

**AMGEN®** Biotech Experience

Scientific Discovery for the Classroom

**Classroom-based**

# **Exploring Precision Medicine**



**Student Science Notes**

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Science Note 1.1

# 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?
- Make a list of these traits in the first column, below.
  - Decide whether each trait is controlled by genetics only, by the environment only, or by both, and put a check in the appropriate column.
  - 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 1.2

# 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. After 72 hours, however, Sarah calls Dr. Washington to complain that her pain relievers don't seem to be working as 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 that might help.

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

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.





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

- Control taste paper
- PTC taste paper
- 1 cup of water
- 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.

7. As your teacher calculates the class totals, record them in your table. Calculate the percentage of students with each phenotype.

Name	Strong-taster	Weak-taster	Non-Taster
Remainder of class (number)			
<b>Total</b>			
<b>Percentage</b>			

8. Make a bar chart showing the number of students with each phenotype below.

**OUR CLASS'S TASTER STATUS BAR CHART**









## Science Note 3.1

**COPY THAT DNA! HANDS ON**

You are going to model the process of PCR in this activity using pop beads—beads that pop together to form a chain. PCR amplifies (copies) fragments of DNA (or even the entire genome) so that there are millions of copies of the fragment, allowing scientists to take a very small sample of DNA and amplify it to be a large enough amount to study in detail.

PCR (and subsequent sequencing) is also used to diagnose inherited genetic diseases (such as Huntington’s disease), to explore a patient’s risk for developing a disease (such as certain types of cancer), and to help determine the best medications to use for that patient. Amplifying a small segment of DNA rather than the entire genome allows researchers and medical professionals to more easily examine and analyze it.

**MATERIALS**

- 1 bag black pop beads
- 1 bag red pop beads
- 1 bag blue pop beads
- 1 bag green pop beads

**PROCEDURE**

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

1. Create the strand of DNA shown on the next page of this science note using the key above. This will be your “template” DNA strand—the section of DNA that we are interested in analyzing.
2. Create the complementary strand of DNA and line up the two strands.

Your forward primer is:



3. Using pop beads, create six copies of your forward primer.

Your reverse primer is:



- Using pop beads, create six copies of your reverse primer.
- Using your primers, make the products of your first cycle of PCR. One forward primer should “bind” to one strand of the template DNA. The reverse primer should “bind” to the other strand of template DNA. Extend the two primers to the end of the strands.
- Using the strands you just created and the original template DNA, make four new strands (the reverse primer should bind to a portion of the strand created when you used the forward primer and vice versa). You should now have six strands of DNA.
- Complete one more cycle of PCR, and create eight new strands of DNA.

## QUESTIONS

- How many strands of DNA did you end up with?
- How many strands of the desired length did you end up with?
- How many strands of the desired length will you have if you complete another cycle?

## TEMPLATE DNA



## Science Note 3.2

# MAPPING OUR GENES

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.

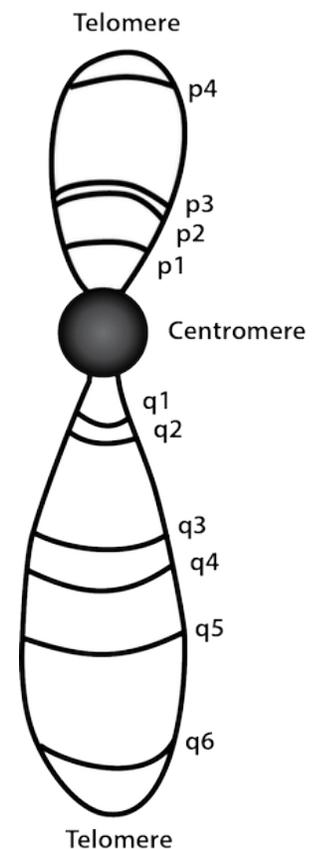
## PROCEDURE

1. 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.

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.

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:

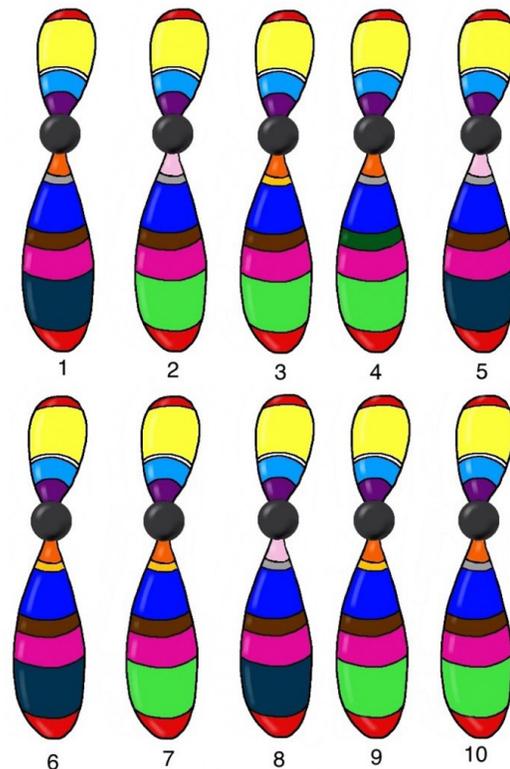
- A chance of eliminating the tendency to sneeze uncontrollably when angry
- No chance of eliminating the tendency to speak in rhymes

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:

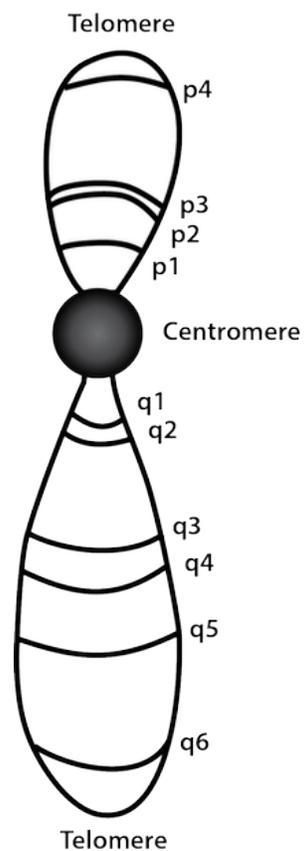
- Only subjects 2, 5, and 8 are terribly afraid of carrots.
- Only subjects 3, 6, 7, and 9 have abnormally deep belly buttons.
- All subjects have very sweaty eyelids.



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

**GENE LOCATION**

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

- 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.

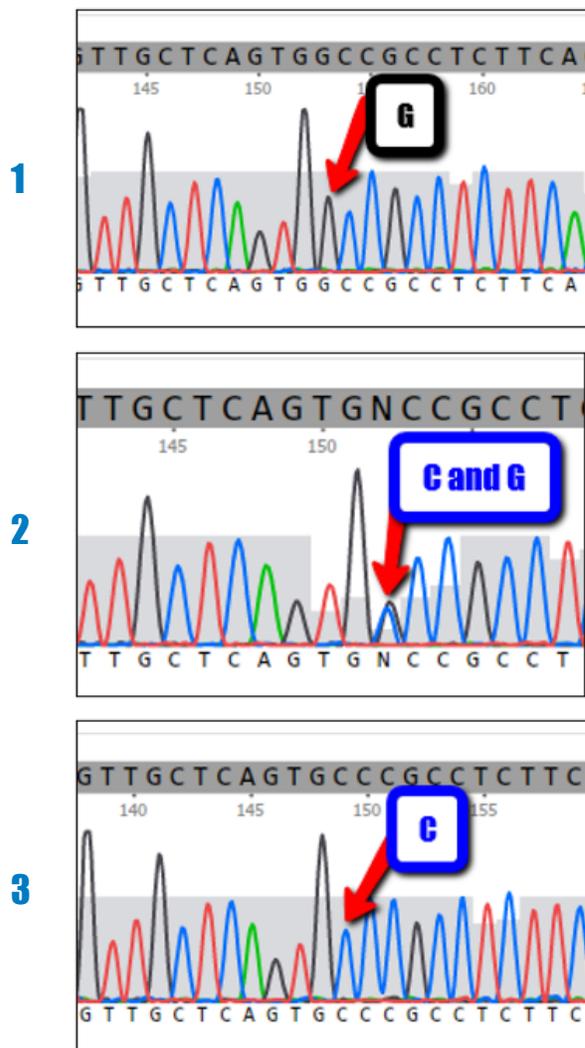
### EXAMPLE CHROMATOGRAM



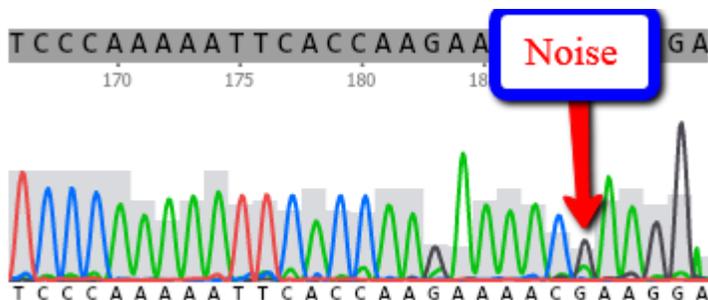
- 4. In a “good” chromatogram, the peaks are spaced evenly (for example, the peaks after #70 highlighted in green). Peak heights may vary, which is normal. Usually, the first 20–40 base pairs of a sequence are not very good (as shown below in yellow), and you can see many “N” placeholders at positions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



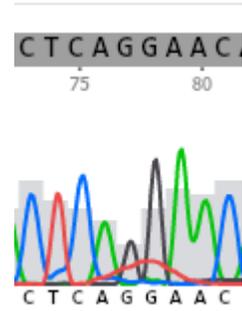
5. If a person is heterozygous for a particular base pair, you should see two peaks at that location. The images below represent sequences that are (1) homozygous G, (2) heterozygous G and C, and (3) homozygous C.



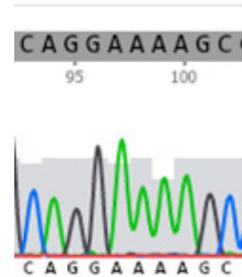
6. There is always some background “noise” from the other trace strands. The computer program can usually determine what is “noise” from what is a heterozygous base pair at a position. For the purpose of this chromatogram analysis, peaks that are less than half the height of the main peak at a nucleotide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



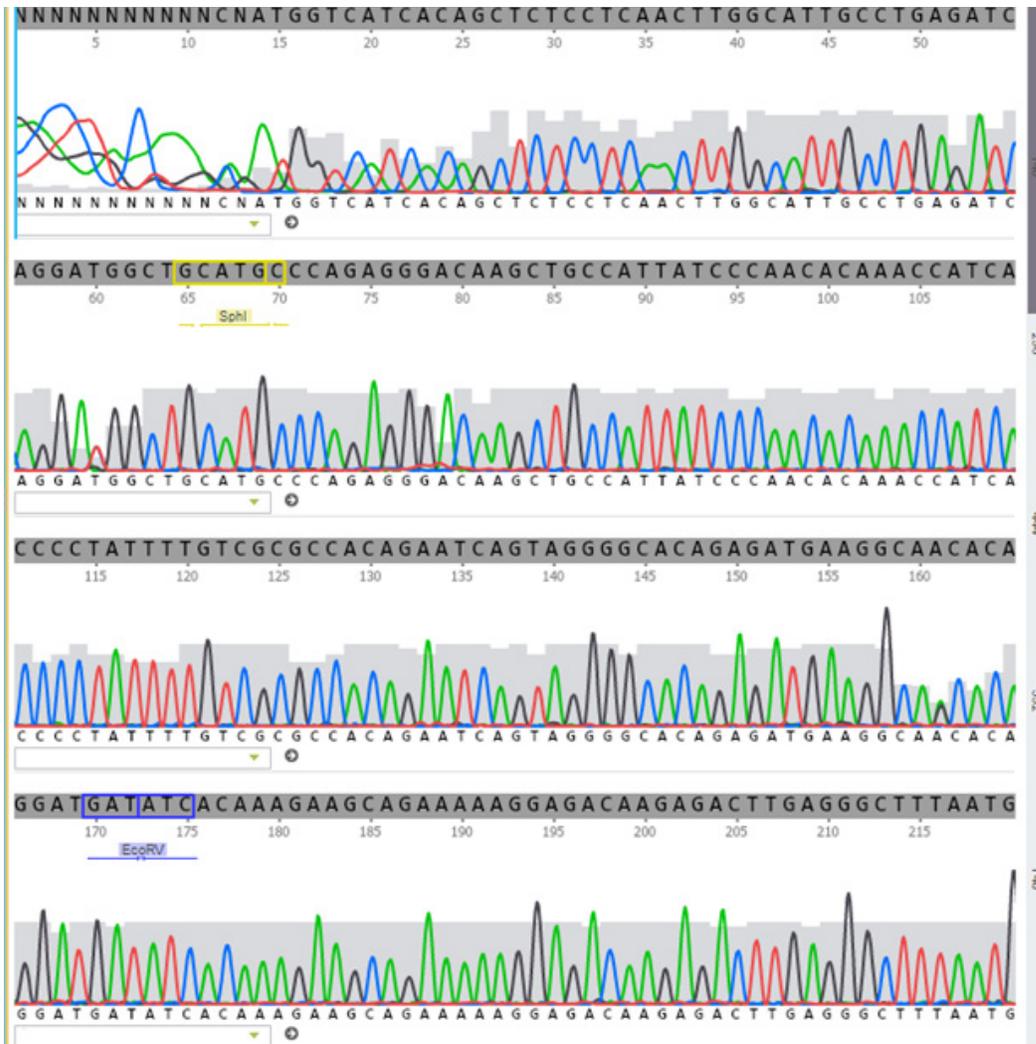
7. Sometimes there are single broad peaks that span several nucleotide positions. These can also be considered "noise."



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 on the following page and answer the questions.



### QUESTIONS

1. Where does the sequence start to look "good"? (**Hint:** Consider the size of the gray area behind the peaks.)
2. What base pair 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

What is the sequence of the TAS2R38 gene? 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

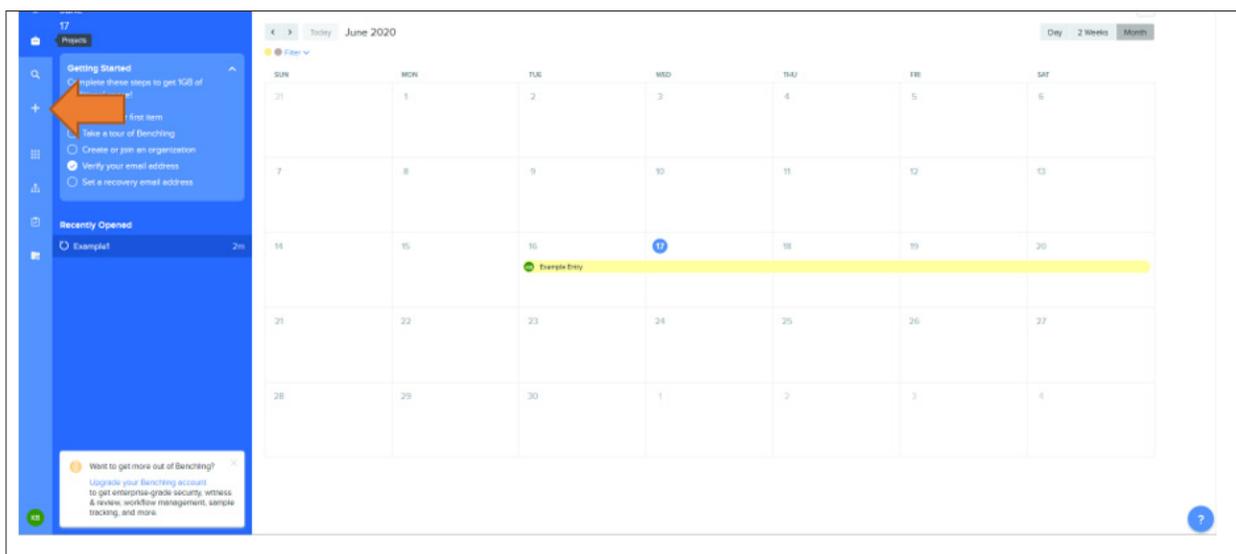
## PROCEDURE

### 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.



- The “Create project” window will open.

Name

Owner

Description

Manage collaborators

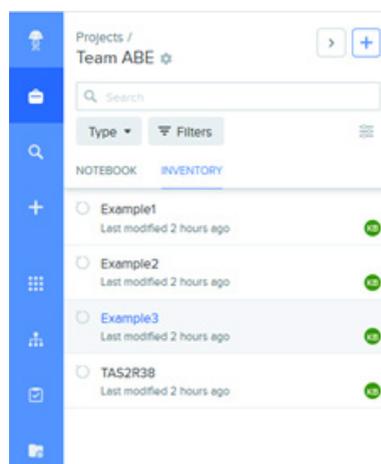
Add collaborator

Collaborators

User / Organization	Permissions	Auditor
	ADMIN	<input checked="" type="checkbox"/>

Create project

- In the “Name” field, type your team’s name.
- In the “Manage collaborators” field, add each of your teammate’s names. In the Permissions dropdown, change each person’s permissions to “ADMIN.”
- Click “Create project” to finish setting up.
- Navigate back to your Project main list by clicking “Projects” above your team name.
- Import the TAS2R38 reference sequence.seq file and then the example sequence files that your teacher provided. You will eventually be comparing these sequences to the reference sequence and looking for differences. Make sure that you note whether the sample came from a strong-taster, a weak-taster, or a non-taster.
- Open one of the example sequence files (in the Inventory list on the left side of the screen) by clicking on it.



- 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.



- 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 a particular sequence and the sequence of the TAS2R38 gene. The software allows you to easily compare multiple sequences and spot differences between them.

- Click on the TAS2R38 file in the Inventory list.
- Click on the "Alignments" icon (☰) on the right side of the screen. In the "Choose File(s)" field, type the name of one of the example sequence files that your teacher provided. When the name of the file appears, click on it.
- Type the name of the second example sequence file in the "Choose File(s)" field.
- In the menu bar, click the green "Create Alignment" button.
- 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.
- 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.)



19. Analyze the difference: Is it because of poor data at the start or end of your trace file? 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 nucleotides at those positions in the **Differences** table below. Which nucleotide is associated with samples from strong-tasters? weak-tasters? non-tasters?

**DIFFERENCES**

Position of the Difference in the TAS2R38 Gene	Nucleotide at That Position		
	Strong-taster	Weak-taster	Non-taster
76	G		T

## Science Note 4.3

**AN ENCYCLOPEDIA OF SNPs**

Between you and the person next to you, you'll likely find a different nucleotide in your DNA once every 1,200 to 1,500 bases. Most of this variation comes in the form of single nucleotide polymorphisms, or SNPs (pronounced "snips"). As their name implies, these variations occur in just one nucleotide. In SNPs, any nucleotide might be substituted—in other words, an A might become a C, a G, or a T.

While most SNPs occur in the non-coding portions of our DNA, some do produce phenotypic differences. Scientists used large datasets of human genomic data to map our genes and identify the location of these SNPs. The information that scientists have found is stored in a Wiki called SNPedia.

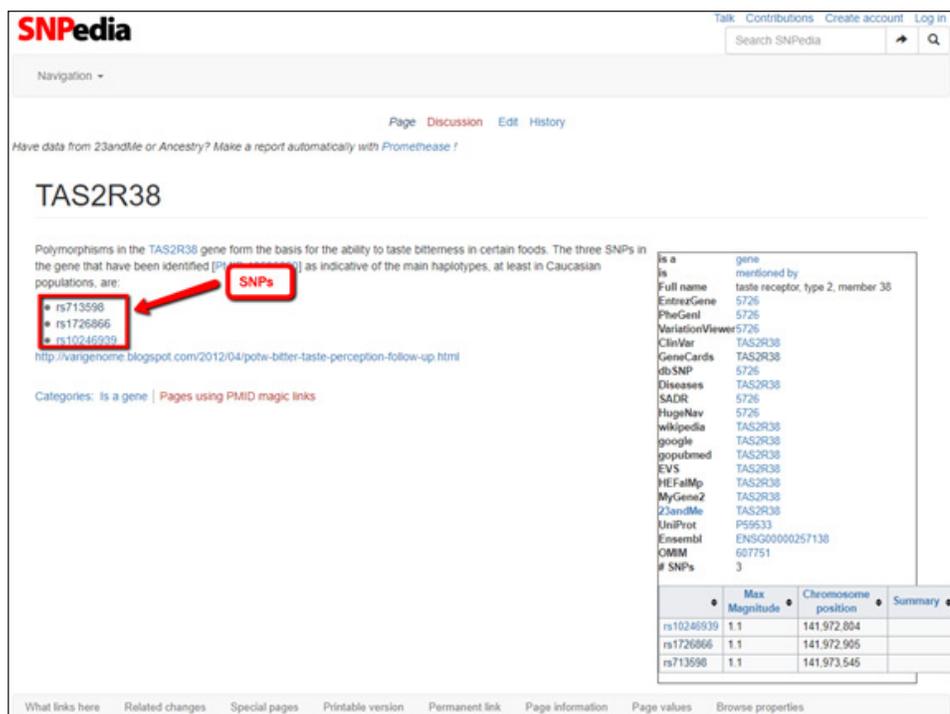
Remember that the ability to taste PTC and other bitter tastes is dependent on SNPs in a gene called TAS2R38, which is found on chromosome 7 and encodes for the TAS2R38 protein—the bitter-taste receptor on the tongue, which triggers a signal to the brain when it binds to PTC. How much of that signal is sent to the brain is dependent on these tiny genetic variants—SNPs—within the TAS2R38 DNA sequence. Similarly, these tiny genetic variations can influence how easily a person metabolizes a particular medication compared with another person.

You'll use three online tools—SNPedia, dbSNP, and the Ensembl project—to find out more about the variations in TAS2R38 and how these differences impact our ability to taste bitter substances. You will also try to determine which SNPs you found in the previous activity.

**PROCEDURE****SNPedia**

1. Watch the [SNPedia tutorial](#).
2. Go to [SNPedia](#).
3. In the "Search SNPedia" box, type "TAS2R38" and hit Enter.

4. The screen will display the SNPs that have been cataloged for the TAS2R38 gene.



- Write the identifiers for the SNPs in the SNP Information Table on the last page of this reproducible master. Identifiers should begin with the letters "rs."
- Click on each identifier to get more information about that SNP, and enter the information you find in the table. Under "Geno," you will find the possible nucleotides.

## rs713598

rs713598 is one of three SNPs that form the main haplotypes behind the ability to perceive as bitter the *taste* of the compound phenylthiocarbamide (PTC) and similar molecules in foods (like cabbage and raw broccoli) or drinks (like *coffee* and dark beers).

Due to it's minus *orientation* and nearly 50/50 split of allele frequency it is easily confused.

In the orientation shown in dbSNP and used in SNPedia

- rs713598(G) is the "non-tasting" allele
- rs713598(C) is the "tasting" allele

In the GRCh37 orientation currently (2017) reported by 23andMe

- rs713598(C) is the "non-tasting" allele
- rs713598(G) is the "tasting" allele

Tasting is dominant, so heterozygotes are able to taste bitterness.

If you are a "taster", you're also likely to carry at least one rs10246939(C) and one rs1726866(C) allele since, along with rs713598(G), these three SNPs form the most common tasting haplotype. If you lack these alleles, you're quite likely (~80%) to be a non-taster of bitterness, meaning that foods that may taste bitter to others taste far less bitter to you.[PMID 12595690]

Geno	Mag	Summary
(C;C)	1.1	Can taste bitter.
(C;G)	1.1	Can taste bitter.
(G;G)	1.1	Possibly unable to taste bitter in some foods.

dbSNP

- Watch the [dbSNP tutorial](#).
- Go to [dbSNP](#).
- Look in the box under the heading "Gene: TAS2R38, taste 2 receptor member 38 (minus strand)," and look at the first entry under the word Change. Note the number after the "c," which tells you the position of the SNP in the TAS2R38 gene and the two possible alleles (T>C). Record the SNP's

position in the SNP Information Table.

dbSNP Short Genetic Variations

Reference SNP (rs) Report **rs1726866** ALPHA Current Build 151 Released July 17, 2018

Organism: Homo sapiens  
 Position: chr7:141972905 (GRCh38.p7)  
 Alleles: G>A  
 Variation Type: SNV Single Nucleotide Variation  
 Frequency: G=0.49845 (122528/245820, GnomAD) G=0.49254 (59719/121246, ExAC) A=0.4874 (15045/30870, GnomAD) (+ 3 more)

Genomic Placements

Sequence name	Change
GRCh37.p13 chr 7	NC_000007.13:g.141672705G>A
GRCh37.p13 chr 7 fix patch HG7_PATCH	NW_003571040.1:g.114856G>A
GRCh38.p7 chr 7	NC_000007.14:g.141972905G>A
TAS2R38 RefSeqGene	NG_016141.1:g.5869T>C

Gene: **TAS2R38, taste 2 receptor member 38 (minus strand)**

Molecule type	Change	Amino acid[Codon]	SO Term
TAS2R38 transcript	NM_176817.4:c.785T>C	V [GTT]> A [GCT]	Coding Sequence Variant
taste receptor type 2 member 38	NP_789787.4:p.Val<..	V [Val]> A [Ala]	Missense Variant

- If you found a difference at that position when you investigated the example sequences using Benchling, circle the SNP. (Refer to the **Differences** table on **Science Note 4.2**, if needed.)
- Use the information here to determine what amino acids are possible with this SNP, and record them in the **SNP Information Table**.

The Ensembl Project

- Watch the [Ensembl tutorial](#).
- Go to the [Ensembl project website](#).
- Search for each SNP by its identifier (rs713598, rs1726866, and rs10246939). Click on the hyperlink in each search result.
- Look for the ancestral allele and the MAF. The **ancestral allele** is the allele that occurs at this locus in closely related species; it is thought to reflect the allele present at the time of speciation. The **MAF (minor allele frequency)** is the frequency of the second most frequent allele in the 1000 Genomes Phase 3 combined population. Add the information you find to the SNP Information Table.

According to its website, the goal of the Ensembl Project is to "automatically annotate the genome, integrate this annotation with other available biological data and make all this publicly available via the web." The website offers a wealth of information about the SNPs in the TAS2R38 gene.

You can learn more about allele frequency in the Khan Academy video [Allele Frequency](#).

## SNP INFORMATION TABLE

From SNPedia				From dbSNP			From the Ensembl project		
Identifier	Chromosome	Orientation	Possible nucleotides	Summary	Position in TAS2R38	Codon	Amino acids	Ancestral allele	MAF
Annotation used to identify the SNP.	Chromosome on which the gene is located.	The same SNP can be defined as being on the forward (plus) or the reverse (minus) strand. In terms of the nucleotides for that SNP, the pairing of A with T and C with G in the DNA double helix means that an A on the plus strand by definition is a T on the minus strand, and vice versa.	Nucleotide bases that appear as polymorphisms: If the orientation of the SNP is minus, then note the complementary bases here.	Phenotypic effect of the SNP.	Where is the SNP located in the TAS2R38 gene?		Amino acid coded for by each SNP.	The allele that occurs at this locus in closely related species and is thought to reflect the allele present at the time of speciation.	Frequency of the second most frequent allele in the 1000 Genomes Phase 3 combined population.
ex123456	75	plus	G T	G;G Purple hair G;T Blue hair T;T Blue hair	199	GTT GAT	Val ASP	T	025 (G)

## Science Note 5.1

## WHICH RESTRICTION ENZYME SHOULD WE USE?

Sending out your genes for sequencing can provide you with a lot of information. However, you might want to learn more about your genome without having to put it in an envelope and send it to another company. Restriction enzyme digestion allows you to do just that: use enzymes that specifically target and cut 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'll need to pick a restriction enzyme that cuts the DNA of one type and doesn't cut the DNA of the other.

### 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 (the sequence of the TAS2R38 gene), and paste it into the box:

```

1 ATGTTGACTC TAACTCGCAT CCGCACTGTG TCCTATGAAG TCAGGAGTAC ATTTCTGTTC
61 ATTTTCAGTCC TGGAGTTTGC AGTGGGGTTT CTGACCAATG CCTTCGTTTT CTTGGTGAAT
121 TTTTGGGATG TAGTGAAGAG GCAGGCCCTG AGCAACAGTG ATTGTGTGCT GCTGTGTCTC
181 AGCATCAGCC GGCTTTTCCT GCATGGACTG CTGTTTCTGA GTGCTATCCA GCTTACCCAC
241 TTCCAGAAGT TGAGTGAACC ACTGAACCAC AGCTACCAAG CCATCATCAT GCTATGGATG
301 ATTGCAAACC AAGCCAACCT CTGGCTTGCT GCCTGCCTCA GCCTGCTTTA CTGCTCCAAG
361 CTCATCCGTT TCTCTCACAC CTTTCTGATC TGCTTGGCAA GCTGGGTCTC CAGGAAGATC
421 TCCCAGATGC TCCTGGGTAT TATTCTTTGC TCCTGCATCT GCACTGTCTT CTGTGTTTGG
481 TGCTTTTTTA GCAGACCTCA CTTTACAGTC ACAACTGTGC TATTCATGAA TAACAATACA
541 AGGCTCAACT GGCAGAATAA AGATCTCAAT TTATTTTATT CTTTCTCTT CTGCTATCTG
601 TGGTCTGTGC CTCCTTTTCT ATTGTTTCTG GTTTCTTCTG GGATGCTGAC TGTCTCCCTG
661 GGAAGGCACA TGAGGACAAT GAAGGTCTAT ACCAGAACT CTCGTGACCC CAGCCTGGAG
721 GCCCACATTA AAGCCCTCAA GTCTCTTGTC TCCTTTTTTCT 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

```

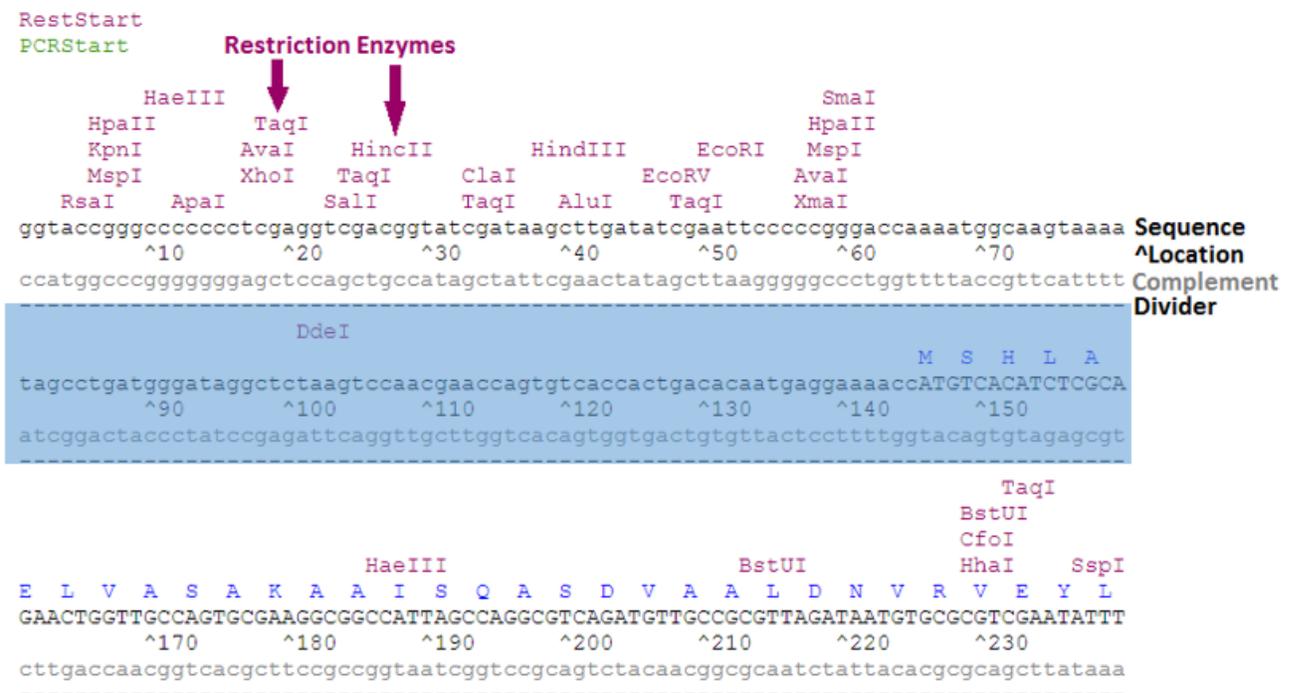
- Select all the text in the box that says, "If there are primers you would like shown on the map, enter each primer as follows: the sequence of the primer, a blank space, and the name of the primer. Use commas to separate multiple primer entries," and delete it. Copy the following (the sequences of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

CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGG forward primer  
 TGAGGCAGGCAGCAAGCCAGAGGTTGGCTTGGTTTGAATCATC reverse primer

- 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 dotted lines.

**QUESTIONS**



- What do you think the blue letters above the sequences represent?
- In what color is the reverse primer shown?
- In what color is the forward primer shown?

4. Roll over the primers to answer the following:
  - o At what position does the forward primer start?
  - o Does the rollover give you any other information about the forward primer?
  - o At what position does the reverse primer start?
  - o Does the rollover give you any other information about the reverse primer?
  - o How long is the fragment that you produced using these two primers? (**HINT: Click on the reverse 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.
  - o Highlight the positions of the primers on the sequence, using two different-colored highlighters.
  - o Note anything else that you've learned about the fragment.
  
  - o Circle the SNP, which is at position 145.

TAS2R38 SEQUENCE EXTRACTOR RESULTS

RestStart

PCRStart

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  
 ATGTTGACTCTAACTCGCATCCGCACTGTGTCCTATGAAGTCAGGAGTACATTTCTGTTTCATTTTCAGTCCTGGAGTTTGC  
 ^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 Q A L S N S D  
 AGTGGGTTTCTGACCAATGCCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCAGGCCCTGAGCAACAGTG  
 ^90 ^100 ^110 ^120 ^130 ^140 ^150  
 TCACCCCAAAGACTGGTTACGGAAGCAAAGAACCCTTAAAAACCCTACATCACTTCTCCGTCGGGGACTCGTTGTAC  
 -----  
 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  
 ATTGTGTGCTGCTGTGTCTCAGCATCAGCCGGCTTTTTCTGCATGGACTGCTGTTTCCTGAGTGCTATCCAGCTTACCCAC  
 ^170 ^180 ^190 ^200 ^210 ^220 ^230  
 TAACACACGACGACACAGAGTCGTAGTCGGCCGAAAAGGACGTACCTGACGACAAGGACTCACGATAGGTGCAATGGGTTG  
 -----  
 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  
 TTCCAGAAGTTGAGTGAACCACTGAACCACAGCTACCAAGCCATCATCATGCTATGGATGATTGCAAACCAAGCCAACT  
 ^250 ^260 ^270 ^280 ^290 ^300 ^310  
 AAGTCTTCAACTCACTTGGTGACTTGGTGTGATGGTTCGGTAGTAGTACGATACCTACTAACGTTTGGTTGGGTTGGA  
 -----  
 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  
 CTGGCTTGCTGCCTGCCTCAGCCTGCTTTACTGCTCCAAGCTCATCCGTTTCTCTCACACCTTCTGATCTGCTTGGCAA  
 ^330 ^340 ^350 ^360 ^370 ^380 ^390  
 GACCGAACGACGGACGGAGTCGGACGAAATGACGAGGTTTCGAGTAGGCAAAGAGAGTGTGGAAGGACTAGACGAACCGTT  
 -----  
 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  
 GCTGGGTCTCCAGGAAGATCTCCCAGATGCTCCTGGGTATTATTCTTTGCTCCTGCATCTGCACTGTCCCTCTGTGTTTGG  
 ^410 ^420 ^430 ^440 ^450 ^460 ^470  
 CGACCCAGAGGTCTTCTAGAGGTTCTACGAGGACCATAATAAGAAACGAGGACGTAGACGTGACAGGAGACACAAACC  
 -----  
 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  
 TGCTTTTTTAGCAGACCTCACTTCACAGTCACAACCTGTGCTATTTCATGAATAACAATACAAGGCTCACTGGCAGAATAA  
 ^490 ^500 ^510 ^520 ^530 ^540 ^550  
 ACGAAAAATCGTCTGGAGTGAAGTGTGAGTGTGACACGATAAGTACTTATTGTTATGTTCCGAGTTGACCGTCTTATT  
 -----  
 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  
 AGATCTCAATTTATTTTATTCTTTCTTCTGCTATCTGTGGTCTGTGCCTCCTTTCTTATTGTTTCTGGTTTCTTCTG  
 ^570 ^580 ^590 ^600 ^610 ^620 ^630  
 TCTAGAGTTAAATAAAAATAAGGAAAGAGAAGACGATAGACACCAGACACGGAGGAAAGGATAACAAAGACCAAAGAAGAC  
 -----

```

-----
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
GGATGCTGACTGTCTCCCTGGGAAGGCACATGAGGACAATGAAGGTCTATACCAGAAACTCTCGTGACCCAGCCTGGAG
      ^650      ^660      ^670      ^680      ^690      ^700      ^710
CCTACGACTGACAGAGGGACCCTTCCGTGTACTCCTGTTACTTCCAGATATGGTCTTTGAGAGCACTGGGGTCCGGACCTC
-----
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
GCCCACATTAAGCCCTCAAGTCTCTTGTCTCCTTTTCTGCTTCTTTGTGATATCATCCTGTGTTGCCTTCATCTCTGT
      ^730      ^740      ^750      ^760      ^770      ^780      ^790
CGGGTGTAATTTCCGGGAGTTCAGAGAACAGAGGAAAAAGACGAAGAAACACTATAGTAGGACACAACCGGAAGTAGAGACA
-----
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
CGGGGATGACTAAGACACCCGCGCTGTTTTATCCCCACTACCAAACACAACCCCTATTACCGTCGAACAGGGAGACCCGTAC
-----
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
GTCGGTAGGACTAGAGTCCGTTACGGTTCAACTCCTCTCGACACTACTGGTAAGACGAGACCCGAGTCTCGTCCGGACTTC
-----
RestEnd
PCREnd

V R A D H K A D S R T L C Z
GTAAGAGCCGACCAAGGCAGATTCCCGGACACTGTGCTGA
      ^970      ^980      ^990      ^10
CATTCTCGGCTGGTGTCCGTCTAAGGGCCTGTGACACGACT
-----

```

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? 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.

<u><i>EcoRI</i></u>	<u><i>SmaI</i></u>	<u><i>AluI</i></u>	<u><i>HaeIII</i></u>
GAATTC	CCCGGG	AGCT	GGCC
CTTAAG	GGGCCC	TCGA	CCGG

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 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

- 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.
- Go to the [Sequence Extractor](#) website, and paste the sequence in the text area. Delete the text in the primers box, and click "Submit."
- 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 near 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.

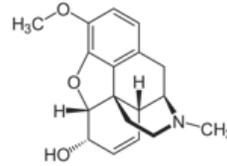
Restriction Enzyme	Restriction Site

## QUESTIONS

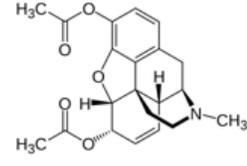
- Which of these restriction enzymes would allow you to distinguish between the base at the SNP that causes a person to have sickle cell disease and a base that does not?
- Scroll down to the bottom and find the table that shows all the sites that match that restriction enzyme within the HBB gene. Is 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?
- 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?



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<sub>18</sub>H<sub>21</sub>NO<sub>3</sub>



Heroin  
C<sub>21</sub>H<sub>23</sub>NO<sub>5</sub>

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?
- o In Dr. Washington's position, what would you do? Why?
  
  - o What questions do you think Dr. Washington should ask Sarah?
  
  - o 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?
  
  - o 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?

- o 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?
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 minute 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.