Taste of Genetics MiniLab

Student's Guide

Cat# M6010/M6012/M6013

Version 031622

Table of Contents

Objectives

This hands-on MiniLab introduces students to the science of human genetic variation through extraction, PCR amplification, restriction digest, and analysis of their own DNA. This lab is ideal for high school biology students, especially honors and advanced placement, and college level biology. This MiniLab contains enough supplies for **10 groups** to perform experiments.

Laboratory Safety

1. Wear lab coats, gloves, and eye protection whenever possible.
2. Use caution with all electrical equipment such as PCR machines and electrophoresis units.
3. The PCR machine has surfaces that can be extremely hot. Use caution when opening and closing the lid and when placing and removing tubes.
4. Heating and pouring molten agarose is a splash hazard. Use caution when handling hot liquids. Wear eye protection and gloves to prevent burns.
5. Wash your hands thoroughly after handling biological materials and chemicals.
6. Dispose of all materials in a biohazard bag or in a wash tub containing a 10% bleach solution.

Introduction

Every human individual perceives the world in a slightly different way. Some differences arise from our past experiences and some are related to our genetic heritage. 99.9% of the human genome is identical between individuals while the remaining 0.1% makes us biologically unique. Genetic differences extend to our sensory systems affecting how we see, hear, and taste the world. In a few cases, we know the specific genes and proteins that underlie differences in perception. In this lab we will explore the molecular genetics of taste. You will determine your own genotype for a chemical receptor involved in sensing bitter compounds.

History of Research on PTC Tasting

Phenylthiocarbamide (PTC; Figure 1) tasting is one of the most studied examples of inherited variation in tasting ability. In the late 1920s, Arthur L. Fox was working as a chemist at DuPont. As he was pouring PTC powder into a bottle, his co-worker, C.R. Noller, complained that the dust tasted extremely bitter. However, Fox could taste nothing. Both took turns sampling the powder with the same results; Fox tasted nothing while Noller experienced a bitter taste. Curious, Fox started testing other people and found that most people either had strong reactions to the bitter taste, even at low concentrations, or tasted nothing at all.

These findings found immediate interest among scientists studying Mendelian inheritance and human sensory variation. Laurence H. Snyder confirmed Fox's results and studied the trait in several families. He concluded that PTC tasting ability was hereditary. Albert Blakeslee, famous for his work in the genetics of plants, conducted the first large scale investigation involving families and found that a PTC tasting follows a Mendelian inheritance pattern, but sensitivity can vary in magnitude. Based on these findings, Blakeslee suggested that other genes may be involved making the genetic basis for PTC tasting more complicated than originally thought. For this lesson we will treat PTC tasting as a simple Mendelian trait exhibiting simple, or complete, dominance.

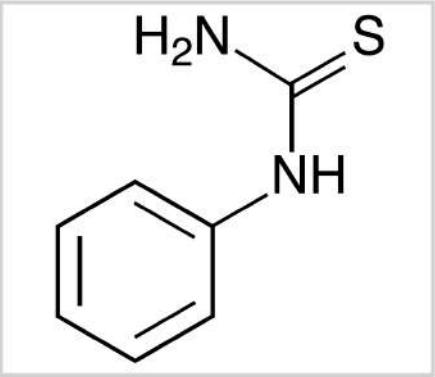


Figure 1. The chemical structure of phenylthiocarbamide (PTC)

Human Taste Physiology

Human taste is a complex phenomenon emerging from interactions between substances in our food and chemical receptors in our mouth and nose, and the processing of signals in the brain. The tongue is covered in bumps called papillae (Figure 2). Each papilla contains taste buds filled with gustatory cells. Each gustatory cell has proteins on its surface with shapes specialized for binding to chemicals associated with different flavors (sweet, salty, sour, bitter, and umami). When a chemical binds to its receptor on the surface of a gustatory cell, a response inside the cell is triggered. If the response is strong enough, the gustatory cell releases neurotransmitters onto the dendrites of sensory neurons. The sensory neurons send a signal to the brain that is processed as the perception of flavor.

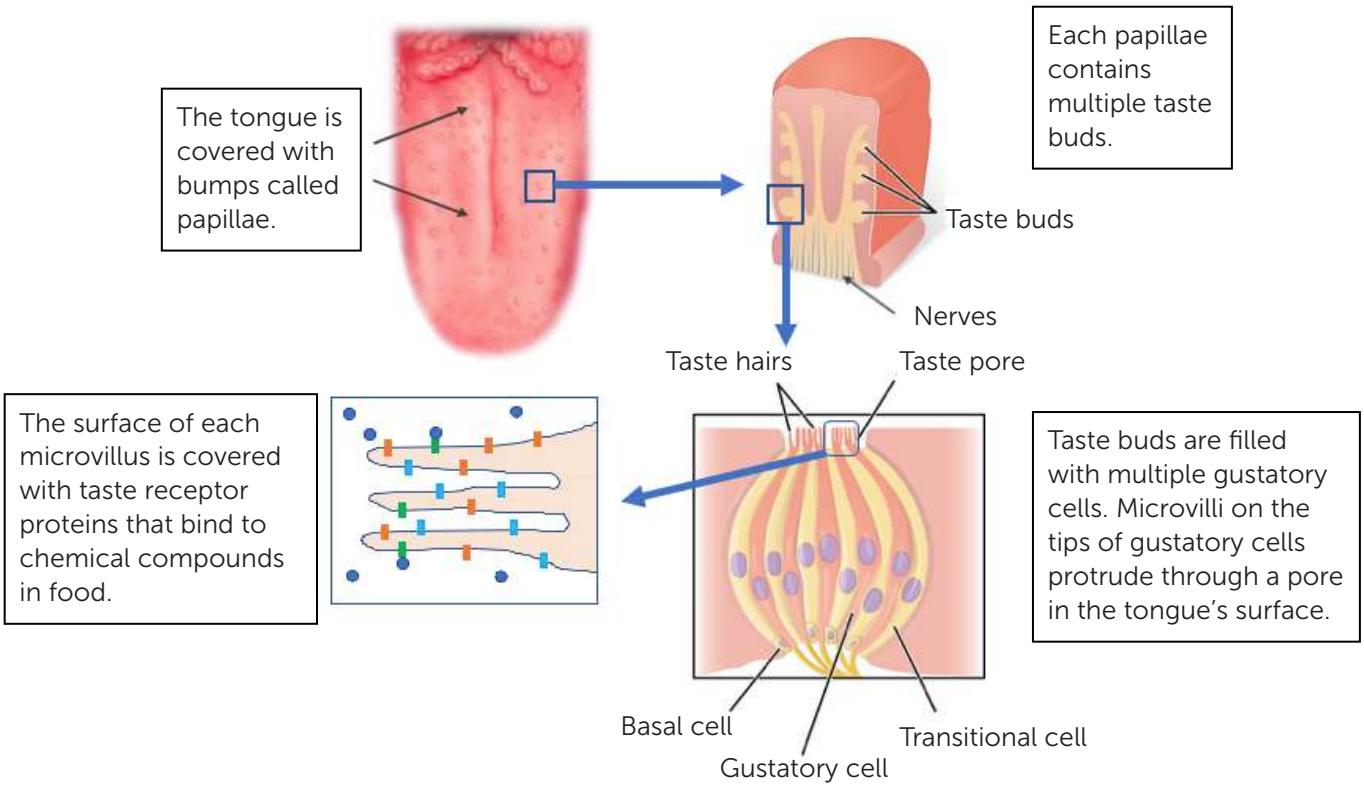


Figure 2. Physiology of human taste. Papillae on the tongue contain taste buds, which are filled with gustatory cells. Each gustatory cell has taste receptor proteins on its surface, which can bind to molecules associated with different flavors.

Image credits: Tongue image: By gabymichel [CC BY-SA 3.0 (https://creativecommons.org/licenses/ by-sa/3.0) or GFDL (http://www.gnu.org/copyleft/fdl.html)], from Wikimedia Commons, Papillae and taste bud images: By OpenStax [CC BY 4.0 (https://creativecommons.org/licenses/by/4.0)], from Wikimedia Commons.

Molecular genetics of PTC tasting

In genetic studies, an observable trait, such as the ability to taste PTC, is called a phenotype. A genotype is the genetic basis of a trait, the genetic information that codes for the phenotype. An allele is one of two or more distinct forms of a gene located at a specific position on a chromosome. Since you have two sets of homologous chromosomes, one inherited from your mother and one from your father, you have two copies of every gene. The two copies can be the same allele or different alleles. In basic studies, the ability to taste PTC is inherited as a simple Mendelian trait. As with other simple Mendelian traits, such as cleft chin or widow's peak, the gene associated with the ability to taste PTC exists in two allelic forms, dominant (T) and recessive (t). Investigations of the PTC phenotype in families concluded that the ability to taste PTC is a dominant trait. Since a dominant allele masks the presence of the recessive allele, the genotype of a taster can be either homozygous dominant (TT) or heterozygous (Tt). If a person is a non-taster then their genotype is homozygous recessive (tt).

The TAS2R38 gene was identified and sequenced in 2003 by Un-kyung Kim and colleagues. This gene codes for a cell-surface protein that binds to PTC and initiates an intracellular signaling cascade. The gene is 1143 nucleotide base pairs (bp) long and located on the long arm of chromosome 7 along with nine other genes for bitter taste receptors. By comparing DNA sequences between tasters and non-tasters, scientists have determined that there are three single nucleotide polymorphisms (SNPs) that differentiate the taster allele (T) from the non-taster allele (t). A SNP is a type of genetic variation where the nucleotide at a single position differs between individuals. To be considered a SNP rather than a mutation, the variant must exist in at least 1% of the general population. The human genome contains roughly 10 million SNPs and each individual's genome has a unique SNP pattern. Since they occur at known positions in the genome, SNPs are useful as molecular markers for diseases whose precise genetic cause is unknown.

Table 1 lists the three SNPs associated with the taster and non-taster alleles of the TAS2R38 gene and the nucleotide position in the gene where the SNP appears. Note that these SNPs are associated with codon changes that alter the amino acid sequence of the protein, altering the protein's function. There are eight possible combinations of these three SNPs. However, the SNPs are genetically linked, meaning they are inherited together, and therefore not all combinations are equally likely. A recent study of 1156 Americans found that 53.1% of the study participants had the AVI (non-taster) combination and 42.3% of the participants had the PAV (taster) combination. Other combinations were less common—2.5% had AAV and 1.2% had AAI, for example.

Your Personal Genome

Analyzing the genetic variations between individuals gives clues to the causes of complex diseases like diabetes, cancer, and heart disease, and can reveal an individual's family history and heritage. With the rapid accumulation of genetic data, personalized medicine, the tailoring of medical treatments for an individual's unique genetic background, may soon become a standard part of health care.

Table 1: Three SNPs in the TAS2R38 gene control the ability to taste PTC. AVI is the non-taster (recessive) variant and PAV is the taster (dominant) variant.

Nucleotide Position (bp)	Nucleotide Change		Codon Change		Amino Acid Change	
	Non-taster	Taster	Non-taster	Taster	Non-taster (AVI)	Taster (PAV)
145	G	C	GCA	CCA	Alanine (A)	Proline (P)
785	T	C	GTT	GCT	Valine (V)	Alanine (A)
886	A	G	ATC	GTC	Isoleucine (I)	Valine (V)

The goal of molecular genetics is to connect an observable phenotype, such as a disease or physical characteristic, with the genetic sequence that determines that phenotype. In this lab you will have an opportunity to observe whether you can taste the compound PTC. You will then analyze your DNA to determine whether you are a homozygous non-taster (tt: AVI/AVI), homozygous taster (TT: PAV/PAV), or heterozygous taster (Tt: PAV/AVI). Before the TAS2R38 gene was identified, it was impossible to know the genotype of a taster without analyzing the phenotypes of close relatives. Now that we know the genetic sequence we can uncover your genotype with a few simple tools. Figure 3 illustrates the Mendelian inheritance of the PTC tasting trait.

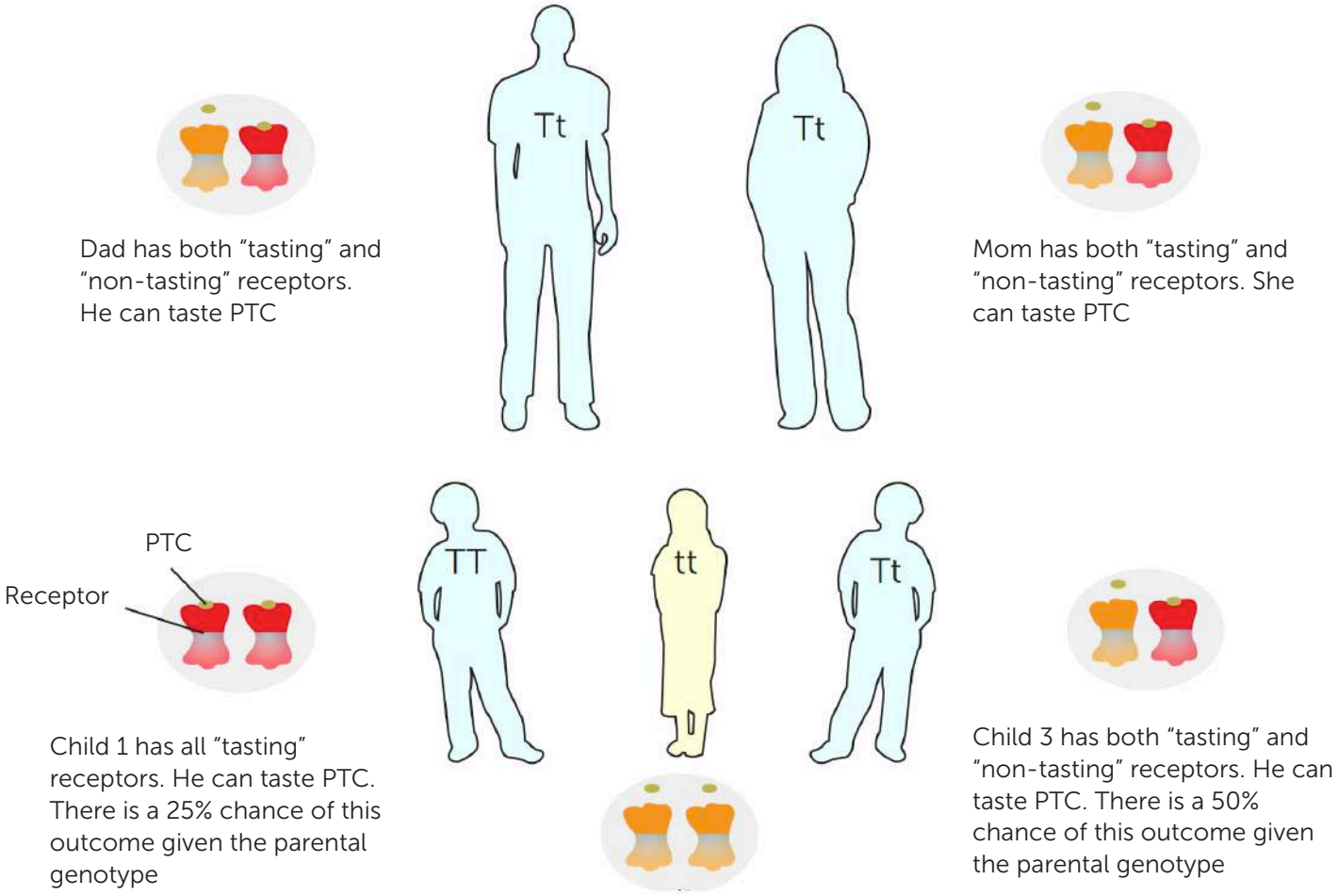


Figure 3. Mendelian inheritance of the PTC tasting trait in a family.

Genotyping with a Restriction Digest

Figure 4 is an overview of the methods you will use to determine your genotype. The first step is to extract genomic DNA from your own cheek cells. Since this extract contains complete chromosomes and far too few copies of the gene to analyze, you will use polymerase chain reaction (PCR) to make billions of copies of a 221 bp region of the TAS2R38 gene that contains the SNP at nucleotide position 145. For details on how PCR works, see Appendix B.

The T and t alleles can be distinguished with a restriction digest assay. Restriction enzymes are known as "molecular scissors" because they cut DNA at a specific nucleotide sequence, called the restriction site. The cutting of DNA with a restriction enzyme is called a restriction digest. These enzymes are made naturally by bacteria as a defense against invading viruses.

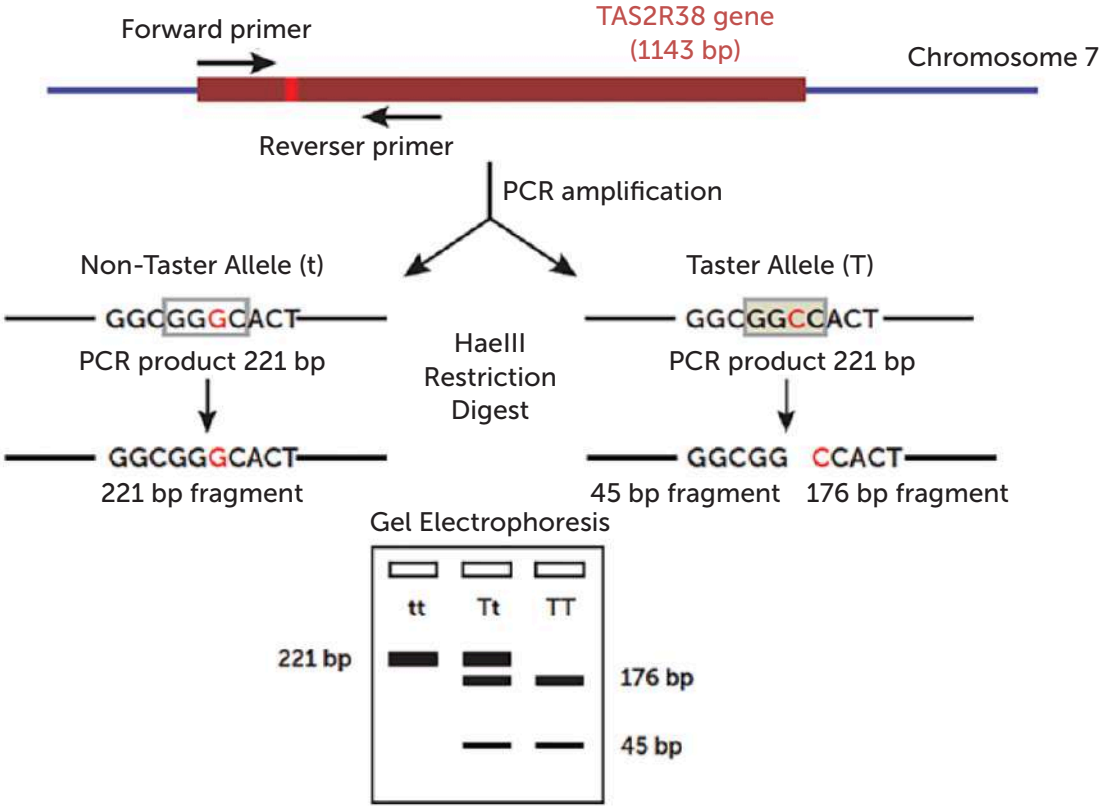


Figure 4. Overview of PTC taster genotyping using PCR, HaeIII restriction analysis, and gel electrophoresis.

In this lab we will use a restriction enzyme called HaeIII, which recognizes the sequence GGCC. When the HaeIII enzyme encounters this recognition sequence it will cleave both DNA strands between the G and C nucleotides, resulting in two DNA fragments. Fragments that are cut give two bands on an agarose gel and fragments that are not cut give one band. This leads to a unique pattern of bands for each genotype. The PCR product of non-tasters will have a single band. The PCR product of tasters that are homozygous dominant will have two bands and tasters that are heterozygous will have three bands (Figure 4). For details on how gel electrophoresis is used to separate DNA fragments of different sizes, see Appendix C.

Day 1 - PTC Taste Test and DNA Extraction

An extraction yielding high quality DNA is the essential first step for analyzing your DNA sequence. Today you will collect your own cells then use heat and a high pH solution to break open the cells to release genomic DNA into solution. You will amplify a segment of this DNA during the rest of the lab to determine your TAS2R38 genotype.

Materials for Common and Student Workstations

Common workstations

Benchtop microcentrifuge

MiniOne® PCR System

Mobile device with MiniOne® PCR App

Gloves, Goggles, Lab Coats

Each student group and workstation

MiniOne 2-20 µL micropipette

2-200 µL Universal micropipette tips

3 mL saline solution (1 cup per student)

Extraction solution (250 µL)

Taste Control and PTC taste papers

0.2 mL PCR tubes (2 per student)

Fine point permanent marker

Waste container for pipette tips and tubes

PCR tube rack

Microcentrifuge tube rack

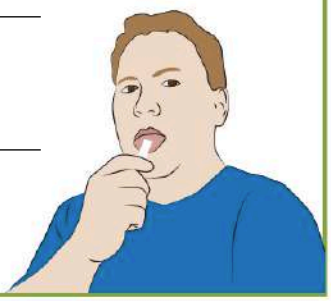
Day 1 - Experimental Procedure
PTC Taste Testing & DNA Extraction

1 Place the Taste control test paper on your tongue.

What happened? _____
(observation)

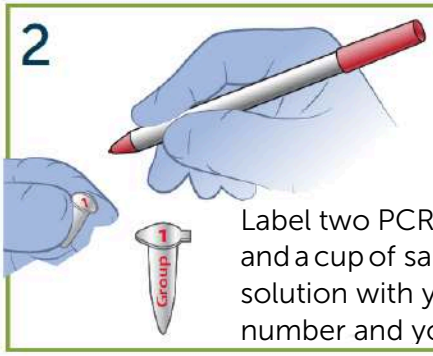
Place the PTC test paper on your tongue.
What happened? _____
(observation)

Why do some of your classmates taste a bitter taste and others do not?



Working in groups of 4:Check your workstation to make sure you have all the materials.

2

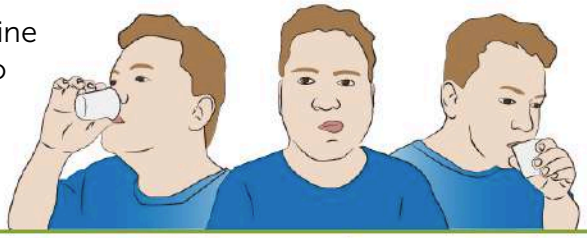


Label two PCR tubes and a cup of saline solution with your group number and your initials.

3

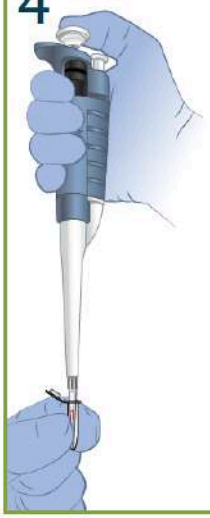
Swish vigorously for 2 minutes

Pour the saline solution into your mouth



Carefully expel the saline solution back into the cup

4



Pipette 200 µL of your spit into one of the labeled PCR tubes.

If you do not have a 20-200 µL micropipette, a 2-20 µL pipette can be used to pipette 10 x 20 µL of spit into the PCR tube

You may want to play the DNA Song listed in Recommended Reading (Appendix D). Tell students to keep swishing until they hear the phrase "spiral staircase" (about 2 minutes).

Discard PTC strips as biological waste.

Day 1 - Experimental Procedure
PTC Taste Testing & DNA Extraction (cont'd)

5

Close the PCR tube tightly but do not squeeze on the thin wall, or otherwise, hairline cracks can occur

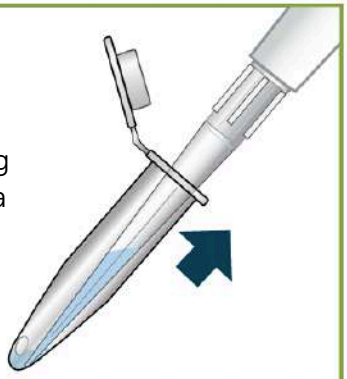
3 min.
8000 RPM

Make sure that the centrifuge is balanced and that PCR tubes are placed in the smallest holes. Use an adaptor if necessary.
**Tip:remember which side of the tube is facing the outside of the centrifuge to make it easier to find the pellet.*

6

Look for a white cell pellet at the bottom of the tube. Carefully remove the supernatant (liquid above the pellet) by either pouring or pipetting the supernatant into a biological waste container.

Be very careful not to disturb the cell pellet!

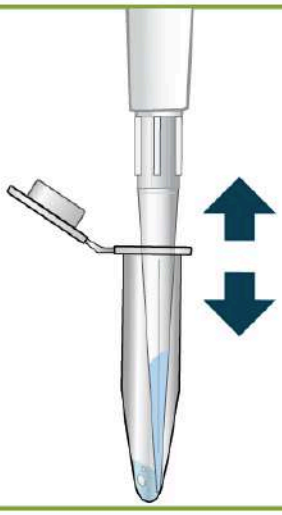


7

Use a 20-200 µL micropipette to add 50 µL of the extraction solution to the PCR tube with the cell pellet. Resuspend the cells by gently depressing and releasing the plunger on the micropipette several times.

Continue to re-suspend until the pellet is broken up and there are no large clumps of cells remaining.

Close the PCR tube tightly.



Day 1 - Experimental Procedure
PTC Taste Testing & DNA Extraction (cont'd)

8 Place the tube in the PCR machine

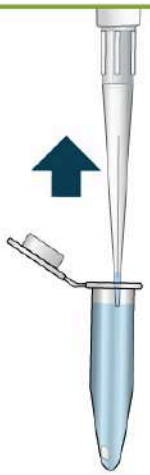
9 Using your mobile device with MiniOne® PCR mobile app, program the PCR machine using constant temperature mode to incubate the samples at **95°C for 300 seconds** (5 minutes) to break open the cells and release the DNA into solution.

Enter 4°C for final incubation temperature. This will keep your samples cold until you are able to pick them up.

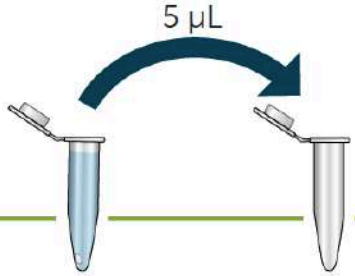
10

Retrieve your tube from the PCR machine and spin the tube 1 minute at minimum 8,000 RPM in a benchtop centrifuge to collect the cell debris at the bottom of the tube.

11



Without disturbing the pellet of cell debris at the bottom of the tube, carefully pipette 5 µL of the DNA-containing supernatant into your 2nd labeled PCR tube. Use the DNA immediately to set up your PCR reaction or store the DNA in the freezer for the next lesson.



Day 1 - Analysis Questions

1. Did the PTC paper taste bitter? Based on your ability to taste PTC what possible genotypes could you have?

2. If a person can taste PTC, what are their possible genotypes?

3. Draw and label a flow chart or diagram of what happens to your DNA sample as a result of the following steps in the extraction protocol. For each step, explain what is happening at the cellular/molecular level.

- Swishing saline solution in your mouth. What does the swishing process do? What does the salt do?

•Heating the sample

•Centrifuging the sample after heating

4. When you extract your DNA using the protocol above, what else is being extracted from the cells besides DNA?

5. Besides studying the TAS2R38 gene, describe two scientific questions that could be explored by studying your DNA sample.

Day 2 - Set up and Run Your PCR Amplification

On Day 1, we extracted total genomic DNA from your cheek cells. Today we will be using PCR to make billions of copies of a small region of the TAS2R38 gene with an SNP that will allow us to distinguish the taster from the non-taster variants with a restriction digest.

Materials for Common and Student Workstations

Common workstations


Benchtop microcentrifuge

MiniOne® PCR System


Mobile device with MiniOne® PCR App


Gloves, Goggles, Lab Coats

Each student group workstation


MiniOne 2-20 µL micropipette


2-200 µL Universal micropipette tips


1 PCR tube per student

45 µL Taq PCR MasterMix (2X)

25 µL PCR Primer Mix

Extracted DNA sample from Day 1 (for each student)


Fine point permanent marker


Waste container for pipette tips and tubes

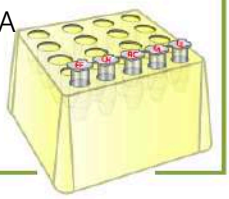

PCR tube rack


Microcentrifuge tube rack

Day 2

Day 2 - Experimental Procedure
Set up and Run Your PCR Amplification

1 Retrieve your extracted DNA sample



2

PCR Setup (one for each student)

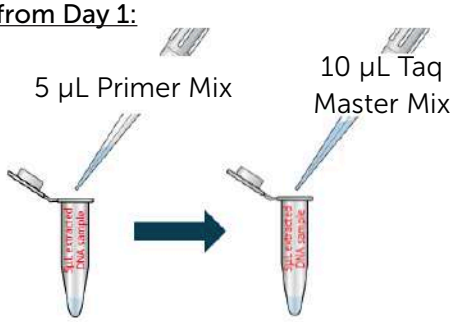
Reagent	Volume
Extracted DNA Sample	5 µL
Primer Mix	5 µL
Taq PCR Master Mix (2X)	10 µL
Total	20 µL

*Tip - add small volumes directly to the bottom of the PCR tube to avoid having bubbles trapped at the bottom of the tube.

Add the following 2 components to your 5 uL of extracted DNA from Day 1:

5 µL Primer Mix

10 µL Taq Master Mix



3 Cap tube tightly and gently flick tube to mix reagents

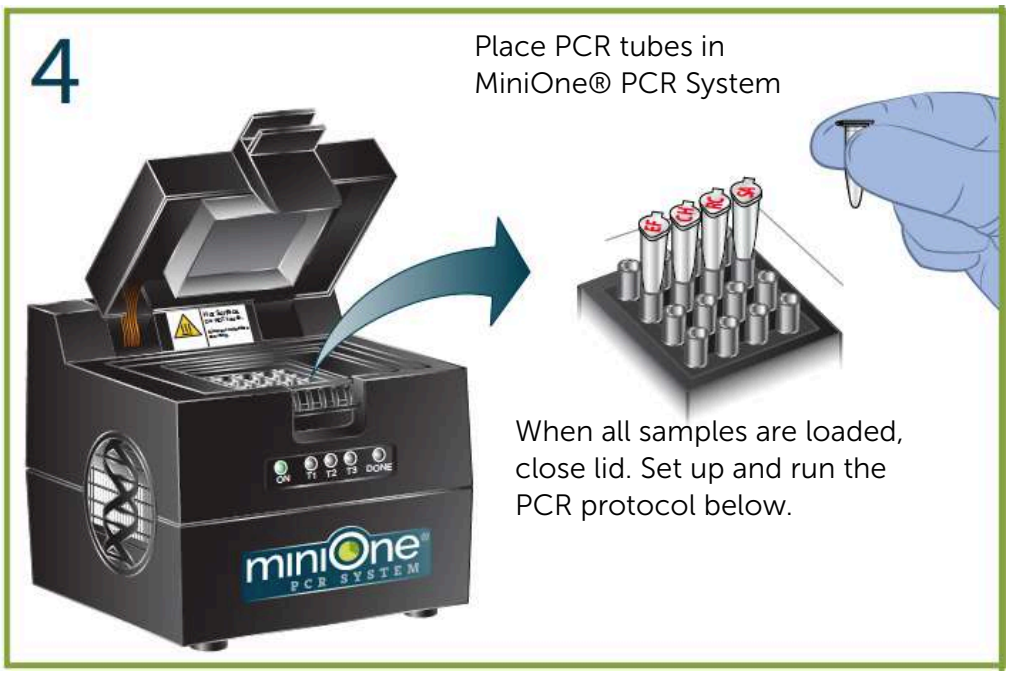
Do not squeeze on the thin wall!

15 sec.
8000 RPM

Be sure centrifuge is balanced

Day 2

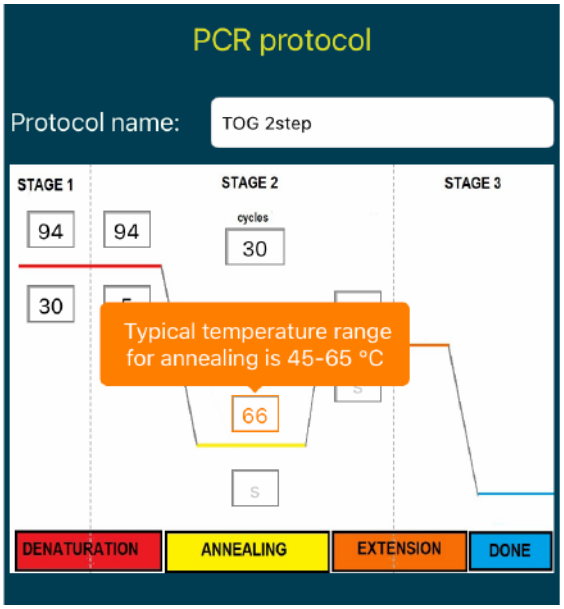
Day 2 - Experimental Procedure
Set up and Run Your PCR Amplification (cont'd)



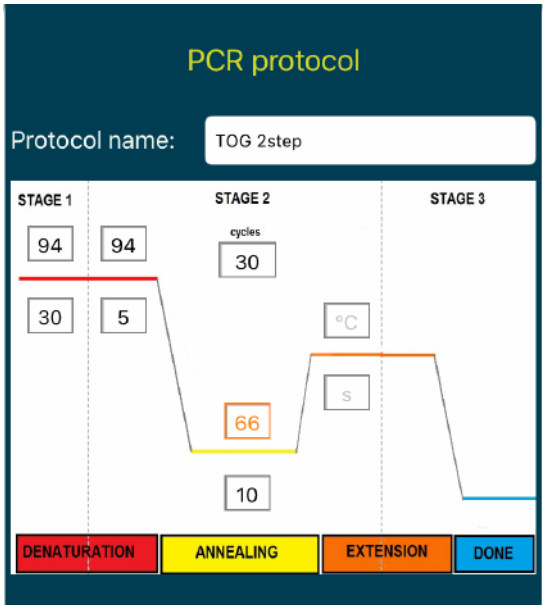
Cycling protocol for fragment amplification:

Step	Duration	Temp	Cycles
Initial Denaturation	30 sec	94°C	
Denaturation	5 sec	94°C	30 cycles
Annealing	10 sec	66°C	
Extension	15 sec	66°C	
Final Incubation	∞	4°C	

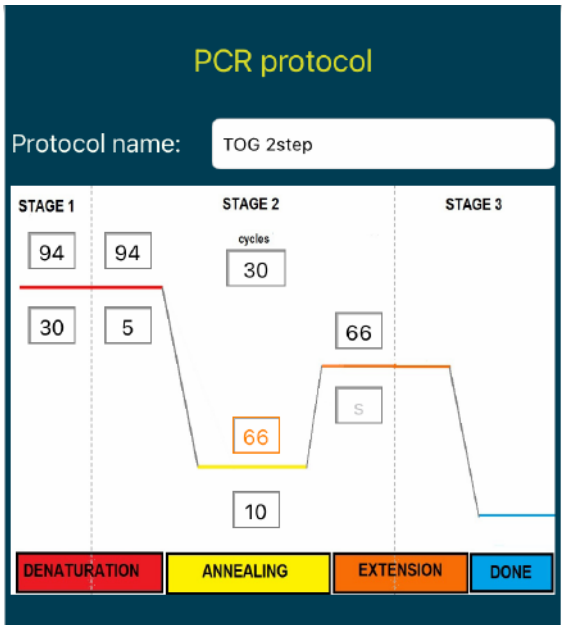
Programming the Annealing and Extension Steps



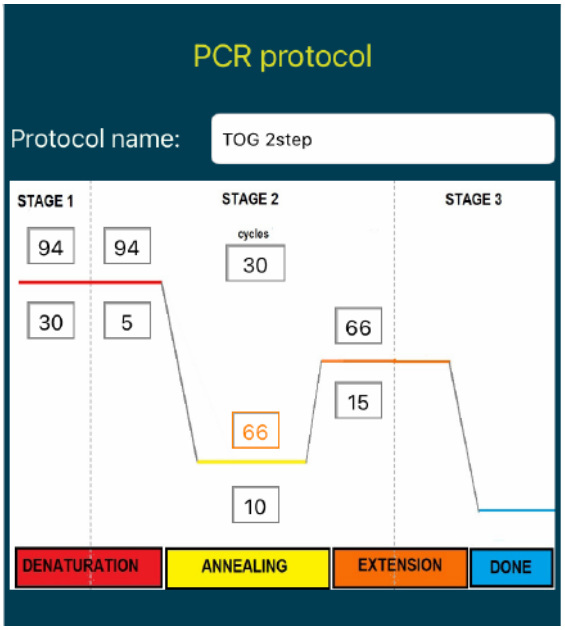
Step 1: Enter 66°C for the Annealing Temperature. When you see a message about the typical temperature range for annealing being 45°-65°C, you can disregard this message and move to the next entry.



Step 2: Enter "10" for the duration of the Annealing Temperature

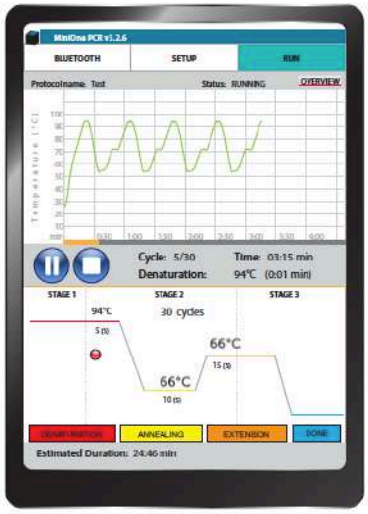


Step 3: Enter 66°C for the Extension Temperature. If you see a message about the typical temperature range, you can disregard this message and move to the next entry



Step 4: Enter "15" for the duration of the Extension Temperature

5 Use the MiniOne® PCR mobile app to monitor the progress of the reaction



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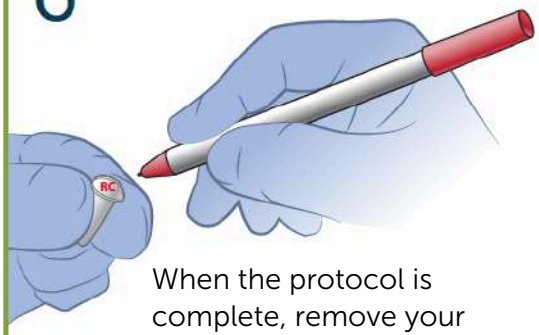
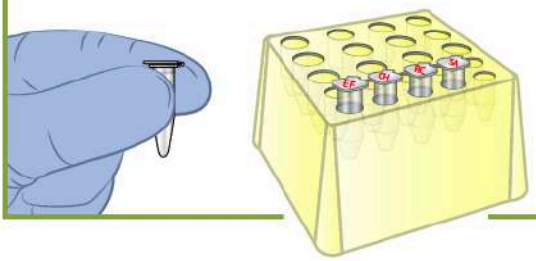


When the protocol is complete, remove your sample from the MiniOne® PCR System. Re-label the tube if needed.

7 Give your amplified DNA sample to your teacher to store until the next lesson.



Day 2 - Analysis Questions

1. What is the purpose of PCR? Describe three situations in which scientists would use PCR.

2. What is the reagent that makes the PCR reaction specific for the TAS2R38 gene? Describe the function of this PCR reagent.

3. Why is the PCR step necessary? Why can't we go directly from DNA extraction to analysis?

4. Sometimes during PCR, the forward and reverse primers stick to each other forming a "primer-dimer" which is then copied in subsequent cycles and may appear as an extra band on your gel. Is primer-dimer more or less likely to happen in a PCR reaction that uses long primers? Why?

Day 3 - Restriction Digest and Gel Electrophoresis

Today you will do a restriction digest of your amplified DNA and analyze the products with gel electrophoresis on an agarose gel. You will run digested and undigested PCR products next to each other on the gel. The pattern of bands in the digested sample will indicate your genotype for the PTC taster gene. Think carefully about what pattern of bands you would expect to see for each genotype. Running the undigested PCR product will confirm the success of your PCR reaction, show which bands are PCR artifacts, and provide a visual benchmark for the size of the 221 bp PCR product.

Materials for Common Workstations

Common workstations

Benchtop microcentrifuge

MiniOne® PCR System

Mobile device with MiniOne® PCR App

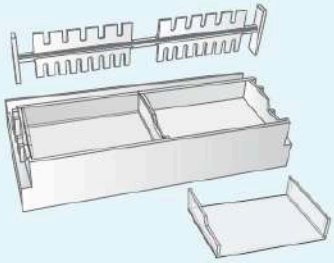
Gloves, Goggles, Lab Coats

Day 3

Day 3 - Student Group Workstations
Restriction Digest and Gel Electrophoresis

Each student group workstation

MiniOne® Electrophoresis System



MiniOne® Casting System

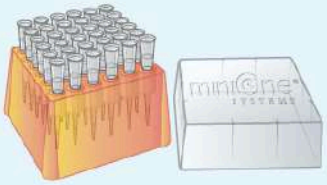
1X TBE running buffer (135 mL)

2% agarose GreenGel™ Cup, TBE

Waste container for pipette tips and tubes

12 µL MiniOne® DNA Marker

MiniOne 2-20 µL micropipette



2-200 µL Universal micropipette tips

1 PCR tube per student

25 µL enzyme dilution buffer

Diluted 25 µL HaeIII restriction enzyme

30 µL Sample Loading Dye (5X)

Fine point permanent marker

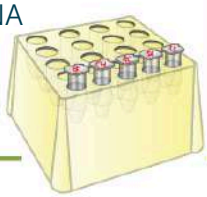
PCR tube rack

Microcentrifuge tube rack

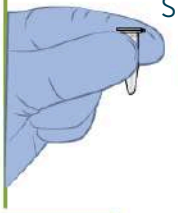
Day 3

Day 3 - Experimental Procedure
Restriction Digest and Gel Electrophoresis

1 Retrieve your amplified DNA sample.



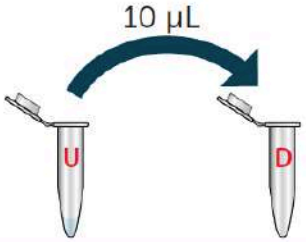
2



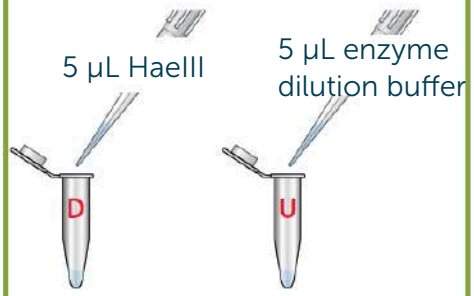
Spin down briefly

3 Pipette 10 µL of your PCR product into a clean PCR tube. Label the clean PCR tube as "D" and the tube containing the rest of the amplified DNA sample as "U".

Also mark your initials on the tubes.



4 Add 5 µL HaeIII restriction enzyme to tube "D" and 5 µl enzyme dilution buffer to tube "U".



5 Cap tubes tightly and flick tubes to mix reagents.

Do not squeeze on the thin walls.

15 sec.
8000 RPM

Be sure centrifuge is balanced

Day 3

Day 3 - Experimental Procedure
Restriction Digest and Gel Electrophoresis (cont'd)

6

Place PCR tubes in MiniOne® PCR System

Incubate restriction digest at 37°C for 900 seconds (15 minutes) using constant temperature mode. Enter 4°C for final incubation temp.

While you are waiting for your digest, prepare the MiniOne agarose gel

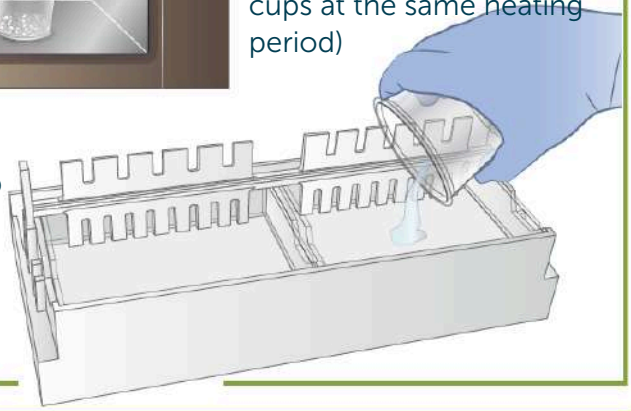
7

Vent gel cup by peeling back slightly prior to microwaving

Prepare 2% agarose gel using MiniOne® Casting System (microwave for 20 seconds, no more than 5 cups at the same heating period)

Use 9-well side of comb

Allow gel to solidify for 10-15 min.



8

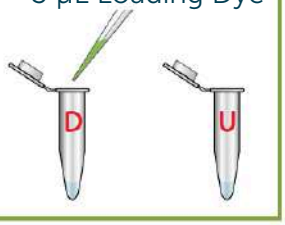
When incubation is complete, remove your sample. If you are doing electrophoresis in a separate lab session give your digests to your teacher to store.

Day 3

Day 3 - Experimental Procedure
Restriction Digest and Gel Electrophoresis (cont'd)

9 Add 3 µL LD to each tube containing your "D" or "U"

3 µL Loading Dye

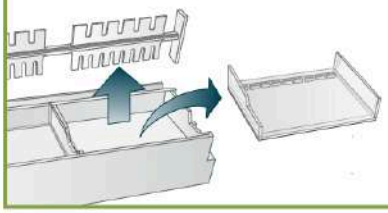


10 Cap tubes tightly and flick tubes to mix reagents

15 sec.
8000 RPM

Be sure centrifuge is balanced

11 Carefully remove comb from gel.



Remove gel tray with solidified gel.

Wipe off excess agarose from bottom of tray.

12 Connect the power supply to the back of the MiniOne® Electrophoresis carriage. Plug the power supply into the wall.

Place gel and gel tray into tank.

Make sure wells are aligned with marks on black viewing platform on negative end.

13 Pour 135 mL TBE running buffer er into one side of tank, allowing liquid to push the air out from under gel tray, creating an even background without trapped air bubbles for clear imaging of results.

Pour the remaining buffer into the other side of the tank.

Day 3

Day 3 - Experimental Procedure
Restriction Digest and Gel Electrophoresis (cont'd)

14 Turn on the low intensity blue light

Load 10 µL of the MiniOne® DNA marker into one well per gel

Load 10 µL of your undigested sample "U" and 10 µL of your digested sample "D" into two adjacent wells

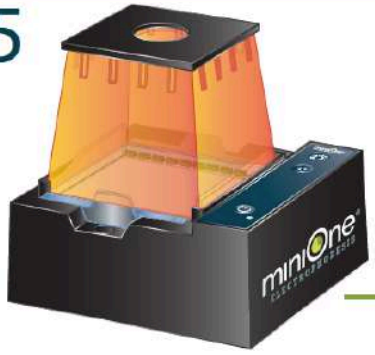
Use the Table 5 to keep track of which group member loads their sample

Table 5. Record the sample loaded into each lane

Well	1	2	3	4	5	6	7	8	9
Sample									

15 Turn on blue light, place photo hood on the carriage unit, and press the power button.

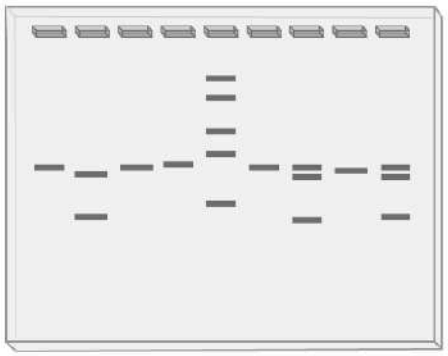
Run MiniOne® Electrophoresis System for 25 minutes or until bands have clearly separated.



16 At the end of the run, turn on the high intensity blue light

Use your phone or camera to take a picture of your gel

Sketch or paste a picture of your gel on the Gel Analysis Worksheet or lab notebook

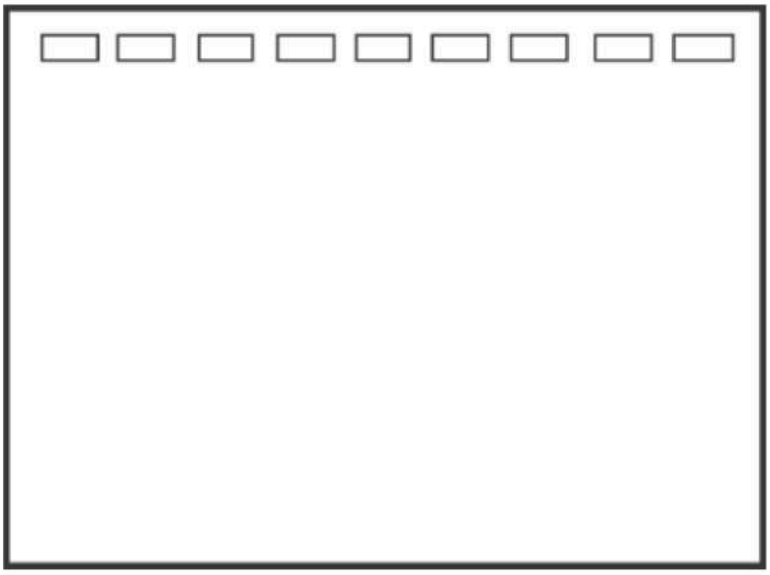


Day 3

Gel Analysis Worksheet

Directions: After completing the gel electrophoresis portion of A Taste of Genetics, record an image of your gel and draw the results on the template below.

Well	Sample
1	
2	
3	
4	
5	
6	
7	
8	
9	



Post-Lab Analysis Questions

1. Explain how gel electrophoresis is used to separate DNA fragments and create a distinct pattern of bands for each PTC genotype.

2. The sizes of the bands in the MiniOne® Molecular Weight Marker are 100, 300, 500, 1000, and 2000 bp. Estimate the sizes of the bands in your PCR lanes by comparing the molecular weight marker lane. Are your estimates consistent with the sizes expected by the digest? If not, what do you think accounts for the difference?

3. Did all your team's samples produce PCR product? If not, explain what could have gone wrong.

4. Did you observe any other bands on the gel besides the expected PCR products? If yes, explain what could have caused the unexpected bands?

5. What is your TAS2R38 genotype based on your gel electrophoresis results? Does this match what you predicted based on your ability to taste the PTC paper?

6. Based on your understanding of Mendelian genetics, is it possible for two parents who both cannot taste PTC to have a child who can taste PTC?

7. If one parent is homozygous for the non-taster variant of TAS2R38 and the other parent is heterozygous, what is the probability that their next child will be a taster?

8. PTC is a compound that does not exist in nature. Do you think there is any evolutionary advantage associated with having the taster allele? Could there be an advantage to not having the taster allele?

9. Aside from genetics, what other reasons might explain why one person is able to taste PTC while another person cannot?

10. Most frequently, the three SNPs on the TAS2R38 gene are inherited together. Describe a situation where a child could inherit a set of SNPs from their mother that is different than the set of SNPs on either of their mother's chromosomes.

Appendix A - Glossary

Term	Definition
Allele	One of two or more distinct forms of a gene located at the same position on homologous chromosomes.
Annealing	As the temperature of a PCR reaction is lowered, short pieces of DNA, called primers, bind to specific sequences within the genome targeting this region to be copied. Annealing temperature is specific to the primers used in your reaction–typical settings are 45-65°C for 5-30 seconds per cycle.
Buffer	A salt added to aqueous solution that helps maintain a consistent pH. Buffers are essential in PCR because the DNA polymerase's function is sensitive to pH changes.
Cycle	A cycle refers to one round of denaturation, annealing, and extension steps of the PCR reaction. The number of cycles needed for a particular reaction will depend on how much DNA you are starting out with and how much DNA you are trying to produce. With high starting concentration, 20-25 cycles is sufficient to produce enough DNA to visualize on a gel. Where the starting concentration is low or large quantities of product are needed, 35-40 cycles can be used.
Denaturation	Denaturation uses high temperature to break the bonds between bases on opposing strands. Double-stranded DNA is split into single- stranded DNA exposing the bases so they can be copied. Typical settings are 90-98°C for 5-30 seconds per cycle.
dNTPs	Nucleotides, the molecular building blocks of DNA.
Enzyme	An enzyme is a biological catalyst that speeds up a chemical reaction without changing the products or being consumed by the reaction. Most enzymes are proteins and they control a wide range of reactions in cells, from copying DNA to extracting energy from food.
Extension	At around 70°C the polymerase gets to work and starts adding nucleotides (dNTPs) to the 3' end of the annealed primers, copying the complementary strand. Typical settings are 72°C for 5 seconds – 5 minutes per cycle.
Final Extension	In some protocols an additional extension step is used. This ensures that the polymerase can add the final base pairs onto the end of the strands, which is necessary in some applications. The typical duration is 2-10 minutes.
Genotype	The genetic makeup of an individual organism.
Initial denaturation	When copying a piece of genomic DNA, an initial denaturation step is often used to make sure the long strands of DNA are fully separated and freed from bound proteins before thermal cycling begins. Typical settings are 90-96°C for 30 seconds-10 minutes.
Monomer	A molecule that can be bonded with other similar molecules to form a polymer.
Phenotype	The set of observable characteristics of an individual organism arising from the genotype and the environment.
Polymer	A molecule that consists of many similar units bonded together.
Primers	Short pieces of DNA with sequences complementary to the sequences flanking the region to be copied. Primers are designed specifically for every PCR reaction taking many variables into account, including length, nucleotide content, and structural features. Many computer tools are available to assist in primer design.
Recognition Site	The specific DNA sequence that a restriction enzyme recognizes and binds to.
Restriction Enzyme	An enzyme produced by bacteria that can cut a DNA strand at a specific sequence.
Single Nucleotide Polymorphism	A type of genetic variation where the genome sequence of two individuals differs at a single nucleotide position. SNPs are the most common form of genetic variation in humans, occurring once every 300 base pairs on average.
Template	DNA containing the sequence that will be copied in a PCR reaction. Can be a short fragment or a whole genome.
Thermal Cycler	Also called a PCR machine, a thermal cycler is an instrument that automatically changes the temperature of the PCR reaction according to a program set up by the user. It heats and cools the reaction between denaturation, annealing, and extension temperatures over a specified number of cycles.

Appendix B - Polymerase Chain Reaction

Polymerase chain reaction (PCR) addresses two major challenges in molecular biology producing usable quantities of DNA for analysis and isolating a specific region of the genome. First, the DNA that scientists want to analyze is often collected in extremely small quantities (for example, a single drop of blood from a crime scene). To have usable quantities of DNA we must make many copies using the original DNA as a template. Second, among the whole genome we must find only the part we want to analyze, whether it is a gene that causes a disease or a sequence that can help identify a species. This is no small matter since the human genome is over 3 billion base pairs (bp) long and we are often interested in regions less than 300 bp long.

The History of PCR

As with many ideas in biotechnology, nature provides us with most of the tools we need. Every time a cell divides it makes two replicates of its entire genome with the help of specialized enzymes. The phenomenon of complementary base pairing should give you a hint as to how a specific region can be targeted. In the late 1970s Frederick Sanger developed a method for copying DNA in vitro that used short pieces of DNA, called **primers** to initiate replication by a DNA polymerase, similar to the RNA primers in your model of cellular DNA replication. An artificial primer used in the Sanger method has a sequence that allows it to bind at only one location in the target DNA sequence.

Using only one DNA primer, the Sanger method can only produce one copy of the target at a time. In 1983 Kary Mullis proposed a modification to this method where a second primer is used to initiate replication using the first copy as a template. Recall that DNA has an antiparallel structure where one strand runs in the opposite direction relative to the second strand. To use the first copy as a target for replication, the second primer that Mullis added needed to initiate replication in the opposite direction, thus one primer is called the forward primer and the other is called the reverse. With the original sequence and its copy serving as templates, two copies are produced instead of one. Further, these two copies can each be used as templates in a second round of copying, which produces four copies. After each round, or cycle, the number of copies has doubled. Over multiple cycles, billions of copies of the DNA sequence between the two primers are generated. This method is called **Polymerase Chain Reaction (PCR)** – polymerase because of the enzyme that is used to copy DNA and chain reaction because the products of one cycle serve as templates for the next round of copying. This rapid and efficient technology for generating usable quantities of DNA won Mullis the Nobel Prize in Chemistry. The first application of PCR was a test for sickle cell anemia.

How PCR Works

Instead of trying to recreate the cell's intricate biochemical machinery in a test tube, scientists rely on heat to control the various steps of the PCR reaction. For DNA to be copied the nucleotide bases must be exposed, which means double stranded DNA must be separated into single strands. Just as heat applied to an ice cube weakens the hydrogen bonds between water molecules and causes a phase transition to liquid water, heat applied to double-stranded DNA weakens hydrogen bonds between bases causing separation of the strands into single-stranded

DNA. As with ice, this is sometimes called melting, but is commonly referred to as **denaturation**. In a PCR cycle, denaturation is performed at 90-98°C for 5-30 seconds. This temperature is just below the boiling point of water.

Once the template DNA has been separated into single strands, the temperature is lowered to encourage primers to bind to their target sequences. Just as in the water analogy, lower temperature favors the formation of hydrogen bonds between molecules, in this case the primer and the template DNA. This step, called **annealing**, is typically between 45 and 65°C for 5-30 seconds. The ideal temperature and duration of the annealing step depends on the sequences of the primers being used. It must be low enough that hydrogen bonds are able to form between the primer and specific complementary sequence, but not so low that nonspecific, or random, binding between primer and template occurs.

DNA polymerase binds to the primer-template complex and begins to add nucleotides (dNTPs) onto the 3' end of the primer. This step, called **extension**, results in a new copy attached to the template as double- stranded DNA. The duration of the extension step depends on the length of the DNA segment being copied and can be anywhere from 5 seconds to 5 minutes.

At this point you may have noticed a problem – DNA polymerase, which the entire PCR process relies on, is a protein enzyme that needs a very specific three-dimensional structure to copy DNA. Before getting to the extension step, the entire PCR mixture, including the polymerase, has already been through the denaturation step where the reaction was heated almost to boiling. Anyone who has cracked an egg into a frying pan will know what high heat does to proteins!

In the early days of PCR, fresh polymerase was added to the reaction tube every cycle to replace the polymerase that had been destroyed by the denaturation step. Having to open the tube and add new enzyme for up to 30 cycles was expensive, inefficient, and increased the chances of contaminating the reaction. The innovation that would remove these obstacles came from an unusual source – Yellowstone National Park.

In the 1970s, scientists had isolated a species of bacteria called *Thermus aquaticus* from a hot spring in Yellowstone. *T. aquaticus* thrives at 75-80°C and can survive much higher temperatures. Its enzymes are similarly heat-tolerant. *E. coli*, the original source for the polymerase used in PCR, thrives at 37.5°C, the same temperature as the gut of mammals.

Since the biochemistry of DNA is similar across the tree of life, polymerase from *T. aquaticus* (called Taq Polymerase) can replace *E. coli* polymerase in PCR, with the modification that the extension step is performed at 70-75°C.

This innovation led to PCR becoming the ubiquitous, inexpensive, and efficient tool that it is today. Reactions can be set up once, sealed inside a tube, and placed in an automated thermal cycler. The thermal cycler (or PCR machine) is a specialized instrument that accurately and rapidly changes the temperature of the tube between denaturation, annealing, and extension. After 20-40 cycles of these three temperatures, billions of copies of the desired DNA product can be produced. The amplified DNA can be analyzed with gel electrophoresis at the end of the experiment or detected as they are being formed using more advanced equipment.

Appendix C - What is Gel Electrophoresis?

Gel electrophoresis is a technique used in many areas of science to analyze the components of complex chemical mixtures. Mixtures of DNA, RNA, proteins, or dyes can be separated into their individual components based on molecular size and electrical charge using a separation matrix within an electric field.

The gel used in gel electrophoresis is a tangle of polymers forming a three-dimensional matrix with water-filled pores through which molecules migrate. A higher density of polymers creates smaller pores. Like the holes in a sieve or colander, the size of the pores has to be the appropriate size for the molecules being separated. Gels can be made from different substances depending on the application. One of the most commonly used and effective materials is agarose, a polymer extracted from seaweed. Agarose gels are formed (or cast) by pouring molten (melted) agarose into a tray where it solidifies into the desired shape as it cools. A comb is placed while the agarose is molten and then removed after it solidifies to create wells where the samples are loaded.

After the gel solidifies it is placed in an electrically conductive buffer between parallel positive ((+) anode) and negative ((-) cathode) electrodes

A voltage is applied between the electrodes, creating a uniform electric field within the gel. Molecules in the wells begin to move under the influence of the electric field: positively charged molecules migrate toward the (-) cathode and negatively charged molecules migrate toward the (+) anode.

The speed of a molecule's movement in an electric field is determined by the strength of its electric charge relative to its molecular weight. This is quantified as the charge to mass ratio. Speed of movement within a gel is also influenced by the size of the molecule relative to the pores in the gel. The polymers in the gel are like an obstacle course: smaller molecules maneuver easily through the pores, traveling faster and farther than large, bulky molecules. However, a large molecule can move faster through a gel than a smaller molecule when the strength of its charge relative to its mass is significantly higher. Shape can also affect how a molecule moves through the gel. Long spaghetti-like molecules will move slower than compact molecules, which slip easily through the pores. Molecules of the same size, shape, and charge will move together and form a distinct band. If multiple types or sizes of molecules are present in the sample, they will separate from each other and each will form a distinct band.

Appendix D - Recommended Reading

Scitable by Nature Education: Background reading about Restriction Enzymes:
https://www.nature.com/scitable/topicpage/restriction-enzymes-545

Explanation of palindromic restriction sequences, from Science Primer:
http://scienceprimer.com/palindromic-sequences

3D Animation of a restriction enzyme binding and cutting DNA from
DNA Learning Center:
https://www.dnalc.org/view/15488-Restriction-digest-3D-animation-with-no-audio.html

DNA electrophoresis sample loading: Video from Kirkwood Community College showing proper technique and some common mistakes:
https://www.youtube.com/watch?v=tTj8p05jAFM

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