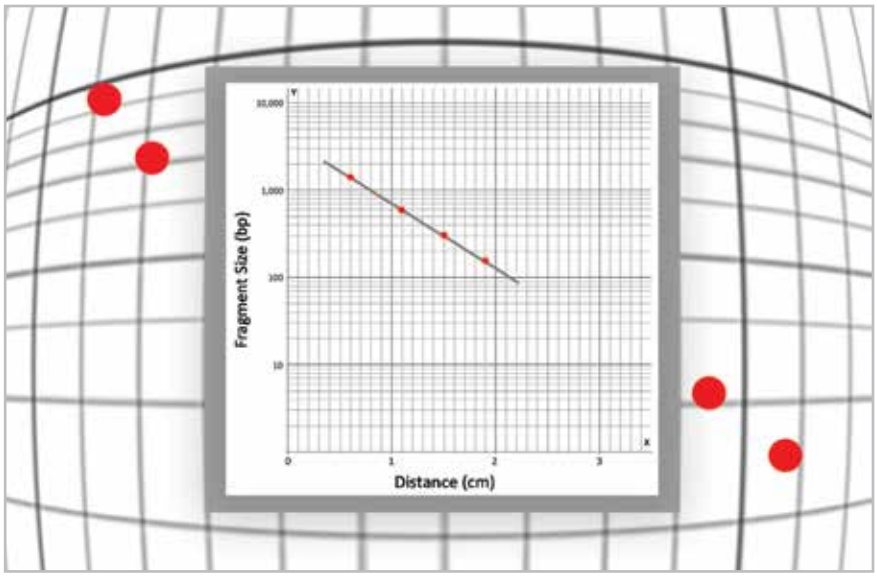
PTC Inheritance and Graphical Analysis MiniLab

Student's Guide

Cat# M3012

Version 032020



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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 understand and use Mendelian inheritance to analyze data and to determine a probable conclusion.
- To use a family's pedigree to observe patterns and make predictions about the inheritance of the ability to taste PTC.
- To develop a basic understanding of how using PCR and restriction digestion can provide data to determine genotype.
- To develop an understanding of electrophoresis principles to confirm predictions and explain patterns observed using scientific data.
- To develop an understanding of how to construct a standard curve.
- To develop an understanding of how to use a standard curve to determine the size of unknown fragment sizes in a gel.

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 bind 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. (https://www.sciencedaily.com/releases/2004/06/040627223325.htm).

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 MiniLab 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. This sample can be obtained by extracting DNA from an individual's cells. The polymerase chain reaction (PCR) can then be used to make millions of copies of just the area of the gene in which we are interested. As mentioned above, the functional allele and the mutant allele only differ by one base pair. To distinguish between these two alleles students would follow PCR with a restriction digest and gel electrophoresis.

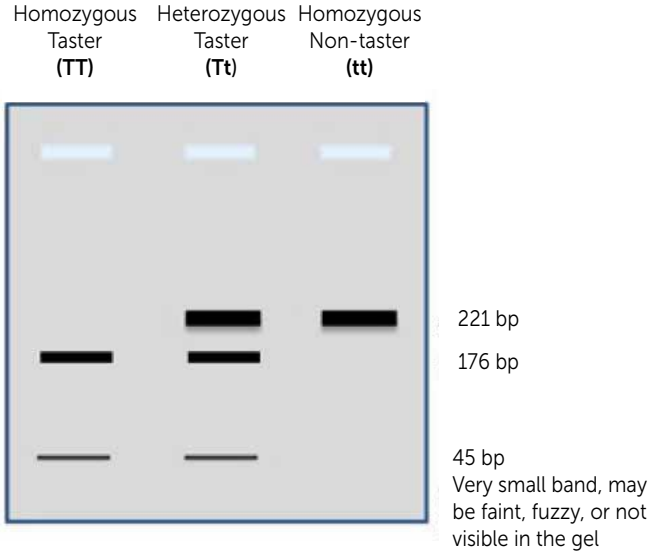
PCR

In order to make copies of only the area of the TAS2R38 gene you are interested in, you must first obtain a sample of DNA. The easiest way to do this is to do a simple saline mouth rinse. Cells from the inside of the mouth will be suspended in the solution. The next step is to lyse the cells and recover DNA, then use the Polymerase Chain Reaction (PCR) to amplify the DNA. PCR is a powerful tool because it enables scientists to target exactly what part of a gene they wish to study by selecting specific primers. PCR mimics how DNA replication takes place in the cell in a tube. Instead of using enzymes to make the DNA single stranded, the sample is heated to a high temperature causing the DNA to become single stranded because the high temperature causes the hydrogen bonds holding the strands together to fall apart. The sample is then cooled, allowing short pieces of DNA called primers bind to each side of the desired segment of DNA. The sample is then heated to a temperature where the special polymerase Taq will add nucleotides to the specifically selected primers. The process is repeated over and over again to make sufficient quantities of the small segment of DNA that will be cut or not cut by a restriction enzyme to determine genotype.

Restriction Digest

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.

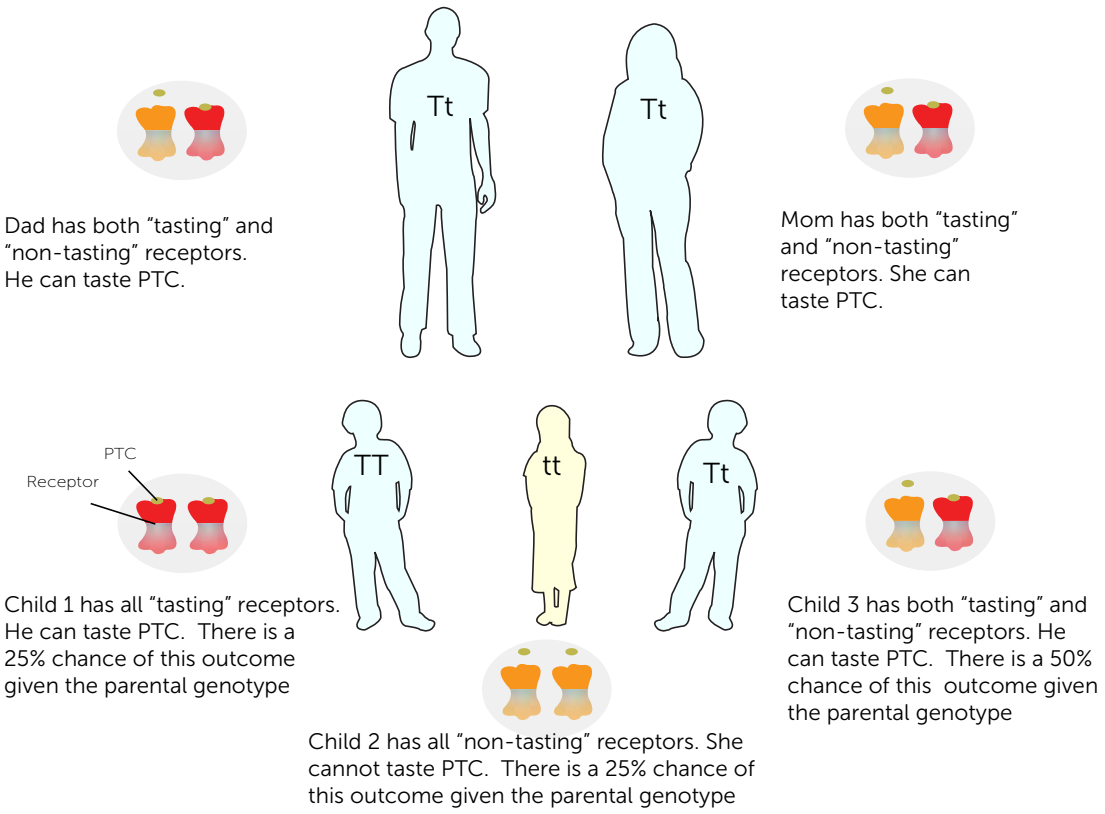


This diagram illustrates the different patterns possible after electrophoretic separation on a 2% agarose gel. As you can see, a homozygous taster will have two bands, a heterozygous taster will have three bands, and a homozygous non-taster will only have one band.

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	HaeIII 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 *Note: Due to the very small size of the 45 bp fragment, this band may be faint, fuzzy, or not visible in the gel*
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

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. How you could test this hypothesis?

Hypothesis

Jillian of course could be the only person in her family to have both of the recessive genes and therefore not a taster. One hypothesis is that Jillian is not really related. (Using electrophoresis to look at the gene will allow you to confirm parentage, as well the genetics of PTC.)

Predict what you would get based on your hypothesis. If Jillian is recessive, draw Punnett squares. (Alternatively, draw the Punnett squares at the end of the lab.)

Pre-Lab Activity

1. Are you a PTC taster? If so, what does PTC taste like?

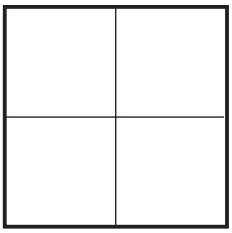
2. Record how many people in your class are tasters and how many people in your class are non-tasters.

3. Why do you think some people can taste the PTC and others can't?

Pre-Lab Questions

1. Explain the relationship between genes and alleles.

2. What is the probability of a heterozygous taster and a homozygous non taster having children that will be tasters?



3. You will be using simulated PCR product that would or would not be cut by the restriction enzyme HaeIII. You will verify DNA fragment sizes using gel electrophoresis. What properties of DNA allows you to do this? (see Appendix B).

Part I: Electrophoresis

Materials

1 MiniOne® Casting System
1 MiniOne® Electrophoresis System
1 agarose GreenGel™ Cup (2%)
MiniOne® DNA Marker
6 x HaeIII Digested DNA samples
TBE running buffer (135 mL)
1 micropipette (2-20 µL) and
7 pipette tips

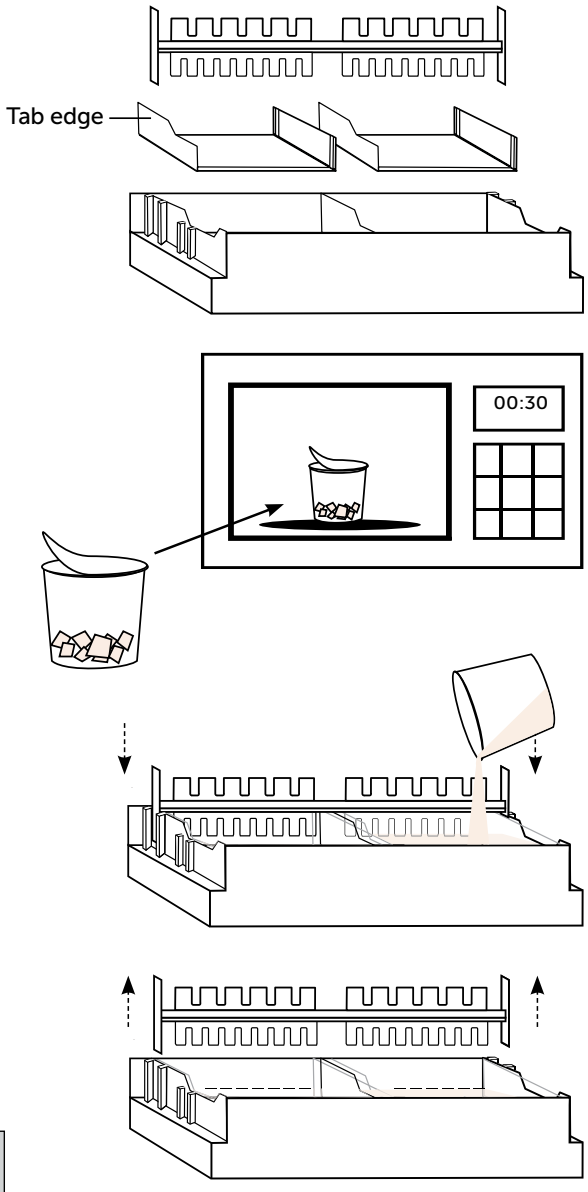
SAMPLE CHART:

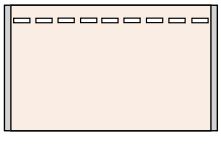
Lane #	Sample Name	Loading Volume
1	MiniOne DNA Marker (from top to bottom 2kb, 1kb, 500bp, 300bp, & 100bp)	10 µL
2	DNA M	10 µL
3	DNA D	10 µL
4	DNA J	10 µL
5	DNA B1	10 µL
6	DNA B2	10 µL
7	DNA G	10 µL
8		
9		

MiniOne Visual Instructions

How to Cast a Gel

1. 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 9-well side facing down.
2. Partially peel the film off a GreenGel™ Cup and microwave for 30 seconds. Allow to cool for 15 seconds. **DO NOT microwave more than 5 gel cups at a time.**
3. **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 at least 10 minutes or until opaque. **DO NOT disturb the gel until time is up.**
4. 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. **Make sure the wells are aligned with the marks on the platform on the negative end.**

2. Plug the power supply into the wall and carefully insert the other end into the back of the MiniOne® Carriage.

3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.

4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.

5. Turn the low intensity blue LED on by pressing the button on the carriage.

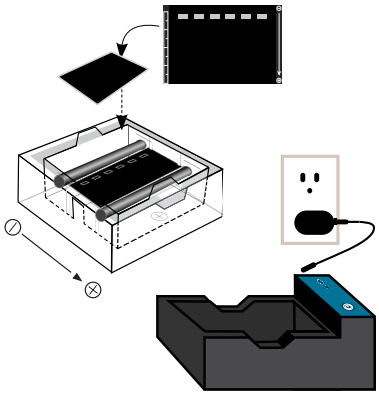
6. Measure 135 mL of TBE running buffer and pour into **one side** of the tank. Watch the air push out between the gel tray and viewing platform. Once air has been removed from under the gel tray, pour remaining buffer into the other side of the gel tank.

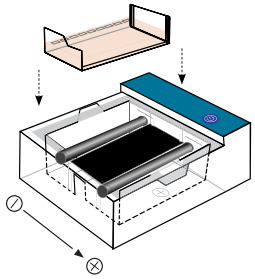
7. Place photo hood on the carriage.

8. Press the power button which should now be a solid green light. If **green light is solid,** turn off the unit and proceed to loading gels.

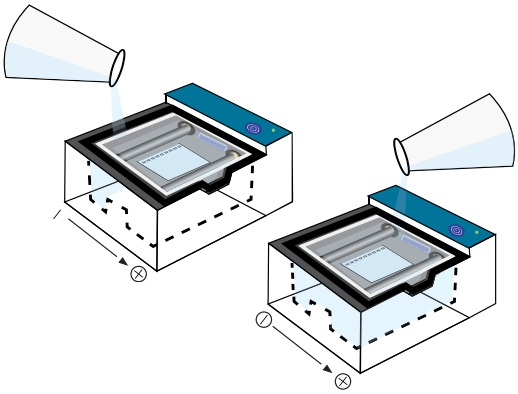
9. Turn the low intensity blue light on by pressing the button on the carriage to help visualize the wells when loading.

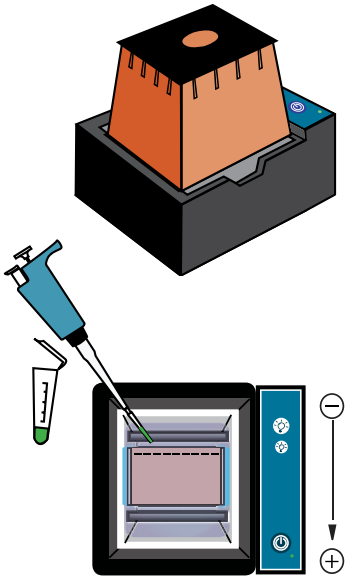
10. Load 10 µL per well. Remember to change pipette tips for each sample. **Load your samples according to the order given in the sample chart.**



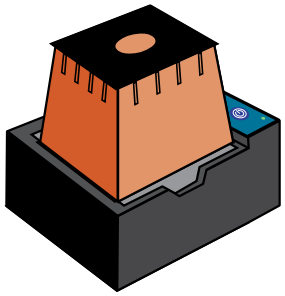


Note: Place gel tank with the gel on the gel tray into carriage **before** pouring buffer in.





Run, Visualize and Capture Image

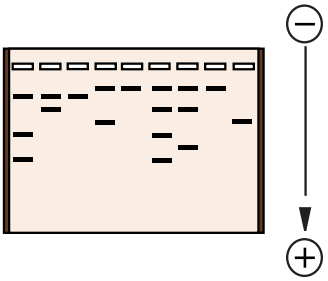


1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the button. The green LED next to the button will turn on.

   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**

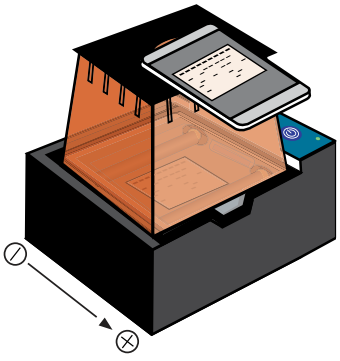
2. Check the migration of the bands (~every five minutes).



3. 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.

4. **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 will result in blurry pictures. (The photo hood is already at the optimal focal length for a smart device.)

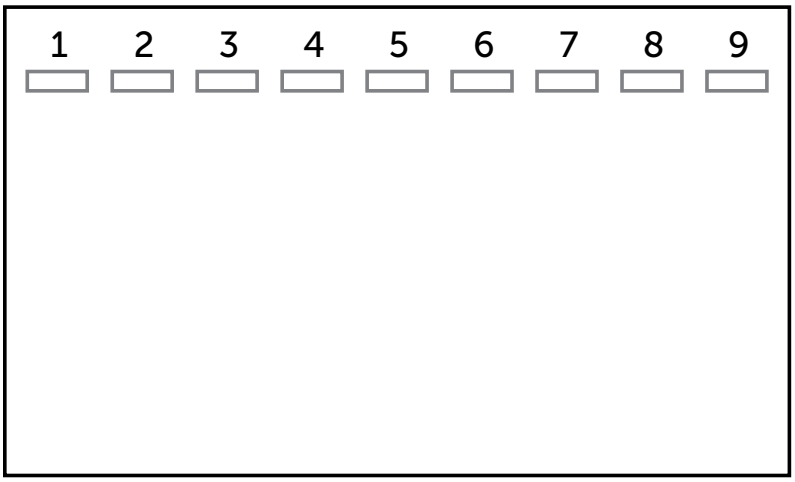


5. Clean up. Follow teacher's instructions on disposal and clean up.

Part II: Results

Gel Analysis Worksheet

Directions: After running the DNA samples on the gel, record an image of the gel and draw the results or tape the image on the template below:



Lane 1: _____

Lane 2: _____

Lane 3: _____

Lane 4: _____

Lane 5: _____

Lane 6: _____

Lane 7: _____

Lane 8: _____

Lane 9: _____

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?

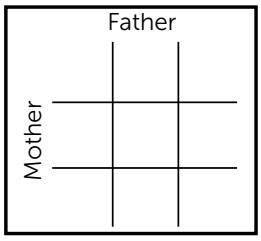
2 How many bands of DNA do you see in Jillian's Mom and Dad samples? Jillian's Mom and Dad are tasters, how many different PTC alleles do they have?

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.



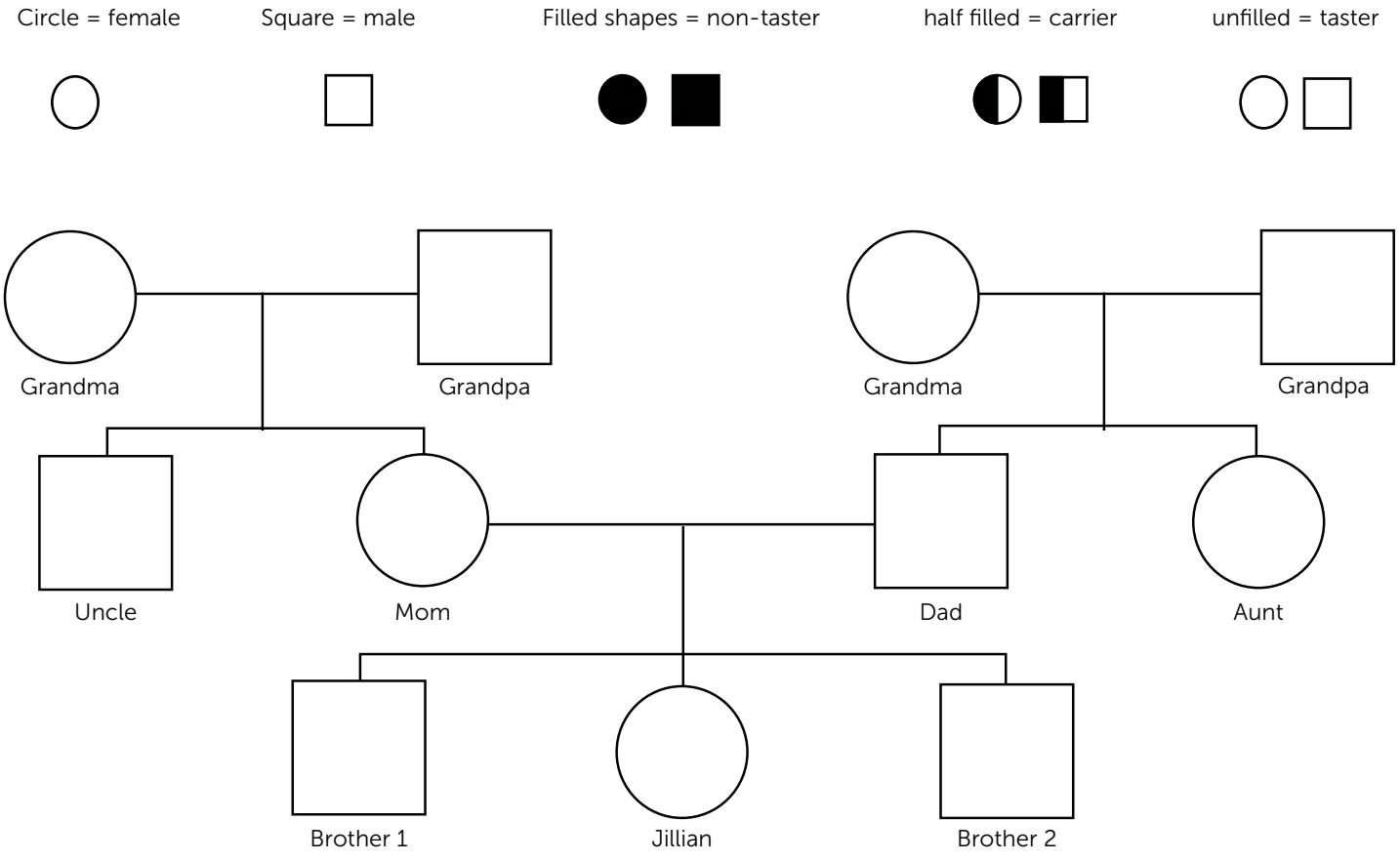
Part III: Analyze Your Data (continued)

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?

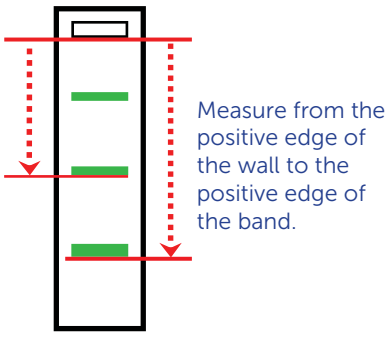
10. Based on what you have learned from this analysis, fill in as much of the family tree as you can. If you do not have the genotype data, fill in what you can with the phenotype data.



Provide Evidence to Support your Conclusions

You examined the patterns in your gel to predict the genotypes of Jillian's family. In your background reading you learned the size of the PCR product of the TAS2R38 gene is 221 bp. That product was then incubated with the restriction enzyme HaeIII. There are 2 alleles of this gene. Tasters have the restriction site HaeIII recognizes. The PCR product of that allele was cut into 2 pieces, 176 bp and 45 bp. The non-taster allele is not cut by the restriction enzyme and will be 221 bp. You need to verify the sizes of the bands in your gel to provide evidence to support your conclusions about your prediction. In order to provide evidence to support your reasoning you will need to construct a standard curve using your Molecular Marker. We know the sizes of the bands of the MiniOne® DNA Marker. The band closest to the well is 2,000 bp, followed by 1,000, 500, 300, and 100. To construct your standard curve:

1. Open the image you took of your gel.
2. Use the ruler along the side of the gel to measure the distance of the known bands in the molecular weight marker in Lane 2.
3. Measure from the positive edge of the well to the positive edge of the band.
4. Record the known fragment sizes in base pairs (bp) and the distance the band traveled, measured in centimeters (cm) in the data table below.



Size (bp)	Migration distance (cm)

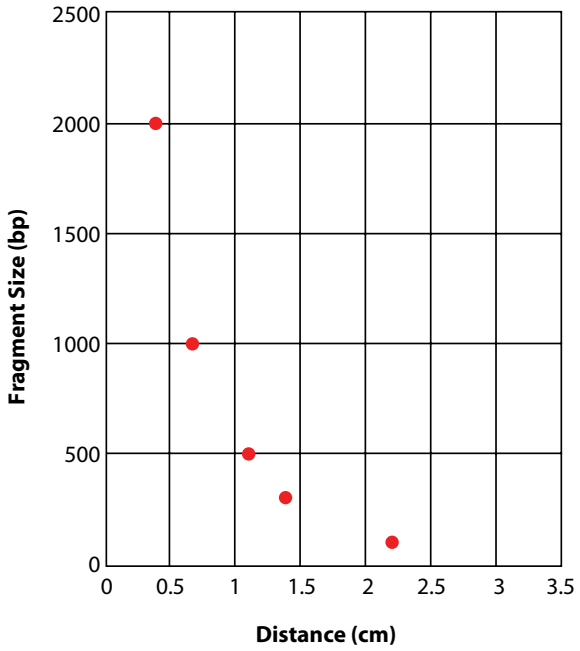
Now consider the sample data below:

Sample Data:

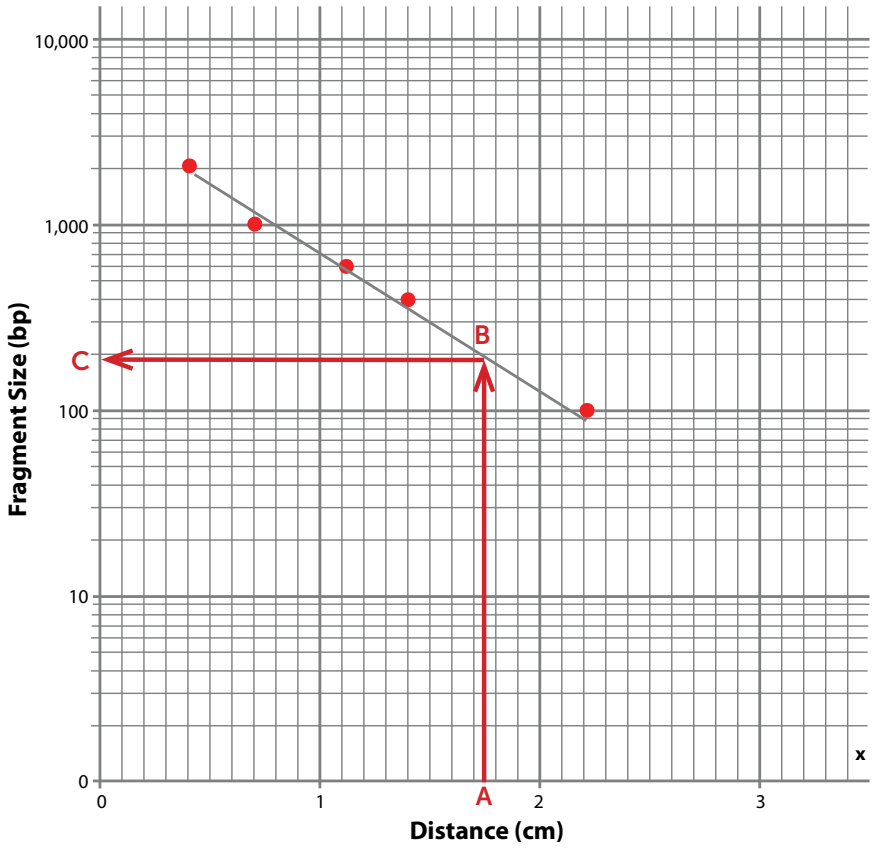
Size (bp)	Migration distance (cm)
2000	*0.4*
1000	*0.7*
500	*1.15*
300	*1.4*
100	*2.2*

5. You will use Log Y graph paper to construct your standard curve. Why?

   On Log Y graph paper, the X-axis is linear and the Y-axis is logarithmic. If you plot the data on linear graph paper for both the X- and Y-axis your graph would look something like this:



Predicting unknown band sizes would be difficult using this graph. When you see a pattern of data with that shape on linear graph paper it may be a hint it follows a logarithmic relationship. You can transform the data using Log Y graph paper. The transformation occurs because the ticks on the log axis are not evenly spaced out. They get closer together to represent the logarithm of the numbers (notice spacing for the Y-axis).



6. Use Log Y paper to graph your data. Use the graph to estimate the molecular sizes of the unknowns. Move along the x-axis to find the distance your unknown travelled (point A). Draw a line vertical from point A to where it intersects the standard curve (point B). Draw a line horizontal from point B to where it intersects the y-axis (point C). This is the estimated fragment size for your unknown. Record the size in the "Estimated Size" column.

Sample	Distance migrated (cm)	Estimated size (bp)
Mom	1.75 2.0 2.75	
Dad	1.75 2.0 2.75	
Jillian	1.75	
Brother 1	2.0 2.75	
Brother 2	1.75 2.0 2.75	
Grandpa	2.0 2.75	

7. How does your actual data compare to your predictions?

   Calculate percent error to determine if your estimates are acceptable. A percent error of less than 5% is acceptable in this case. To calculate percent error use the following formula:

   [|(observed-expected)| /expected] X100%

8. Extrapolate your data beyond the linear range. Calculate percent error for the 45 base pair fragment. Is it still within the acceptable range? Explain.

9. Provide a summary paragraph outlining your prediction (claim), how the data you collected from your gel supports your claim, and how calculations from your standard curve provides evidence for your reasoning.

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/
https://learn.genetics.utah.edu/content/labs/pcr/

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 wont 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 - Optional Activity: Linear or Log Y Graph

1. Examine the data in Table 1. Notice the migration distance is linear (there is no large change in value) while the number of base pairs is not.

Size Number of Base Pairs	Distance Migrated (cm)
2000	.4
1000	.7
500	1.15
300	1.1
100	1.0

Table 1

2. Look closely at the relationship between the distance and the size. The larger the fragment (in bp) the lower the distance migrated. What type of graph would provide you with the best data to determine an unknown from your standard, linear or Log Y? To help you decide, examine Figure 1.

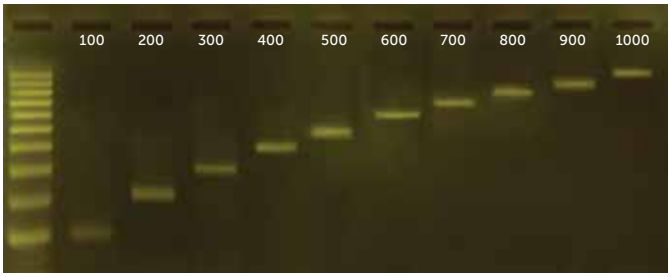


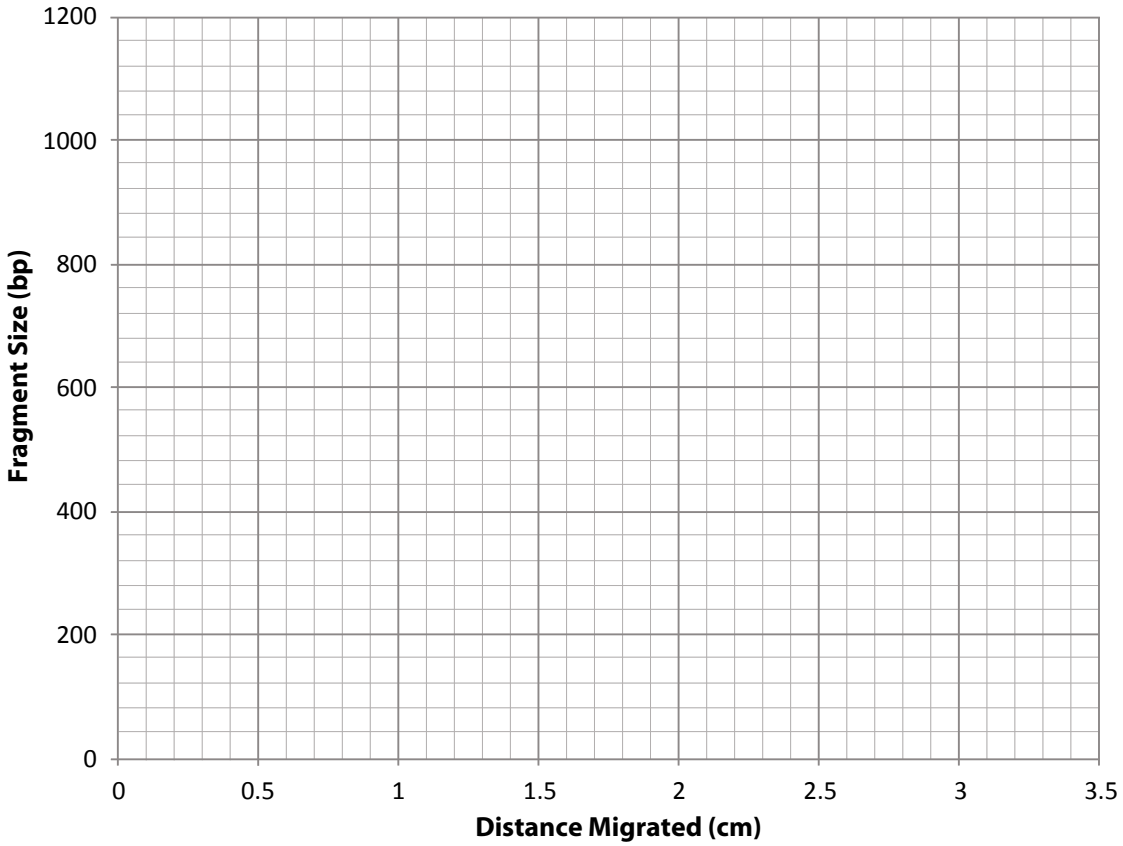
Figure 1. Image of a gel with a universal ladder in Lane 1 and fragments ranging from 100 to 1,000 in Lanes 2–9.

3. Measure the distance each band migrated and record it in Table 2.

Size Number of Base Pairs	Distance Migrated (cm)

Table 2

4. Plot your data on linear graph paper.



5. Did you get a straight line?

6. Compare your graph to Figure 2. Does your graph resemble the picture?

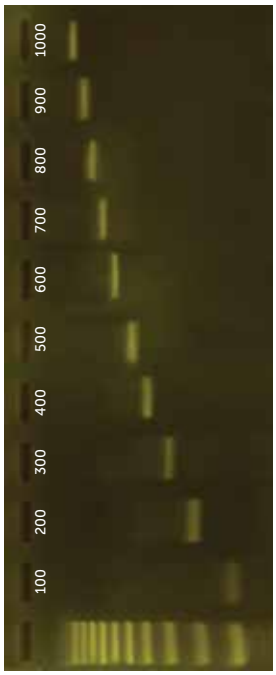
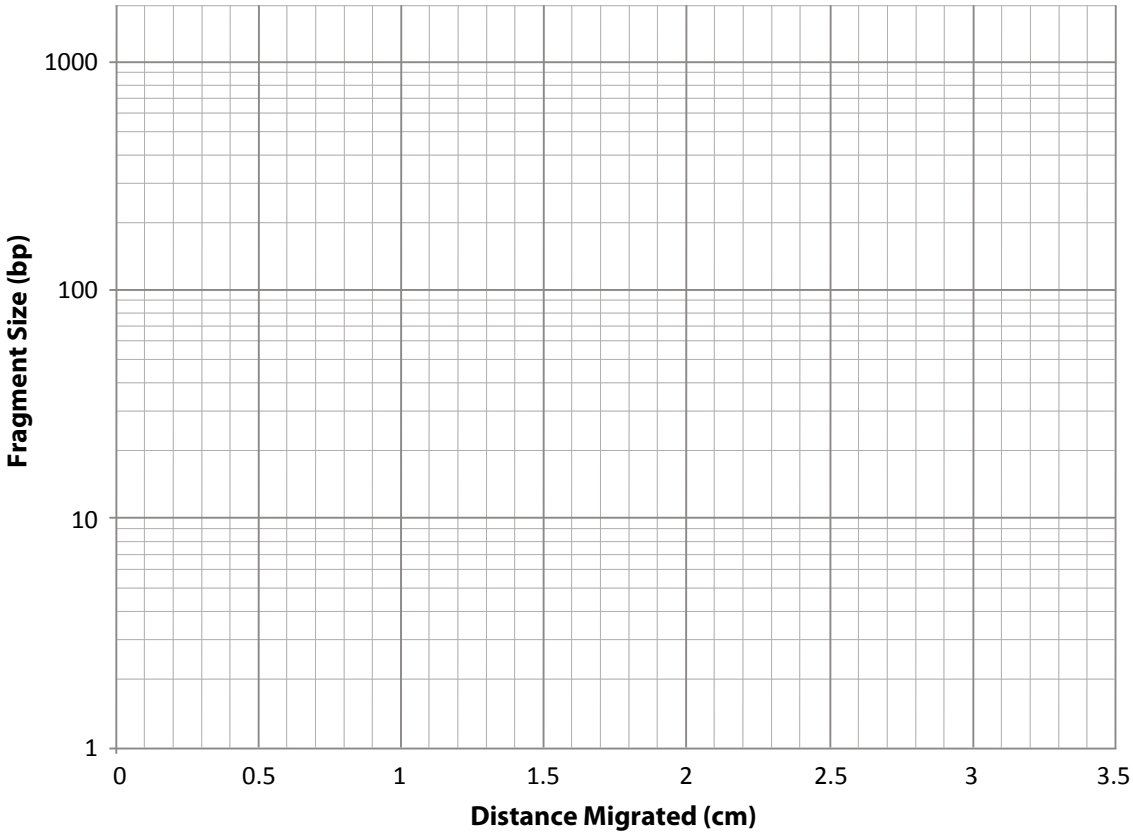


Figure 2. Image of gel in Figure 1 rotated 90 degrees

7. Plot your data on Log Y graph paper.



8. Did you get a straight line?

9. Compare the graphs. Which type of graph provides the best data?

10. How is the linear graph paper and Log Y paper different?

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