



PTC Genetics MiniLab

Student's Guide

Cat# M3003

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Laboratory Safety

1. Exercise caution when heating or melting reagents.
2. Exercise caution when working with electrical equipment.
3. Gloves and eye protection should be used whenever needed as part of good laboratory practice.
4. Always wash hands thoroughly after handling biological materials or reagents.

Objectives

To formulate a hypothesis and an experimental method to test it. To develop an understanding of electrophoresis and genetic principles. To analyze results and to deduce genotypes from given phenotype and pedigree information.

Background

In 1931, a chemist named Arthur Fox started to measure some powdered phenylthiocarbamide (PTC). Pouring hastily, Fox accidentally caused some of the chemical to blow into the surrounding air. Fox's lab mates nearby complained of the bitter taste in the air due to the chemical. Yet, Fox was perplexed- he tasted nothing. Since that day, PTC has been used to show genetic variation in tasting abilities. When people sample PTC, some people taste a strong bitterness, others taste a slightly bitter taste, and others taste nothing at all. Using genetics, we can try to understand why some people can taste this chemical and others can't (refer to Appendix A for more information on genetic inheritance).

Taste and Genetics

The sensation of taste can be categorized into five basic types: sweet, sour, salty, bitter, and umami (the taste of monosodium glutamate). These five tastes serve to classify compounds into potentially nutritive and beneficial (sweet, salty, umami) or potentially harmful or toxic (bitter, sour). The ability to taste is due to the presence of chemically sensitive, specialized taste receptor cells on the surface of the tongue and throat. When we eat something sweet, the soluble molecules in the food dissolve in saliva and binds to specific receptor proteins on the surface of the receptor cells that detect sweet taste. The stimulated receptor cells send nerve impulses to gustatory region of the brain where the sense of taste is interpreted. Different types of taste receptors are activated by different chemicals, and the nerve impulses they send to the brain are interpreted as different tastes.

Scientists have shown that the TAS2R taste receptor proteins are responsible for the human ability to taste bitter substances. These taste receptor proteins are encoded by about 30 different genes. One of the best-studied genes, the TAS2R38, codes for a G protein-coupled receptor (GPCR) which contributes to the tasting of the chemical Phenylthiocarbamide (PTC). When molecules of PTC bind to the TAS2R38 receptor protein, some people can taste the bitterness while others taste nothing at all – we call them "Tasters" and "Non-Tasters", respectively. PTC is a man-made chemical that resembles toxic alkaloids found in some poisonous plants. Although PTC is not found in nature, the ability to taste it correlates strongly with the ability to taste other bitter substances that do occur naturally, many of which are toxins.

This genetic variation in the ability to taste PTC has been of great interest to those who study genetics. The variation in PTC sensitivity is determined by two common alleles of the TAS2R38 gene: the functional allele and the mutated allele. The DNA sequence between these two alleles is only different at a single base pair. This type of polymorphism within a DNA sequence is termed "Single Nucleotide Polymorphism (SNP)". Just a single point mutation changes the DNA coding sequence, the protein produced, and the protein's function. Any person with a single functional allele can make the PTC receptor protein and therefore can taste the bitterness of PTC. Some studies have shown that when homozygous tasters (who carry two functional alleles) attempt to taste PTC, they experience a more intense bitterness than heterozygous tasters do (who only carry one functional allele). On the other hand, homozygous non-tasters (with two mutant alleles) cannot taste the bitterness of PTC at all.

In this kit, the provided DNA samples include only a short region of the TAS2R38 gene. This region of the TAS2R38 gene is 221 base pairs (bp) in length. As mentioned above, the functional allele and the mutant allele only differ by a single base pair. To distinguish between these two alleles, we could either 1) sequence the DNA fragments to find the exact nucleotide sequence, or 2) combine the techniques of restriction enzyme digestion and gel electrophoresis to reveal the difference. In our PTC kit, we will focus on the second method which is an easier and more affordable method for college and high school students. This method does not require the use of expensive equipment to analyze the DNA sequence.

Restriction Enzyme Digestion

What is a restriction enzyme? A restriction enzyme (an endonuclease) is an enzyme that can scan double-stranded DNA (dsDNA) molecule for a specific sequence of base pairs known as a restriction site and then make a cut at or near the restriction site of the dsDNA.

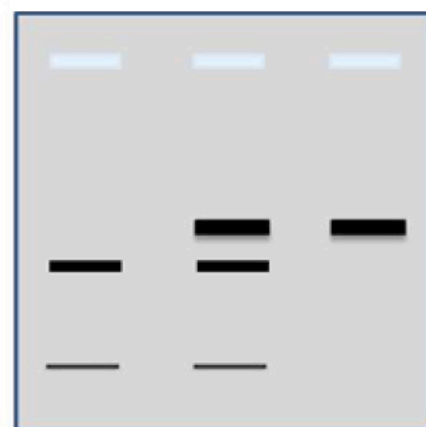
We used the restriction enzyme *HaeIII* to cut the DNA samples (the short 221 bp region of the TAS2R38 gene). *HaeIII* scans for the 4 base-long restriction site "GGCC" which is only present in the TAS2R38 functional allele. In this case, *HaeIII* cuts the dsDNA of the functional allele into two fragments (176 bp and 45 bp) but cannot cut the dsDNA of the mutant allele. After incubating the DNA samples with *HaeIII*, the cut and the uncut DNA fragments can be separated by size using the gel electrophoresis technique.

Based on the function of the restriction enzyme *HaeIII*, you know why one sample has two bands and the other has only one, however you may be wondering why the middle one has three bands. This is because heterozygotes have one copy of each allele: one that can be cut and one that cannot. The mutated allele cannot be cut, so the 221 bp fragment remains intact, but the normal allele can be cut, resulting in a 176 bp fragment and a 45 bp fragment. Since they are all present in the same sample, the final result is three fragments of distinct sizes.

The comparisons between the different types of PTC tasters and the corresponding DNA patterns on the gel are shown in the table below:

Taster Type	TAS2R38 Genotype	<i>HaeIII</i> Restriction Site	DNA Pattern on Gel
Homozygous Taster	TT (2 functional alleles)	Yes, present on both functional alleles	2 bands 176 bp - fragment of functional 45 bp - fragment of functional <i>Note: Due to the very small size of the 45 bp fragment, this band may be faint, fuzzy, or not visible in the gel</i>
Heterozygous Taster	Tt (1 functional allele, 1 mutant allele)	Yes, present on the one functional allele	3 bands 221 bp - uncut mutant 176 bp - fragment of functional 45 bp - fragment of functional
Homozygous Non-Taster	tt (2 mutant alleles)	Not present on either mutant allele	1 band 221 bp - uncut mutant

Homozygous Taster (TT) Heterozygous Taster (Tt) Homozygous Non-taster (tt)

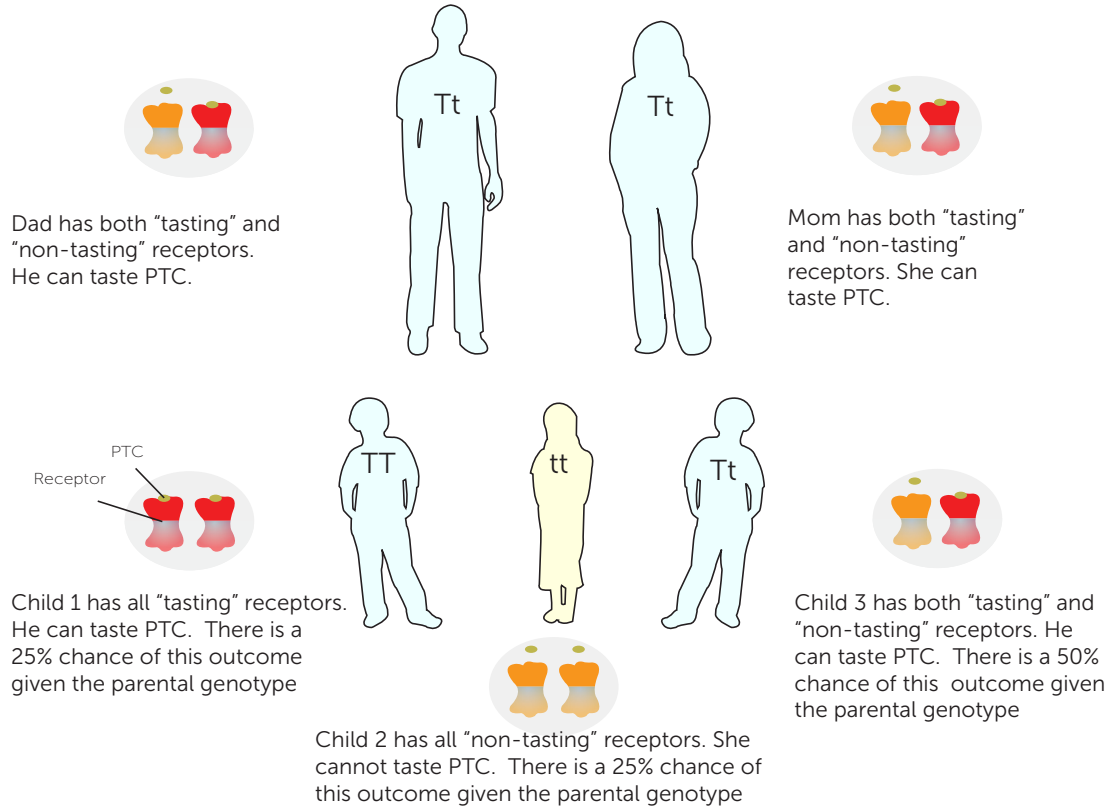


221 bp
176 bp

45 bp
Very small band, may be faint, fuzzy, or not visible in the gel

This diagram illustrates the different patterns possible after electrophoretic separation on a 2% agarose gel. As you can see, a homozygous taster will have two bands, a heterozygous taster will have three bands, and a homozygous non-taster will only have one band.

PTC sensitivity is often used as an example of a simple Mendelian inheritance (for more information refer to Appendix A). Below is an example of simple Mendelian inheritance.



Scenario

Jillian is a student at Cactus High School in Peoria. Her class learned about PTC tasting when her class learned about inherited genetic traits. As it turned out, she was not a taster. Jillian decided to get some PTC paper and have her family do the taste test, and draw a family tree based on the tasting data. Surprisingly, everyone in her family is a taster, her mother, her father, both her brothers, and even her grandparents, aunts and uncles. Jillian was quite perplexed. How is it possible that Jillian cannot taste PTC when everyone else in her family can taste?

Based on what you know about genetics come up with a hypothesis and how you could test this hypothesis?

Hypothesis

Pre-Lab Activity

1. Are you a PTC taster? If so, what does PTC taste like?
2. How many people in your class are tasters? How many people in your class are non-tasters?
3. Why do you think some people can taste the PTC and others can't?

Pre-Lab Questions

1. What is DNA and what does it do?
2. What are chromosomes and how many copies do you have in each of your cells? Who did they come from?
3. How much DNA do you share with each of your parents? How much DNA do you share with your siblings?
4. What are some characteristics or properties of DNA?

Part I: Electrophoresis

Materials

- 1 Minione[®] Casting System
- 1 MiniOne[®] Electrophoresis System
- 1 agarose GreenGel[™] cup (2%)
- 6 DNA samples
- TBE running buffer (135 mL)
- 1 micropipette (2-20 μ L) and 6 pipette tips

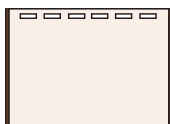
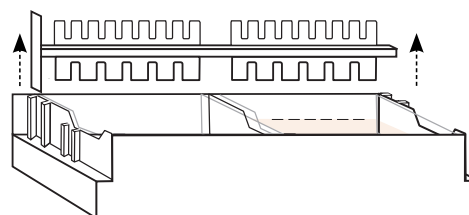
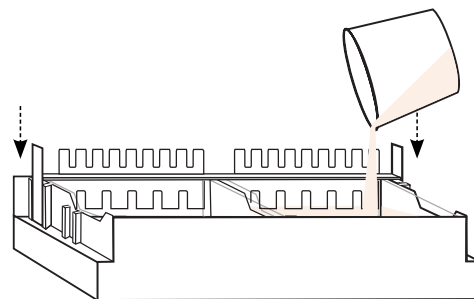
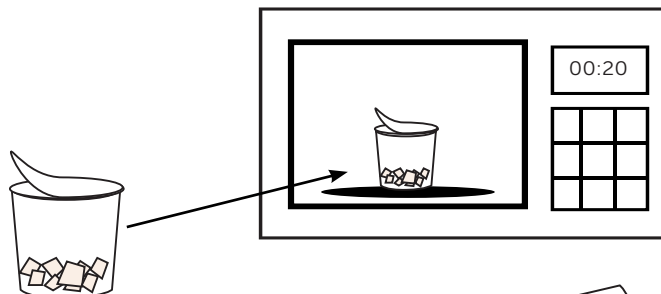
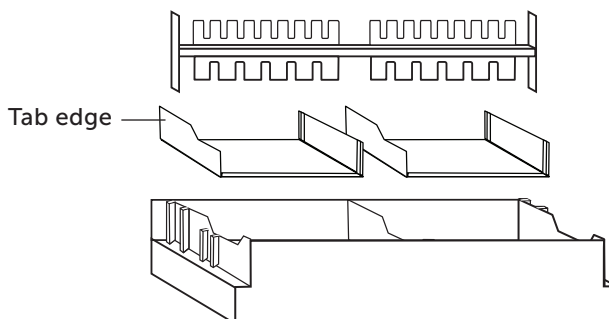
SAMPLE CHART:

Well	Sample Name	Volume
1	DNA M	10 μ L
2	DNA D	10 μ L
3	DNA J	10 μ L
4	DNA B1	10 μ L
5	DNA B2	10 μ L
6	DNA G	10 μ L

MiniOne Visual Instructions

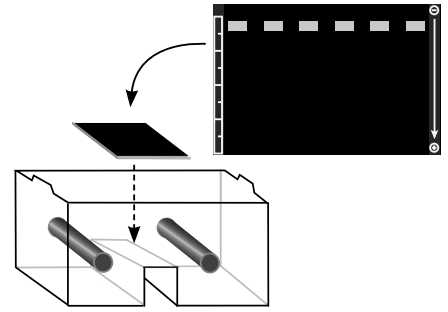
How to Cast a Gel

- Place the MiniOne[®] Casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 6-well side facing down.
- Partially peel the film off a GreenGel[™] cup and microwave for 20 seconds. Allow to cool for 15 seconds. **DO NOT microwave more than 5 gel cups at a time.**
- One gel cup is for making one agarose gel!** Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 minutes or until opaque. **DO NOT disturb the gel until time is up.**
- Carefully remove comb when gel is ready. Remove gel tray with solidified gel from Casting Stand and wipe off any excess agarose from the bottom of the tray.

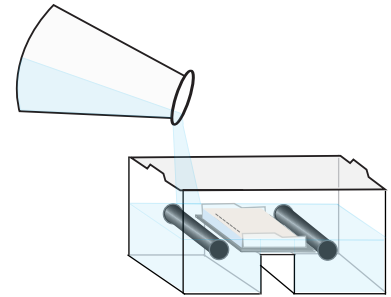


How to Load a Gel

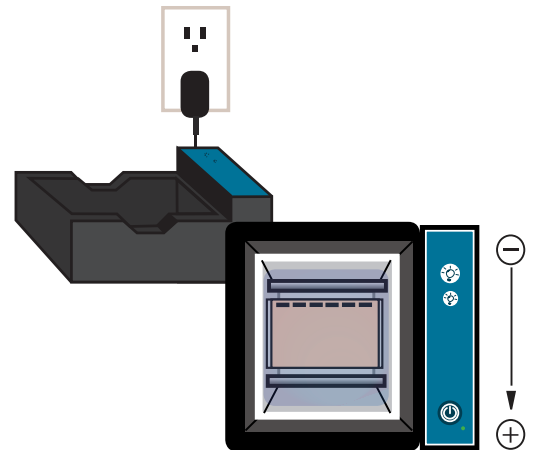
1. Ensure the black viewing platform is in the tank if it is not already installed and put the gel (along with the gel tray) into the tank. **Make sure the wells are aligned with the marks on the platform on the negative end.**




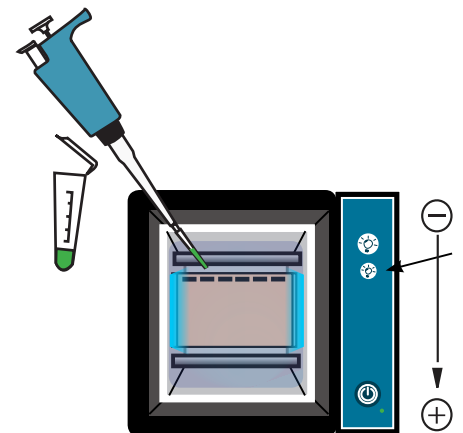
2. Measure 135 mL of TBE running buffer and pour into **one** side of the tank to push out the air, creating a nice even background without air bubbles or air trapped for imaging later.




3. Plug the power supply into the wall. Place the tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.



4. Turn the low intensity blue light on by pressing the  button on the carriage to help visualize the wells when loading. Load 10 μ L per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**

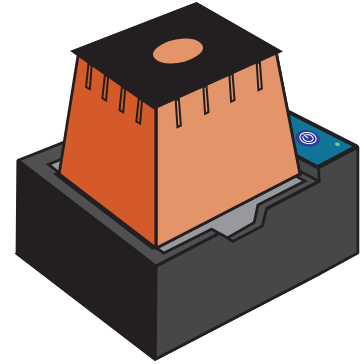



Run, Visualize and Capture Image

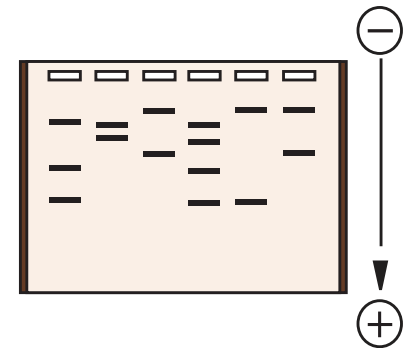
- Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the  button. The green LED next to the button will turn on.

The green power LED will not turn on if:

- The tank is not properly placed inside the carriage
- There is no buffer in the tank
- The buffer is too concentrated or too diluted
- The photo hood is not on the carriage
- There is too much or too little running buffer
- The power supply is not plugged in. Check by turning on the blue LEDs

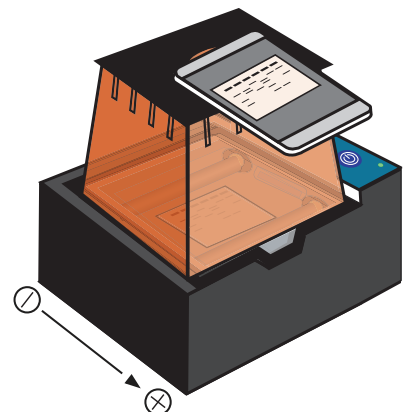


- Allow the gel to run approximately **25 minutes** or until DNA separation is sufficient. After your run is complete, turn off the power by pressing the  button. Use the low intensity for viewing during the run. Light will weaken the fluorescent DNA signal.



- Document your results.**

Wipe off the condensation from the inside of the hood with a soft cloth if necessary, then place the hood back on the carriage. **Turn on** the high intensity light. Place your cell phone or camera directly on the photo hood to take a picture of the DNA. **DO NOT** zoom in as this will result in blurry pictures. (The photo hood is already at the optimal focal length for a smart device).



Clean Up

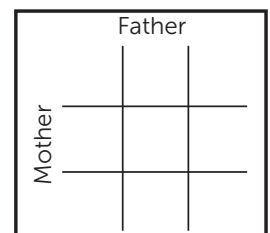
1. After collecting data and documenting results, remove the photo hood and unplug the power supply from the wall and from the back of the MiniOne[®] Carriage. Remove the clear running tank from the carriage and remove the gel and tray from the running tank.
2. Pour the used running buffer down the drain or into a waste beaker. Throw the gel away. Rinse the clear plastic running tank, gel tray, comb, and casting system with DI or distilled water. Allow the tanks to fully air dry before storing.
3. Use a paper towel or kimwipe to gently wipe the gold rivets in the carriage (where the electrodes connect) to ensure all moisture is removed. Wipe up any buffer that may have spilled into the black carriage. Follow any additional directions the instructor gives for cleanup and storage.

Part II: Results

What does your gel look like? Record images of the gel.

Part III: Analyze Your Data

1. How many DNA bands do you see in Jillian's sample? Jillian is a non-taster, how many different PTC alleles does Jillian have?
2. How many bands of DNA do you see in Jillian's mom and dad samples? Jillian's mom & dad are tasters, how many different PTC alleles do they have?
3. Based on the DNA pattern on the gel and the genetics of the PTC genes that you have learned, can you determine which DNA band corresponds to the normal PTC allele and which one corresponds to the mutant PTC allele?
4. What is the genotype for Jillian, her mom and dad?
5. Can you determine which PTC gene Jillian got from her mom and dad?
6. Compare Jillian's Mom's and Dad's PTC genes to Jillian's. Based on the data, is your hypothesis supported or refuted as to why Jillian is not a PTC taster and Jillian's parents are PTC tasters? Draw a Punnett square to explain your results.



Part III: Analyze Your Data (continued)

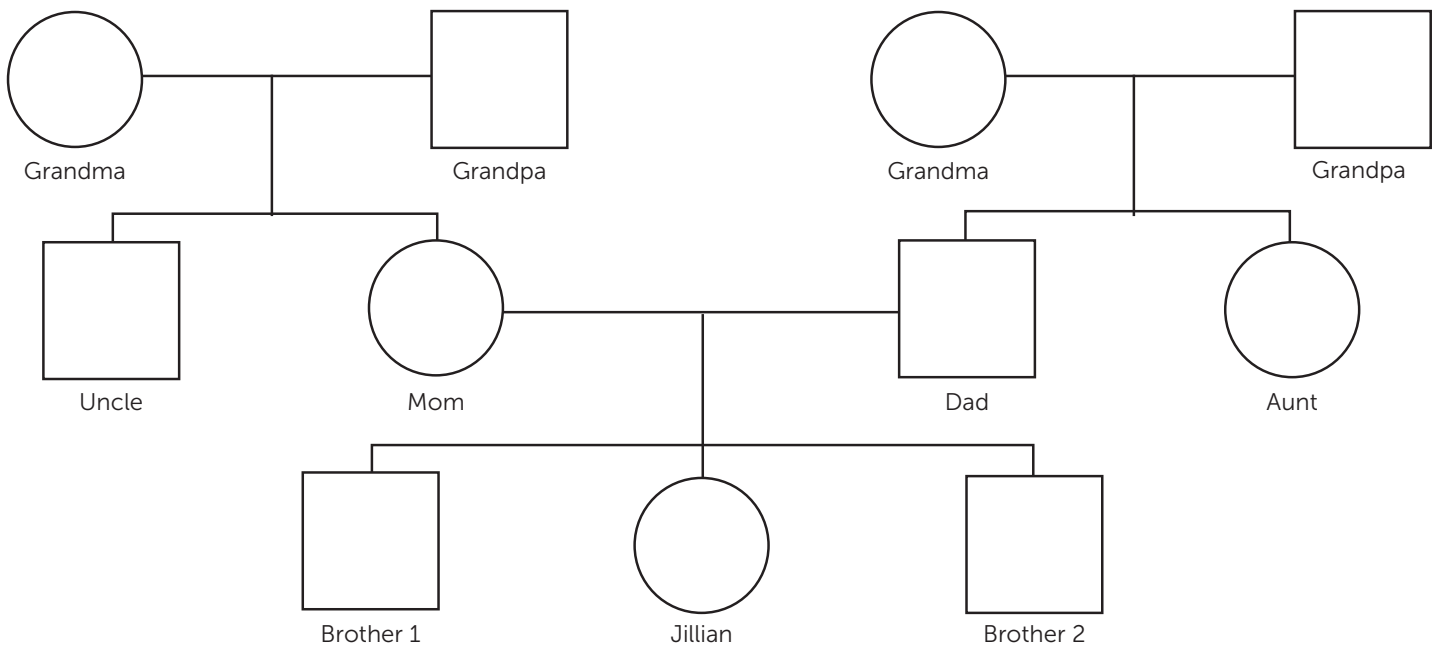
7. Are the DNA patterns seen in Jillian's brother's samples consistent with their ability to taste PTC?

8. What does seeing the DNA patterns in Jillian's maternal grandpa's sample tell you about her maternal grandma's PTC gene?

9. What percent chance of Jillian's parents having another baby who cannot taste PTC? How about one that is like Brother 1, homozygous for the PTC taster gene? Or like Brother 2 heterozygous?

10. Based on what you have learned from this analysis, fill in as much of the family tree as you can. If you do not have the genotype data, fill in what you can with the phenotype data.

Circle = female Square = male Filled shapes = non-taster half filled = carrier unfilled = taster



Appendix A - Mendelian Genetics - A Brief Overview

Background

During the 1860's, an Austrian monk by the name of Gregor Mendel was studying pea plants. As a result of his experiments, he developed and introduced a new theory of genetic inheritance. The typical notion of inheritance at the time was that traits were a result of parental "essences" mixing together, rather like blending yellow and red paint to get orange. Mendel believed heredity was the result of distinct units of inheritance, and every individual unit (gene) acts independently in one's genome. Mendel thought that all traits are controlled by single genes, however we now know that many traits are determined by multiple different genes, as well as environmental factors.

Simple Mendelian Inheritance Pattern

According to Mendel's theory, the inheritance of a specific trait or characteristic depends on these units being passed on from parent to offspring, and he developed two laws to describe the process. There are different forms of genes, and these different forms are called alleles. Mendel's Law of Segregation states that the genes of a parent split into their constituent alleles and that a person inherits one allele from each parent for any given gene, resulting in a complete gene. It also states that the allele that gets passed on is completely up to chance. The other law is the Law of Independent Assortment which states that alleles are passed to the offspring independently of one another. This means that inheritance of a gene or genes at one location in the genome will not affect the inheritance of other genes elsewhere. If the alleles inherited from both parents are the same, the person is considered to have a homozygous genotype for that trait. If the alleles are different, their genotype is heterozygous.

Genotype vs Phenotype

Your genotype is the actual genetic code you possess, whereas your phenotype is the expression of your genotype in an observable way, like your hair or eye color, your ability to taste certain chemicals, etc. Your phenotype is affected by the alleles in your genotype. Alleles have different classifications based on how they interact. They can be considered dominant, co-dominant, incompletely dominant, recessive, or a combination of these (it's all relative).

Dominant and recessive alleles are straight-forward. Dominant alleles are typically denoted with a capital letter (example: T). A dominant allele completely masks the expression of the other allele. Both heterozygotes and homozygous dominant people will express this phenotype. Recessive alleles are typically denoted with a lowercase letter (t). A recessive allele is masked by any allele that is dominant to it. Homozygotes would be the only ones to express the phenotype of these alleles.

Co-dominance and incomplete dominance are two distinctly different things, but both are a result of a combination of different alleles (heterozygotes). The big difference between these two categories is how the phenotype is affected. In both of these cases, the denotation can be arbitrarily defined, with one allele represented by a capital letter and the other lowercase, or two different letters may be used all together.

Co-dominant alleles express both phenotypes simultaneously. An example of this would be a flower with red and white petals where one parent had white petals and the other had red. The colors are both present, not mixed together to make a new color. Another example of allele co-dominance is the human ABO blood typing system. Type O is recessive to both types A and B, however types A and B are co-dominant. This is how a person can have AB blood; both A and B alleles are being expressed. However if their genotype is AO, the allele for A is the only one that will be expressed, so the phenotype would be Type A.

Appendix A - Mendelian Genetics (continued)

Incomplete dominance also describes simultaneous expression of both alleles, however it is more like a blending of the two phenotypes of the homozygotes instead of both homozygous phenotypes being present. An example of this would be a flower with pink petals (Rr), with one parent having red petals (RR) and the other with white (rr). The phenotypes mixed together to produce a third intermediate phenotype.

References and Additional Resources

More background information

<http://learn.genetics.utah.edu/content/inheritance/ptc/>

Sample protocols and teaching guides

This sample teacher's guide and the accompanied student protocol are adapted from examples written by and shared by the Biotech Project at University of Arizona.

<http://biotech.bio5.org>

References:

N. Chaudhari and S. Roper (2010) The Cell Biology of Taste. JCB 190(3) 285 – 296.

<http://jcb.rupress.org/content/190/3/285.full>

Genetic Science Learning Center. PTC: Genes and Bitter Taste.

<http://learn.genetics.utah.edu/content/inheritance/ptc/>

Cold Spring Harbor Laboratory. Dolan DNA Learning Center.

<http://www.dnalc.org/>

<http://knowgenetics.org/mendelian-genetics/>

Appendix B - What is Gel Electrophoresis?

Looking at a sample of green dye, how can you know if it is really green? Could it be a mixture of blue and yellow dyes? Electrophoresis is a technique used in many areas of science to analyze and separate samples by applying a constant electric field. Biologists or forensic scientists can use this technology to separate mixtures of DNA or dyes into each component based on size and electrical charge.

The gel in gel electrophoresis is essentially a matrix through which particles travel. Gels can be made from different substances depending on what is being separated (DNA, RNA, proteins, etc.), but it should be both conductive and have the ability to form a uniform matrix with appropriate pore sizes. The matrix is like a sieve or collander: if the holes are too big or too small it won't work very well. One of the most commonly used and effective reagents for DNA separation is agarose. Agarose gels are usually cast in a tray with molten (melted) agarose. A comb is placed while the agarose is molten and then removed after the gel solidifies to create wells in which to load samples. A DNA stain is added to the gel to enable visualization of the DNA, either before casting or after the run if DNA is going to be observed.

As an electric field is applied to the agarose gel, the particles in the wells will begin to move. The direction that particles migrate depends on their charge. DNA has a negative charge, so it will be attracted to a positive electrode. Some dyes and other particles have a positive charge and will thus migrate toward a negative electrode. The relative speed of migration is determined mainly by the size of the particle but also by the strength of the particle's charge. Like an obstacle course, larger particles have more difficulty passing through the matrix with their bulk and do not travel very far, while shorter and smaller ones can maneuver much more easily and therefore travel faster and farther.

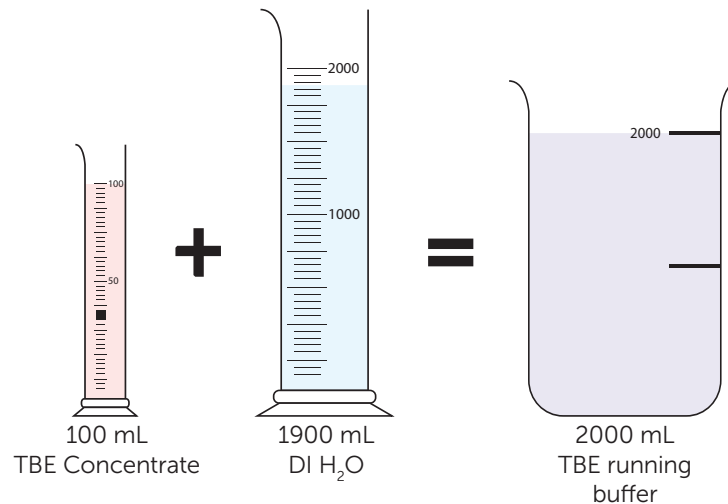
Sometimes a particle with a bigger size migrates faster than a smaller particle. This can happen if the strength of the charge of the larger particle is significantly stronger by comparison to the charge on the smaller particle. An example of this phenomenon is the loading dye Orange G. This dye often runs faster than the smaller DNA fragments and other relatively small particles because it is more negatively charged and has a stronger attraction to the electrode than the smaller particles.

Both particle size and electrical charge can affect the results of gel electrophoresis experiments. In general however, gel electrophoresis separates charged particles and fragments by size.

Appendix C - TBE Concentrate Dilution Instruction

Dilute **1** part TBE Concentrate with **19** parts deionized or distilled water

- To make 2000 mL TBE running buffer (1X)** = mix 100 mL TBE Concentrate and 1900 mL deionized or distilled water



Limited TBE running buffer is stable to store at room temperature.

- To make various volumes of TBE concentrate use the formula:**

$$C_1 \times V_1 = C_2 \times V_2$$

Where:

C_1 = Original TBE Concentrate

V_1 = Volume of the Original TBE Concentrate needed

C_2 = Final concentration

V_2 = Total final volume desired

Once you have calculated the volume of TBE Concentrate needed, **SUBTRACT** that amount from the total volume of TBE running buffer desired to find the volume of water needed.

We recommend diluting buffer in batches for accuracy.

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 theminione.com

 (858) 684-3190

 info@theminione.com

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