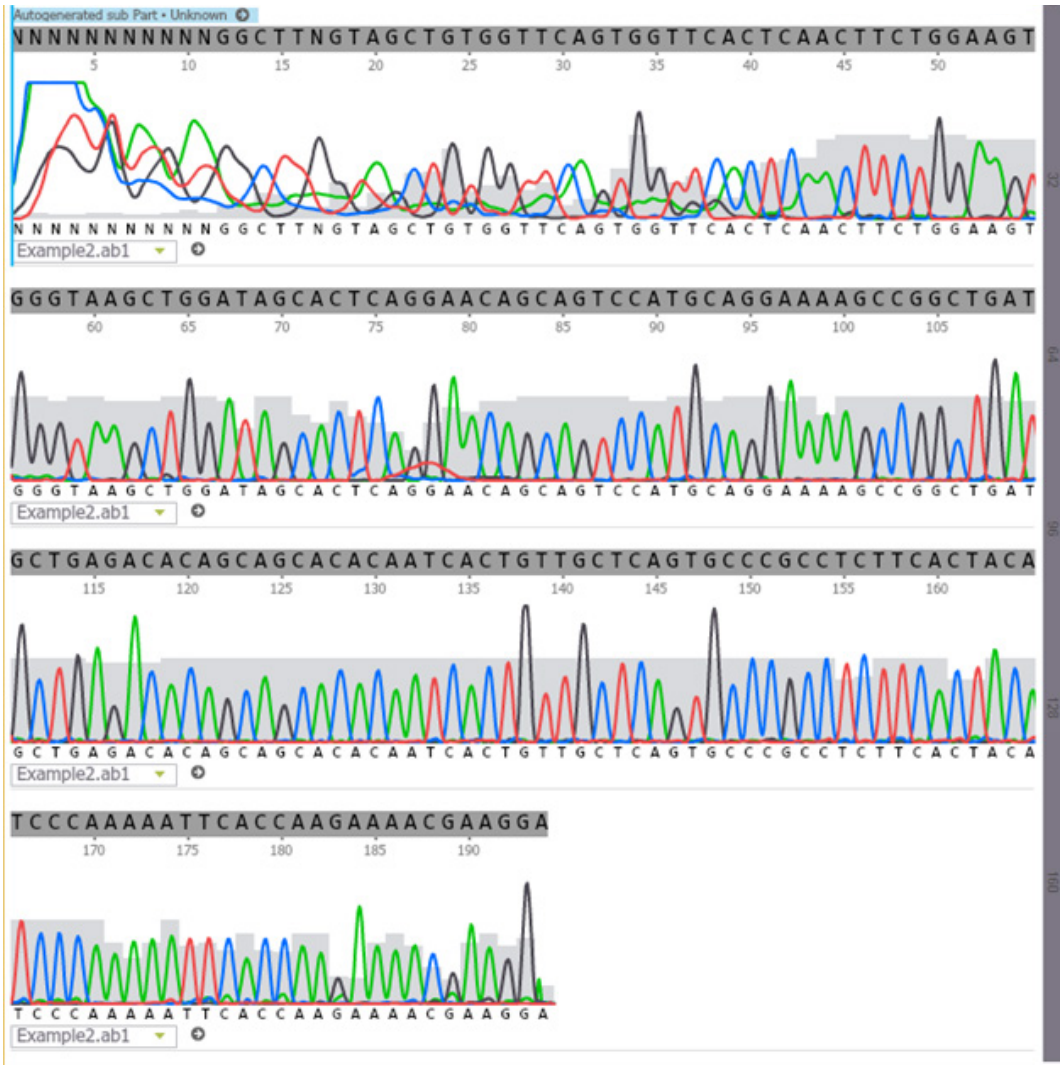
7. Sometimes there are single broad peaks that span several nucleotide positions. These can also be considered "noise."



8. A broad peak base is also seen at locations where the sequence has the same nucleotide multiple times. However, the trace will show defined peaks for each nucleotide position; the computer reads these as defined signals and will assign the proper nucleotide bases.



9. Now try it yourself. Explore the chromatogram below and answer the questions on the following page.



QUESTIONS

1. Where does the sequence start to look "good"? (**HINT: Consider the size of the gray area behind the peaks.**)

2. What nucleotide is at position 177?

3. ... position 115?

4. ... position 148?

5. Can you find any positions that look like they might be heterozygous? If so, what positions are heterozygous?

Science Note 4.2

FINDING TAS2R38 DIFFERENCES

Now that you have explored how to "read" the files created when DNA is sequenced, you can use software to compare the DNA sequences of different individuals to see how they differ and how the differences might be related to their phenotypes. In this activity, you are going to use some sequences of TAS2R38 genes to make comparisons and look for differences related to the expression of the TAS2R38 gene. Specifically, you will be comparing the sequences of tasters and non-tasters to the reference sequence (an accepted representation of the human genome sequence used by researchers) to look for differences. How do the gene sequences of people with different tasting phenotypes differ from one another?

NOTE: When you extracted your DNA, you extracted a small fragment. In this activity, you will be exploring a fragment that is almost the length of the complete 1002 bp TAS2R38 gene.

MATERIALS

- Computer, Chromebook, or tablet with internet connection
- TAS2R38 reference sequence.txt file
- TAS2R38 example sequence files provided by your teacher

PROCEDURE

Get Familiar with Benchling

Watch the ABE Benchling tutorial.

Import Files into Benchling

1. Your teacher will direct you to where the sequencing data files are located. Download the appropriate Trace file (.ab1).
2. Open the Benchling homepage and sign in.
3. Click on the + in the menu ("Create") and select the "Project" option.
4. The "Create project" window will open.
5. In the "Name" field, type your team's name.
6. In the "Manage collaborators" field, add each of your teammate's names. In the Permissions dropdown menu, change each person's permissions to "ADMIN."
7. Click "Create project" to finish setting up.
8. Navigate back to your Project main list by clicking "Projects" above your team name.
9. Import the TAS2R38 reference sequence.seq file and then three example sequence files. You will explore three example files, one from a strong-taster, one from a weak-taster, and one from a non-taster. Using Benchling, you will be able to see differences between the genotypes that correspond to these phenotypes.

10. Open one of the example sequence files (in the Inventory list on the left side of the screen) by clicking on it.
11. Open the chromatogram by clicking on the "Alignments" icon ( ) on the right side of the screen. Click on the name of your example sequence under "Saved Alignments" to display the chromatogram of the example sequence.
12. Explore the chromatogram. At what position do you think the data becomes "good"? Do you see any places where the sequence might be heterozygous? Make note of any heterozygous locations below .

Align Multiple Sequences

Sequence alignment allows you to see the differences between the sequences of a taster, a weak-taster, a non-taster, and the reference sequence of the TAS2R38 gene. The software allows you to easily compare multiple sequences and spot differences between them.

13. Click on the TAS2R38 Reference Sequence file in the Inventory list.
14. Click on the "Alignments" icon ( ) on the right side of the screen. In the "Choose File(s)" field, type "Example1." When the name of the file appears, click on it.
15. Type the name of the second example sequence file in the "Choose File(s)" field.
16. Type the name of the third example sequence file in the "Choose File(s)" field.
17. In the menu bar, click the green "Create Alignment" button.
18. Use the scroll bar at the bottom of the screen to scroll through the alignment. The red-highlighted nucleotides indicate a difference in the sequences.
19. Highlight the differences you find, and note the position of each difference. (It should say "START: x END: x," where x is the position of the difference.)
20. Analyze the difference: Is it because of poor data at the start or end of your trace file? Is it because the algorithm could not determine the nucleotide (is labeled N)? Or is there a well-defined peak in the sequencing data that indicates a nucleotide difference between the sequences? Note the positions of the differences at well-defined peaks and the sequences at those positions in the Differences table on the next page. Which nucleotide is associated with samples from strong-tasters? non-tasters?

DIFFERENCES

Position of the Difference in the TAS2R38 Gene	Sequence Around the Difference (4 nucleotides before and 4 nucleotides after)	
	Strong-taster	Non-taster
188	TCCAGTCGA	TCCATTCGA

Science Note 5.1

WHICH RESTRICTION ENZYME SHOULD WE USE?

In the previous activity, you explored the tiny differences (SNPs) in the TAS2R38 gene. Knowing which SNP you have confirms whether you have a tasting or non-tasting phenotype. To find out your genotype, you can send out your DNA for sequencing. However, you might want to learn more about your genome without having to put it in an envelope and pay someone to sequence it, and you can learn more—by using restriction enzymes. Restriction enzymes are enzymes that cut DNA into fragments at or near specific recognition sites. These enzymes were isolated from bacterial immune systems where they cut up the genetic material of invading viruses.

Restriction enzymes recognize specific 4–8 bp long DNA sequences and typically cut the strands at a particular position within or before the recognition site. Restriction enzyme digestion cuts a specific series of nucleotides, generating DNA fragments of different sizes. These DNA fragments can then be separated using gel electrophoresis based on the size of each fragment.

Choosing the correct restriction enzyme is critical to ensure that the DNA fragments generated during the digestion can be individually identified on the gel. Your task is to choose a restriction enzyme that will cut pieces of DNA that allow you to compare bitter-tasters and non-tasters.

HINT: To tell the difference between bitter-tasters and non-tasters, you will need to pick a restriction enzyme that cuts the DNA of one type and doesn't cut the DNA of the other. In the previous activity, you noted the location of a SNP in the fragment you amplified (145), and you know the sequences of both the tasting and non-tasting genotype. So, consider which restriction enzymes will cut your DNA when one of the possible nucleotides is present but not when the other is present.

MATERIALS

- Computer, Chromebook, or tablet with internet access
- 2 different-colored highlighters

PROCEDURE

1. Watch the Sequence Extractor tutorial.
2. Go to the Sequence Extractor website.
3. Select all the text that appears in the box that says "Paste a sequence into the text area below. *Accepted formats are: raw, GenBank, EMBL, and FASTA*" and delete it. Copy the following sequence (the sequence of the TAS2R38 gene), and paste it into the box.

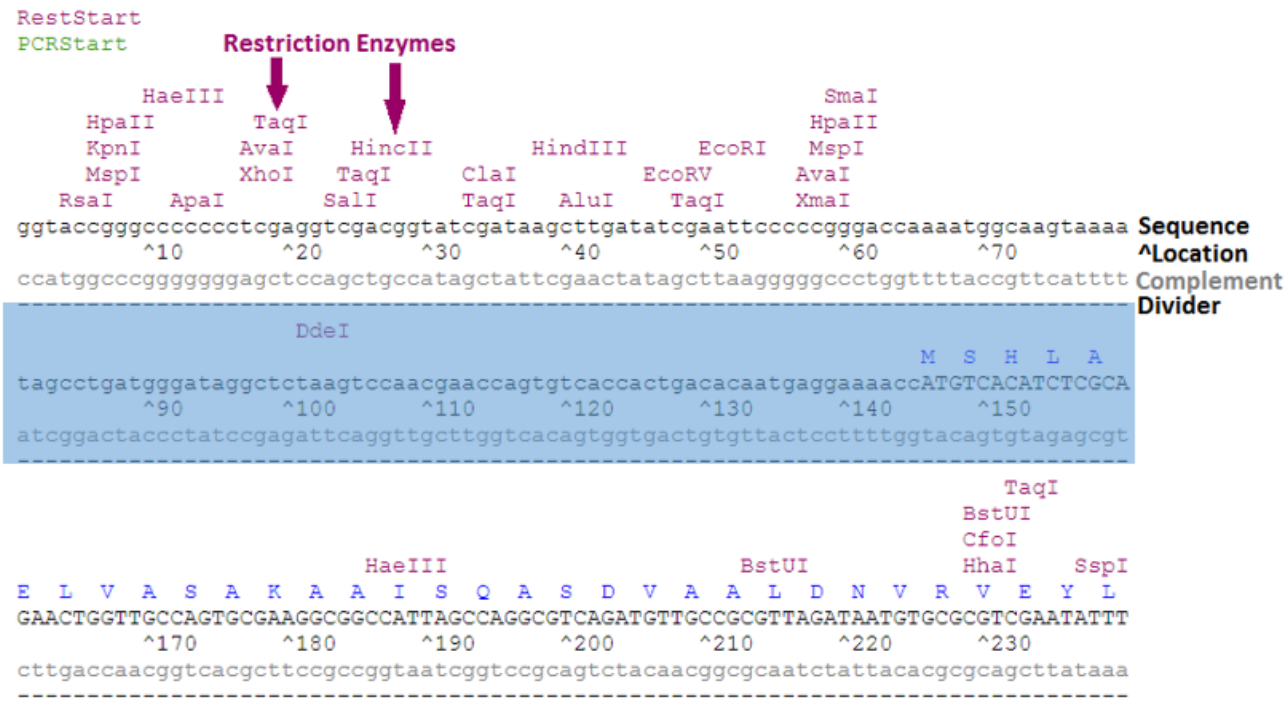
```
1   ATGTTGACTC TAACTCGCAT CCGCACTGTG TCCTATGAAG TCAGGAGTAC ATTTCTGTTC
61  ATTTCAGTCC TGGAGTTTGC AGTGGGGTTT CTGACCAATG CCTTCGTTTT CTTGGTGAAT
121 TTTTGGGATG TAGTGAAGAG GCGGCCACTG AGCAACAGTG ATTGTGTGCT GCTGTGTCTC
181 AGCATCAGCC GGCTTTTCCT GCATGGACTG CTGTTCCTGA GTGCTATCCA GCTTACCCAC
241 TTCCAGAAGT TGAGTGAACC ACTGAACCAC AGCTACCAAG CCATCATCAT GCTATGGATG
301 ATTGCAAACC AAGCCAACCT CTGGCTTGCT GCCTGCCTCA GCCTGCTTTA CTGCTCCAAG
361 CTCATCCGTT TCTCTCACAC CTTCCTGATC TGCTTGGCAA GCTGGGTCTC CAGGAAGATC
421 TCCCAGATGC TCCTGGGTAT TATTCTTTGC TCCTGCATCT GCACTGTCCT CTGTGTTTGG
481 TGCTTTTTTA GCAGACCTCA CTTCACAGTC ACAACTGTGC TATTCATGAA TAACAATACA
541 AGGCTCAACT GGCAGAATAA AGATCTCAAT TTATTTTATT CCTTTCTCTT CTGCTATCTG
601 TGGTCTGTGC CTCCTTTCCT ATTGTTTCTG GTTTCTTCTG GGATGCTGAC TGTCTCCCTG
661 GGAAGGCACA TGAGGACAAT GAAGGTCTAT ACCAGAAACT CTCGTGACCC CAGCCTGGAG
721 GCCCACATTA AAGCCCTCAA GTCTCTTGTC TCCTTTTTCT GCTTCTTTGT GATATCATCC
781 TGTGTTGCCT TCATCTCTGT GCCCCTACTG ATTCTGTGGC GCGACAAAAT AGGGGTGATG
841 GTTTGTGTTG GGATAATGGC AGCTTGTCCC TCTGGGCATG CAGCCATCCT GATCTCAGGC
901 AATGCCAAGT TGAGGAGAGC TGTGATGACC ATTCTGCTCT GGGCTCAGAG CAGCCTGAAG
961 GTAAGAGCCG ACCACAAGGC AGATTCCCGG ACACTGTGCT GA
```

4. Select all the text in the box that says "If there are primers you would like shown on the map, enter each primer as follows: the sequence of the primer, a blank space, and the name of the primer. Use commas to separate multiple primer entries" and delete it. Copy the following (the sequences of the Forward and Reverse primers), and paste it into the box.

```
CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGG Forward primer
TGAGGCAGGCAGCAAGCCAGAGGTTGGCTTGGTTTGCAATCATC Reverse primer
```

5. Click "Submit." What does the resulting screen show?

NOTE: It may be a little difficult to understand the results at first. The following diagram can help you get oriented. Each segment of the strand appears between two dashed lines.



QUESTIONS

1. What do you think the blue letters above the sequences represent?

2. In what color is the Reverse primer shown?

3. In what color is the Forward primer shown?

4. Roll over the primers to answer the following:
   a. At what position does the Forward primer start?

   b. Does the rollover give you any other information about the Forward primer?

   c. At what position does the Reverse primer start?

   d. Does the rollover give you any other information about the Reverse primer?

   e. How long is the fragment that you produced using these two primers? (**HINT: Click on the Forward primer sequence on the screen. The pop-up box or new tab should tell you the length of the fragment that you created.**)

5. Look at the TAS2R38 Sequence Extractor Results diagram on the next two pages.
   a. Highlight the positions of the primers on the sequence using two different-colored highlighters.

   b. Note anything else that you've learned about the fragment.

   c. Circle the SNP, which is at position 145.

TAS2R38 SEQUENCE EXTRACTOR RESULTS

```
M  L  T  L  T  R  I  R  T  V  S  Y  E  V  R  S  T  F  L  F  I  S  V  L  E  F  A
ATGTTGACTCTAACTCGCATCCGCACTGTGTCCTATGAAGTCAGGAGTACATTTCTGTTCATTTCAGTCCTGGAGTTTGC
         ^10        ^20        ^30        ^40        ^50        ^60        ^70
TACAACTGAGATTGAGCGTAGGCGTGACACAGGATACTTCAGTCCTCATGTAAAGACAAGTAAAGTCAGGACCTCAAACG
--------------------------------------------------------------------------------
 V  G  F  L  T  N  A  F  V  F  L  V  N  F  W  D  V  V  K  R  R  P  L  S  N  S  D
AGTGGGGTTTCTGACCAATGCCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGGCCACTGAGCAACAGTG
         ^90        ^100       ^110       ^120       ^130       ^140       ^150
TCACCCCAAAGACTGGTTACGGAAGCAAAAGAACCACTTAAAAACCCTACATCACTTCTCCGCCGGTGACTCGTTGTCAC
--------------------------------------------------------------------------------
  C  V  L  L  C  L  S  I  S  R  L  F  L  H  G  L  L  F  L  S  A  I  Q  L  T  H
ATTGTGTGCTGCTGTGTCTCAGCATCAGCCGGCTTTTCCTGCATGGACTGCTGTTCCTGAGTGCTATCCAGCTTACCCAC
         ^170       ^180       ^190       ^200       ^210       ^220       ^230
TAACACACGACGACACAGAGTCGTAGTCGGCCGAAAAGGACGTACCTGACGACAAGGACTCACGATAGGTCGAATGGGTG
--------------------------------------------------------------------------------
F  Q  K  L  S  E  P  L  N  H  S  Y  Q  A  I  I  M  L  W  M  I  A  N  Q  A  N  L
TTCCAGAAGTTGAGTGAACCACTGAACCACAGCTACCAAGCCATCATCATGCTATGGATGATTGCAAACCAAGCCAACCT
         ^250       ^260       ^270       ^280       ^290       ^300       ^310
AAGGTCTTCAACTCACTTGGTGACTTGGTGTCGATGGTTCGGTAGTAGTACGATACCTACTAACGTTTGGTTCGGTTGGA
--------------------------------------------------------------------------------
 W  L  A  A  C  L  S  L  L  Y  C  S  K  L  I  R  F  S  H  T  F  L  I  C  L  A  S
CTGGCTTGCTGCCTGCCTCAGCCTGCTTTACTGCTCCAAGCTCATCCGTTTCTCTCACACCTTCCTGATCTGCTTGGCAA
         ^330       ^340       ^350       ^360       ^370       ^380       ^390
GACCGAACGACGGACGGAGTCGGACGAAATGACGAGGTTCGAGTAGGCAAAGAGAGTGTGGAAGGACTAGACGAACCGTT
--------------------------------------------------------------------------------
  W  V  S  R  K  I  S  Q  M  L  L  G  I  I  L  C  S  C  I  C  T  V  L  C  V  W
GCTGGGTCTCCAGGAAGATCTCCCAGATGCTCCTGGGTATTATTCTTTGCTCCTGCATCTGCACTGTCCTCTGTGTTTGG
         ^410       ^420       ^430       ^440       ^450       ^460       ^470
CGACCCAGAGGTCCTTCTAGAGGGTCTACGAGGACCCATAATAAGAAACGAGGACGTAGACGTGACAGGAGACACAAACC
--------------------------------------------------------------------------------
C  F  F  S  R  P  H  F  T  V  T  T  V  L  F  M  N  N  N  T  R  L  N  W  Q  N  K
TGCTTTTTTAGCAGACCTCACTTCACAGTCACAACTGTGCTATTCATGAATAACAATACAAGGCTCAACTGGCAGAATAA
         ^490       ^500       ^510       ^520       ^530       ^540       ^550
ACGAAAAAATCGTCTGGAGTGAAGTGTCAGTGTTGACACGATAAGTACTTATTGTTATGTTCCGAGTTGACCGTCTTATT
--------------------------------------------------------------------------------
```

```
 D  L  N  L  F  Y  S  F  L  F  C  Y  L  W  S  V  P  P  F  L  L  F  L  V  S  S  G
AGATCTCAATTTATTTTATTCCTTTCTCTTCTGCTATCTGTGGTCTGTGCCTCCTTTCCTATTGTTTCTGGTTTCTTCTG
         ^570       ^580       ^590       ^600       ^610       ^620       ^630
TCTAGAGTTAAATAAAATAAGGAAAGAGAAGACGATAGACACCAGACACGGAGGAAAGGATAACAAAGACCAAAGAAGAC
------------------------------------------------------------------------------
  M  L  T  V  S  L  G  R  H  M  R  T  M  K  V  Y  T  R  N  S  R  D  P  S  L  E
GGATGCTGACTGTCTCCCTGGGAAGGCACATGAGGACAATGAAGGTCTATACCAGAAACTCTCGTGACCCCAGCCTGGAG
         ^650       ^660       ^670       ^680       ^690       ^700       ^710
CCTACGACTGACAGAGGGACCCTTCCGTGTACTCCTGTTACTTCCAGATATGGTCTTTGAGAGCACTGGGGTCGGACCTC
------------------------------------------------------------------------------
A  H  I  K  A  L  K  S  L  V  S  F  F  C  F  F  V  I  S  S  C  V  A  F  I  S  V
GCCCACATTAAAGCCCTCAAGTCTCTTGTCTCCTTTTTCTGCTTCTTTGTGATATCATCCTGTGTTGCCTTCATCTCTGT
         ^730       ^740       ^750       ^760       ^770       ^780       ^790
CGGGTGTAATTTCGGGAGTTCAGAGAACAGAGGAAAAAGACGAAGAAACACTATAGTAGGACACAACGGAAGTAGAGACA
------------------------------------------------------------------------------
 P  L  L  I  L  W  R  D  K  I  G  V  M  V  C  V  G  I  M  A  A  C  P  S  G  H  A
GCCCCTACTGATTCTGTGGCGCGACAAAATAGGGGTGATGGTTTGTGTTGGGATAATGGCAGCTTGTCCCTCTGGGCATG
         ^810       ^820       ^830       ^840       ^850       ^860       ^870
CGGGGATGACTAAGACACCGCGCTGTTTTATCCCCACTACCAAACACAACCCTATTACCGTCGAACAGGGAGACCCGTAC
------------------------------------------------------------------------------
  A  I  L  I  S  G  N  A  K  L  R  R  A  V  M  T  I  L  L  W  A  Q  S  S  L  K
CAGCCATCCTGATCTCAGGCAATGCCAAGTTGAGGAGAGCTGTGATGACCATTCTGCTCTGGGCTCAGAGCAGCCTGAAG
         ^890       ^900       ^910       ^920       ^930       ^940       ^950
GTCGGTAGGACTAGAGTCCGTTACGGTTCAACTCCTCTCGACACTACTGGTAAGACGAGACCCGAGTCTCGTCGGACTTC
------------------------------------------------------------------------------
V  R  A  D  H  K  A  D  S  R  T  L  C  Z
GTAAGAGCCGACCACAAGGCAGATTCCCGGACACTGTGCTGA
         ^970       ^980       ^990       ^10
CATTCTCGGCTGGTGTTCCGTCTAAGGGCCTGTGACACGACT
 ----------------------------------------
```

6. Try to determine which of the four restriction enzymes will allow you to tell bitter-tasters' sequences from those of non-tasters and mild-tasters. Which one would you choose, and why?

   HINT: Remember, you need to be able to distinguish between a C and a G at position 145, so consider which restriction enzymes will cut your DNA if there is a G at position 145 and not cut it if there is a C there (or vice versa). In other words, the restriction enzyme should match one genotype but not match the other.

   HINT: Use the Differences Table on Science Note 4.2 to help you determine the sequence that will need to be cut.

EcoRI	SmaI	AluI	HaeIII
G|AATTC	CCC|GGG	AG|CT	GG|CC
CTTAA|G	GGG|CCC	TC|GA	CC|GG

Write your answer here:

7. Determine the lengths of the fragments of DNA you would see, depending on your SNP, and fill in the table below. Lengths are measured by the number of base pairs (BP).

Phenotype	Genotype	Allele 1: Cut or uncut?	Allele 2: Cut or uncut?	DNA fragment length		
				____ bp	____ bp	____ bp
Strong-taster	BB					
Weak-taster	Bb					
Non-taster	bb					

Science Note 5.2

GEL ELECTROPHORESIS OF TAS2R38

In Chapter 3, you used PCR to amplify a portion of your TAS2R38 gene. PCR provided you with millions of copies of a fragment of your TAS2R38 gene, so you have sufficient DNA to allow you to perform further experiments. First, you will use a restriction enzyme to cut the fragment you amplified, which will allow you to distinguish between TAS2R38 genotypes. You will then use gel electrophoresis to see whether or not your DNA was cut by the restriction enzyme, which will tell you what your tasting genotype is.

While you are using gel electrophoresis to determine your TAS2R38 genotype, you can also use it as a quality-control measure for your PCR sample before you perform a more expensive procedure. Scientists and researchers use gel electrophoresis to ensure that their PCR procedure was successful in amplifying the correct fragment—by checking that the fragment is present and of the correct size. It is wise to ensure that a sample has been amplified correctly and, at a minimum, appears to be the correct size before proceeding to more time-consuming or expensive techniques.

An agarose gel is a porous matrix, and smaller DNA fragments move through the pores more easily, thus traveling faster and moving farther than larger fragments. As a sample progresses through the gel, the fragments sort into distinct bands based on their sizes.

MATERIALS

Part A: Restriction Digest

FOR EACH STUDENT:

- PCR reaction (PTC MM) from Chapter 3
- 1 pair of gloves

FOR EACH TEAM:

- 1 fine-point permanent, waterproof marker
- 1 P-10 or P-20 micropipette
- Micropipette tips
- Ice and ice bucket
- 1 pink 0.2-mL or 0.5-mL PCR tube containing HaeIII restriction enzyme

FOR THE CLASS:

- Microcentrifuge
- Thermocycler (PCR machine), heat block, or water bath

Part B: Gel Electrophoresis

FOR EACH STUDENT:

- 1 pair of gloves

FOR EACH TEAM:

- 1 electrophoresis chamber and power supply
- Gel running buffer
- 1 P-10 or P-20 micropipette
- Micropipette tips
- 6 µL 100 bp ladder (M)
- 1 transilluminator
- 1 lane of a 2% gel

PROCEDURE

Part A: Restriction Digest

Step	Notes
1. Obtain your PCR reaction (PTC MM) from your teacher and thaw contents on ice.	
2. Put on gloves.	
3. Centrifuge PTC MM once fully thawed to pool the liquid to the bottom of the tube.	
4. Using a micropipette, add 2 µL of HaeIII restriction enzyme to your PTC MM tube and add "RE" to the lid to indicate that HaeIII has been added.	*You will digest your entire sample.*
5. Gently pipette up and down to ensure that the enzyme mixes with your sample.	
6. Centrifuge your sample to pool the reagents to the bottom of the tube.	
7. Place your tube in the thermocycler. Once all of your team's tubes are in the thermocycler, set the thermocycler to 37°C for 5 minutes.	
8. Place the reaction on ice until you are ready to load samples into a gel well. Optional stop point: store your digest reaction at –20°C until the gel electrophoresis lab. Thaw on ice and centrifuge the reaction tube before loading into a gel well.	*The digestion step is performed at 37°C because that is the optimal temperature for HaeIII enzyme activity. This is now your restriction enzyme digest reaction.*

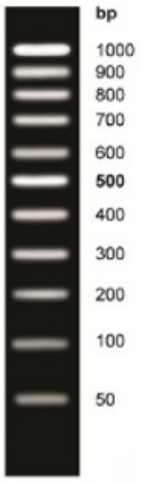
Part B: Gel Electrophoresis

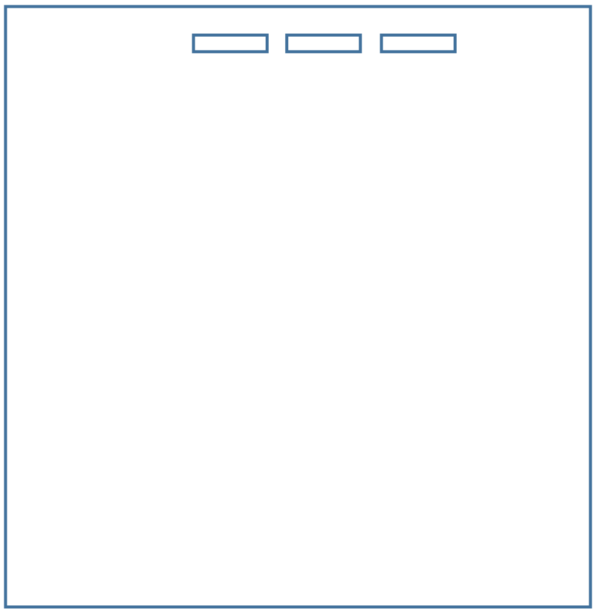
Step	Notes
1. Ensure that your gel apparatus is set up properly. The gel should be positioned so that the wells are toward the anode (–). There should also be enough buffer in the running chamber to completely submerge the gel.	
2. Your teacher will assign you one lane into which to load your sample.	*Record your lane number.*
3. Using a new pipette tip, load 10–15 µL of your restriction enzyme digest reaction (RE) into your assigned lane.	

Step	Notes
4. Gently depress the pipette button to the first stop to slowly expel the sample. Keep the pipette button depressed at the first stop when removing the tip from the well, and release the pipette button only after the pipette tip is in the air.	
5. If your teacher hasn't already done so, load 6 µL of the 100 bp ladder (M) into one outside well per row of samples.	*Each gel needs only **one** ladder. It may be prudent to load the ladder into different lanes to differentiate between different gels.*
6. Run the gel (your teacher will instruct you on voltage and time). After the allotted time, stop the gel and notify your teacher.	

You may not be able to run the full gel. If not, your teacher will turn off the voltage and save your gel for the next class.

7. Place your gel on the transilluminator, and observe the DNA bands in your sample with the blue light of the transilluminator.	*Your gels have been prepared with GelGreen or GelRed dyes that attach to DNA and fluoresce under blue light.* *Do not view your gel on the transilluminator without the amber filter in place when the blue light is on.*
8. Draw the bands that you find on the template below. Compare this to the predictions you made in the class discussion after Part 1 of Chapter 5, and discuss with your team.	





Science Note 5.3

GEL ELECTROPHORESIS AND DISEASE DETECTION

You just explored how you could use gel electrophoresis to determine whether you are a PTC-taster. The restriction enzyme selectively cut the DNA fragment when certain target sites were present.

In this homework, you will consider whether gel electrophoresis can be used to help detect genes that affect whether a person has sickle cell anemia.

MATERIALS

- Computer, Chromebook, or tablet with internet access
- HBB.txt file

PROCEDURE

1. To learn more about sickle cell disease, watch the video *Sickle Cell Anemia: A Patient's Journey* by the American Society of Hematology.
2. Go to SNPedia and type "sickle cell anemia" into the Search SNPedia box.
3. Read about the disease. Below, note the identifier for the common SNP that causes this disease.

4. Click on the hyperlink for that SNP. Make note of the various possible genotypes and their effects in the table below.

Genotype	Summary of their effects

5. Download the sequence of the hemoglobin gene (HBB) that your teacher gave you access to. Open the sequence in software that allows text files to be read, and copy the sequence.
6. Go to the Sequence Extractor website, and paste the sequence in the text area. Delete the text in the primers box, and click "Submit."
7. The sickle cell disease SNP is located at position 2992. Scroll down in the Sequence Extractor output until you find position 2992. Look for any restriction enzymes within a few bases of that position. (Remember to look above the strand.) Hover over the restriction enzyme to see its restriction site. Make note of any possible restriction enzymes below.

Restriction Enzyme	Restriction Site

QUESTIONS

1. Look at the sequence of nucleotides surrounding the SNP and consider which one(s) will allow you to distinguish between the bases that you identified using SNPedia. Which of these restriction enzymes would allow you to distinguish between the base at the SNP that causes a person to have sickle cell disease and a base that does not?

2. Scroll down to the bottom and find the table that shows all the sites that match that restriction enzyme within the HBB gene. Is there more than one site in the gene that matches this restriction enzyme? If so, what fragment of the gene would make sense to cut in order to make it easy to distinguish between people with sickle cell disease and people without?

3. Why might it be important to be able to use gel electrophoresis rather than sequencing technology to determine whether someone has a disease-causing SNP?

Science Note 6.1

PHARMACOGENOMICS AND OPIOIDS

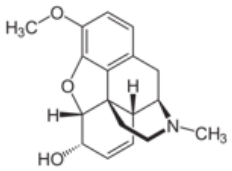
Dr. Russ Altman is a Stanford-based physician studying ***pharmacogenomics***, the relationship between genetics and pharmacology—or, in other words, how DNA influences how patients respond to medications. In the TEDx Talk *Personalized Prescriptions*, he describes how patients can respond quite differently to a very common pain relief medication: acetaminophen with codeine (which is an opiate). After watching a segment of Dr. Altman's TEDx Talk, work with your team to answer the questions below.

QUESTIONS

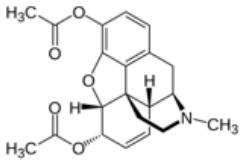
1. According to Dr. Altman, what does the liver enzyme CYP2D6 do to codeine after it enters the body?

2. Minute differences in genetics can greatly impact medication metabolism. Describe how a patient with a gene that codes for a very active form of the CYP2D6 enzyme (an "ultra-rapid metabolizer") might respond to codeine, and how a patient with a less active form of CYP2D6 (a "slow metabolizer") might respond. Which patient do you believe is more likely to become addicted to codeine?

3. We know that over-prescribing of opioids has been a factor in the opioid overdose crisis. Recent research indicates that 21–29% of patients who are prescribed opioids eventually misuse them. How might this new information about the differences in how people metabolize opioids have affected the opioid crisis, had aware of it sooner?



Codeine
$C_{18}H_{21}NO_3$



Heroin
$C_{21}H_{23}NO_5$

RESOURCE: See *Opioid Overdose Crisis* on the National Institute of Drug Abuse website if you would like to learn more.

4. Think back to the case of Sarah Smith, the patient who seemed to need more pain medication than Dr. Washington anticipated. Based on what you have learned, how would you answer these questions now?

   a. In Dr. Washington's position, what would you do? Why?

   b. What questions do you think Dr. Washington should ask Sarah?

   c. It seems that Sarah is in more pain than Dr. Washington expected. Do you think that you feel pain differently than other people? Do some people have lower pain thresholds than others? Why do you think that could be?

   d. Historically, the vast majority of medical research has been conducted on patients who are wealthy, male, and Caucasian. Based on Sarah's experience in the case study, why do you think this might be problematic in terms of evaluating the effectiveness of a particular drug?

e. In what ways might the people on whom medical research is normally conducted be different from Sarah, and how might that affect what the doctor might know about Sarah and how she should be treated?

f. How have your answers changed since you first answered these questions, if at all?

5. If genetics can significantly impact the efficacy of certain medications, do you believe that every patient should be required to have genetic testing? What might the pros and cons be of such a policy?

EXTENSION

As you have learned, opioids are not the only medications that are impacted by genetics. Azathioprine, carbamazepine, dextromethorphan, efavirenz, and suxamethonium chloride are all therapeutic drugs whose efficacy can vary dramatically based on a few tiny genetic differences. Do a Web search to determine what each of these medications treats and how the metabolism of each can vary due to genetics.