Genotype to Phenotype

PTC Taster Lab

Genotype to Phenotype:
PTC Taster Lab
KT-1004-03

Version: 2.4
Release: April 2025

Contents

Getting started

Student guide

Instructor guide

At a glance

Lab overview

Examine the link between genotype and phenotype using yourself as an experimental subject! You will assess your ability to taste the chemical phenylthiocarbamide (PTC) and determine how that ability correlates with your genotype for a gene that encodes a bitter taste receptor protein.

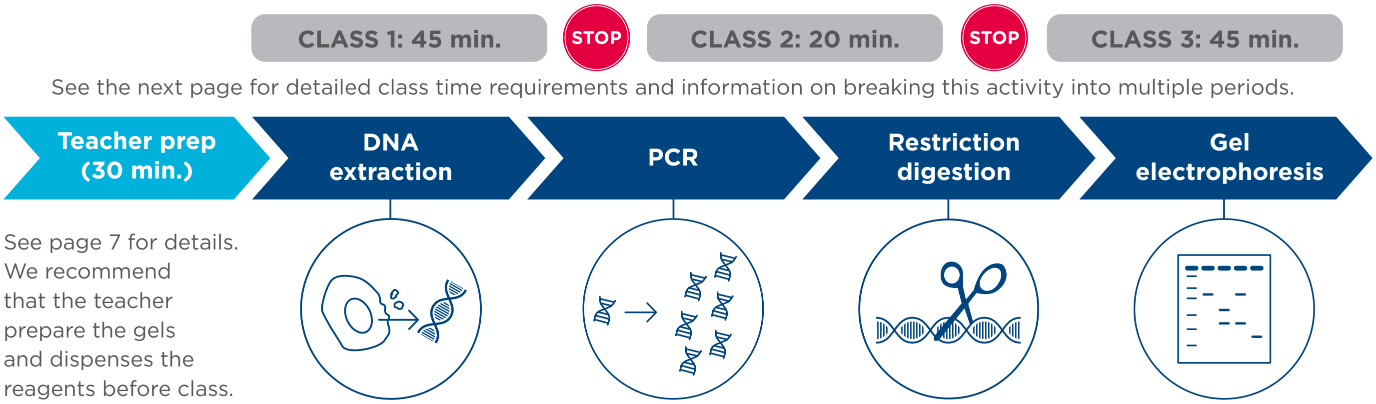
TECHNIQUES	TOPICS	LEVEL
Micropipetting	Genotype to phenotype	General high school
DNA extraction	Inheritance	Advanced high school
PCR	Biotechnology	College
Restriction digestion		
Gel electrophoresis		

Required lab skills

- Students must be proficient in accurately pipetting liquids in the 2-20 µl range.
- Instructional videos, worksheets, and free activities to help students build micropipetting skills can be found at https://www.minipcr.com/micropipetting/.

Planning your time

- This activity has four parts that will likely take place over multiple days.
- The entire experiment can be completed in a single three-hour block.
- The most common classroom implementation timeline is shown below.



CLASS 1: 45 min. | STOP | CLASS 2: 20 min. | STOP | CLASS 3: 45 min.

See the next page for detailed class time requirements and information on breaking this activity into multiple periods.

Teacher prep (30 min.) → **DNA extraction** → **PCR** → **Restriction digestion** → **Gel electrophoresis**

See page 7 for details. We recommend that the teacher prepare the gels and dispenses the reagents before class.

Technical support

If you have any questions about implementing this activity, contact support@minipcr.com.

Class time requirements

This protocol offers some flexibility to help you manage the class time needed.

Steps		Time required
1	**DNA extraction**	15 minutes
STOP	Optional stopping point: The DNA extract can be stored in the refrigerator for up to eight days before proceeding to PCR.	
2	**PCR**	
	A. Set up PCR samples	20 minutes
	B. Run PCR	70 minutes The PCR program can be started during class and left to run without being monitored.
STOP	Optional stopping point: The PCR product is stable at room temperature for several days and can be left in the machine. Transfer tubes to the freezer for long-term storage.	
3	**Restriction digestion**	
	A. Set up reactions	5 minutes
	B. 37 °C incubation	Minimum: 15 minutes Maximum: overnight Longer incubation leads to more complete digestion.
STOP	Optional stopping point: You can freeze the restriction digestion reactions and run them on a gel at a later time.	
4	**Gel electrophoresis**	
	A. Load gel	10 minutes
	B. Run gel	20-30 minutes The gel does not need to be actively monitored during this time.
	C. Interpret results	5 minutes

Materials needed

Supplied in kit (KT-1004-03)

- Kit contains reagents for 32 students.
- Regents can be stored in the freezer for 12 months after receipt.
- Reagents for preparing gels, plastic tubes for distributing reagents to individual groups, plastic tubes for PCR, and pipette tips are sold separately. See below for details.

Contents	Provided	Required	Storage
X-tract™ DNA Extraction Buffer	1000 µl x 2 tubes	50 µl per student	Freezer
2X EZ PCR Master Mix, Load-Ready™	700 µl	15 µl per student	Freezer
PTC Primer Mix	550 µl	15 µl per student	Freezer
Restriction Enzyme Fnu4HI	50 µl	1 µl per student	Freezer
Nuclease Free Water Note: Distilled water can also be used	100 µl	50 µl for an entire class	Freezer
Fast DNA Ladder 2	150 µl	15 µl per group	Freezer

Electrophoresis reagents and plastics sold separately

- This lab requires:
  - 2% agarose gels with a fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®).
  - Plastic tubes for distributing reagents to individual groups and 0.2 ml PCR tubes for running PCR.
- The Learning Lab Companion Kit (KT-1510-01) provides sufficient reagents to make and run eight gels when using the blueGel or Bandit electrophoresis system, as well as plastic tubes for distributing reagents to individual groups and plastic tubes for PCR.
- Alternatively, bulk electrophoresis reagents and plastics (tubes, pipette tips) are available for purchase from miniPCR bio.
- Gel electrophoresis reagents and plastics can also be purchased from other suppliers.

Required equipment

- This lab is compatible with any thermal cycler.
- This lab is compatible with any horizontal gel electrophoresis system in combination with:
    - A fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®).
    - A transilluminator that is compatible with the DNA stain used. Fluorescent DNA stains typically require blue light (~470 nm) or UV (~260 nm) illumination.
- The table below outlines equipment from miniPCR bio that meets these requirements.

AVAILABLE AT MINIPCR.COM

Item	Recommended quantity
miniPCR thermal cycler	Each student will have 1 PCR sample Groups can share machines
Gel electrophoresis and visualization system	
Option 1: blueGel™ OR GELATO™ electrophoresis systems with integrated blue light transilluminator	1 blueGel can be shared by two groups 1 GELATO can be shared by four groups
Option 2: Bandit™ STEM electrophoresis kit paired with the Viewit™ Illumination Kit	1 Bandit + 1 Viewit per group
Option 3: Bandit™ STEM electrophoresis kit paired with a blueBox™ blue light transilluminator	1 Bandit per group + 1 blueBox for the class to share
Micropipettes and tips	
2-20 µl adjustable micropipette	1 pipette per group
20-200 µl adjustable micropipette	1 pipette for teacher

Other materials supplied by user

- Distilled water
- Flat-end toothpicks
- PTC paper
- Microwave or hot plate
- Heat-resistant flask or beaker
- Microcentrifuge (optional)
- Plastic tubes for dispensing reagents (1.5 or 0.2 ml tubes can be used)
- 0.2 ml PCR tubes
- Disposable laboratory gloves
- Protective eyewear
- Fine-tipped permanent marker

Also available

- PTC Taster Lab Control Bands (KT-1004-04): Ready-to-load controls allow students to compare their own genotyping results to known reference bands directly.
- PTC Digital Lab: Interactive companion to our hands-on lab, plus a full lab simulation so students can interpret experimental results no matter what.

Teacher prep

Protective gloves and eyewear should be worn for the entirety of this experiment.

Overview

- Reagents are sufficient for 32 students.
- This activity has four parts that will likely take place over the course of multiple days.
- The table below provides an overview of the teacher prep, and the subsequent pages provide detailed instructions.

Prep	Time required	Timeline
Dispense reagents	10 minutes	Can be completed up to one week before DNA extraction and PCR
Dilute restriction enzyme	5 minutes	Can be completed up to one week before the restriction digestion
Prepare electrophoresis buffer and agarose gels	20 minutes	Varies - If using gel reagents from miniPCR, gels can be prepared and stored for up to five days before use

Dispense reagents

- Reagents are stable at room temperature for 24 hours.
- If you want to dispense reagents further in advance, tubes can be stored in the refrigerator for up to one week.
- This kit provides sufficient reagents for 32 students.

Materials needed for this section

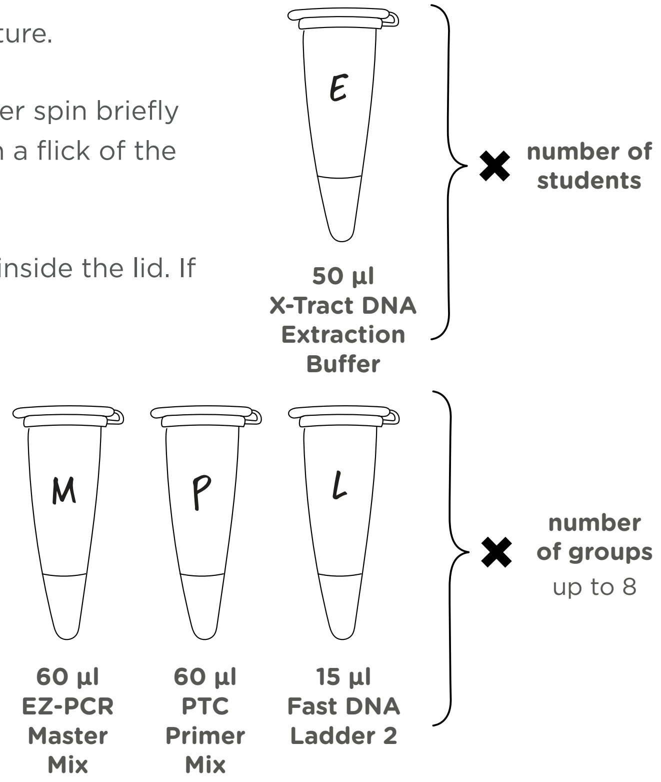
From the lab kit (stored in the freezer):
- X-Tract™ DNA Extraction Buffer
- 2X EZ PCR Master Mix, Load-Ready™
- PTC Primer Mix
- Fast DNA Ladder 2

Leave the Restriction Enzyme Fnu4HI in the freezer

Supplied by user:
- Plastic tubes for dispensing reagents (1.5 ml of 0.2 ml tubes can be used)
- 2-20 µl and 20-200 µl micropipettes and tips
- Fine-tipped permanent marker

1. Thaw reagents by placing tubes at room temperature.
2. Collect the liquid at the bottom of each tube. Either spin briefly in a microcentrifuge or shake the liquid down with a flick of the wrist.
3. When you open each tube, check for liquid stuck inside the lid. If necessary, put the lid back on and repeat step 2.
4. **For each student**, dispense 50 µl of X-Tract DNA Extraction Buffer into a labeled plastic tube that fits your 95 °C heat source. If using a miniPCR thermal cycler for the DNA extraction, use 0.2 ml PCR tubes.
5. **For each group** of four students, dispense the following reagents into labeled plastic tubes. 1.5 ml or 0.2 ml plastic tubes can be used.

- EZ PCR Master Mix (label as tube "M")	60 µl
- PTC Primer Mix (tube P)	60 µl
- Fast DNA Ladder 2 (tube L)	15 µl



Note: Ladder is not needed until Part 4 of the lab, but you can aliquot it now and store in the refrigerator until needed.

6. If you are dispensing the reagents more than 24 hours before class, store the tubes in the refrigerator until use. Reagents can be stored in the refrigerator for one week.

Dilute restriction enzyme

- Because of the small volumes involved, we recommend that the teacher add the restriction enzyme directly to each student's sample.
- We recommend diluting the restriction enzyme to avoid the need to pipette 1 µl volumes. When diluted, 2 µl of restriction enzyme will be added to each student PCR product.
- Diluted restriction enzyme can be stored in the freezer for up to seven days before use.
- If you have access to a 0.5-10 µl micropipette and you are very experienced with pipetting 1 µl volumes, you can skip this step and add 1 µl of undiluted restriction enzyme to each student's sample in Part 3 of the experiment.

Materials needed for this section

From the lab kit (stored in the freezer):

- Restriction Enzyme Fnu4HI
- Nuclease Free Water
  Note: Distilled water can also be used

Supplied by user:

- 20-200 µl micropipettes and tips
- Microcentrifuge (optional)

1. Collect the liquid at the bottom of the tube containing the restriction enzyme. Either spin briefly in a microcentrifuge or shake the liquid down with a flick of the wrist.

2. Carefully open the Restriction Enzyme Fnu4HI tube, making sure no liquid remains trapped inside the screw-top cap. Repeat step 1 if necessary to collect all the liquid in the bottom of the tube.

3. Add 50 µl of Nuclease Free Water to the tube of restriction enzyme.

4. Pipette up and down 10 times to mix very well.

5. If diluting the restriction enzyme the day your students will use it, place the tube on ice until use. If diluting the restriction enzyme in advance, you can store the tube in the freezer for up to seven days before use.

Prepare gel electrophoresis buffer and agarose gels

1. Prepare electrophoresis buffer.
   - Follow the manufacturer's instructions to prepare buffer solution.
   - The volume of buffer needed varies depending on the gel electrophoresis system.
     - For the blueGel and Bandit electrophoresis systems, 600 ml of TBE buffer is sufficient for at least eight gel runs.
     - For other systems, refer to the manufacturer's instructions for:
       (1) The buffer volume needed to prepare agarose gels.
       (2) The buffer volume needed for use as running buffer.

2. Prepare 2% agarose gels with fluorescent DNA stain.
   - You will need one lane for each student, plus one lane for the ladder per gel. If groups are sharing gels, a single lane for the ladder per gel is sufficient.
   - This lab kit is compatible with any molecular grade agarose and fluorescent DNA stain (*e.g.,* SeeGreen™ or GelGreen®).
   - The volume of gel needed varies based on the gel electrophoresis system you are using. Refer to the manufacturer's instructions.
   - If using gel electrophoresis reagents from miniPCR bio, gels can be prepared up to five days in advance. Store prepared gels at room temperature in an airtight container protected from light. Do NOT soak the gels in buffer or wrap them in paper towels.

Detailed instructions for preparing buffer and gels for miniPCR electrophoresis systems

blueGel
https://links.minipcr.com/gelpouring

Bandit
https://links.minipcr.com/BanditDNAgel

Student workstation setup

Part 1: DNA Extraction

	Per student
X-Tract DNA Extraction Buffer (Tube E)	50 µl
Flat-end toothpicks	1
2-20 µl micropipette and tips	
Fine-tipped permanent marker	
Access to a 95 °C heat source (you can use a miniPCR in heat block mode)	

Part 2: PCR

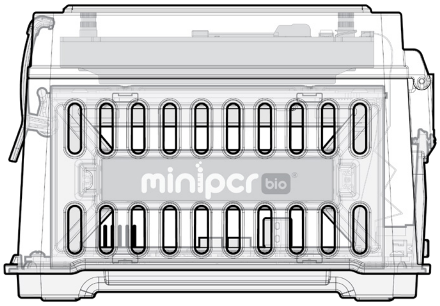
	Per student	Per group of 4
DNA extract from previous step	Each student will have their personal sample	
EZ PCR Master Mix (Tube M)	15 µl	60 µl
PTC Primer Mix (Tube P)	15 µl	60 µl
Empty 0.2 ml PCR tubes	1	4
2-20 µl micropipette and tips		
Fine-tipped permanent marker		
Access to a thermal cycler		

If using miniPCR thermal cyclers:

- Groups will need a miniPCR thermal cycler and power supply.
- Download the miniPCR app from the app store or at www.minipcr.com/downloads.
- Machines can be programmed ahead of time by the teacher or during class by the students.
- Once the program has started, the miniPCR will complete the program even if disconnected from the device running the app.
- If you want to monitor the reaction in real-time during the run, the miniPCR thermal cycler must remain connected to the device running the app.

Detailed instructions for using a miniPCR thermal cycler

https://links.minipcr.com/minipcrRUN



Part 3: Restriction digestion

	Per student
PCR samples from previous step	Each student will have their personal sample
Empty 0.2 ml PCR tube	1
Access to restriction enzyme *We recommend that the teacher add restriction enzyme directly to each student sample	If using diluted restriction enxyme: 2 µl per student If using undilted restriction enzyme: 1 µl per student
2-20 µl micropipette and tips	
Fine-tipped permanent marker	
Access to a 37 °C heat source	

Part 4: Gel electrophoresis

	Per group
Restriction digestion samples from previous step	Each student will have their personal sample
DNA ladder (Tube L)	15 µl per group
Electrophoresis buffer *Volume depends on your electrophoresis system	30 ml TBE if using a blueGel or Bandit
2-20 µl micropipette and tips	
2% agarose gel with fluorescent DNA stain	1 well per student plus an additional lane for ladder

Student guide

Background information

Genetic variation

You might be surprised that for all the variation that you see in people, more than 99% of DNA is the same in all humans. It’s the less than 1% of DNA that is different that makes each of us unique. Those small differences are scattered throughout the genome and can come in a few different forms. The most common type of sequence variation is one where the DNA sequence differs by a single nucleotide (Figure 1). These differences are called *single nucleotide polymorphisms*, or SNPs for short (pronounced ‘snips’).

```
                 *
5’… ATATCATCCTGTGCTGCCTTCATC…3’
3’… ATATCATCCTGTGTTGCCTTCATC…5’
```

Figure 1. Single nucleotide polymorphisms. More than 99% of DNA sequences are the same in all humans. The most common type of genetic difference is a change at a single position in the genome. In the sequence above, the location indicated with an asterisk is a SNP—some people have a cytosine (C) at this location, while others have a thymine (T).

Today we will focus on SNPs in genes. While the term gene can be difficult to define, in this lab we refer to a *gene* as a stretch of DNA that contains the instructions for making a specific protein. Scientists use the term *allele* to describe different versions of a gene. Different alleles for the same gene vary in their DNA sequence, and these sequence differences can affect how the protein is made. In some cases, alleles for the same gene have substantially different sequences due to insertions or deletions of many DNA nucleotides. In other cases, alleles of a gene can vary by a single nucleotide, in other words, a SNP. One of the best-characterized examples of SNPs in a gene influencing a human phenotype relates to a protein involved in our sense of taste.

Genotype specifies phenotype

Humans carry two copies of most genes, one inherited from each parent. We refer to the two copies of any given gene as a person’s genotype for that gene, while we use the word phenotype to describe the person’s observable traits that can be influenced by that gene. Most phenotypes result from a complex combination of both genetic and environmental influences. For example, your height is dependent on both the genotypes of many genes and the quality of nutrition you receive. Other phenotypes have a much more direct link to their underlying genotypes. In this lab we will investigate the ability to taste a particular bitter chemical, called **p**henyl**t**hio**c**arbamide (PTC). In this example, a person’s phenotype (whether or not they can taste PTC) will depend almost entirely on their genotype (which alleles they have for a particular gene).

People detect taste through specialized cells found mostly on the tongue. On their surface, these taste receptor cells contain receptor proteins that bind various chemicals that enter the mouth. The receptor proteins set off a chemical relay within the cell, which then will send signals to the brain, allowing us to perceive tastes (Figure 2).

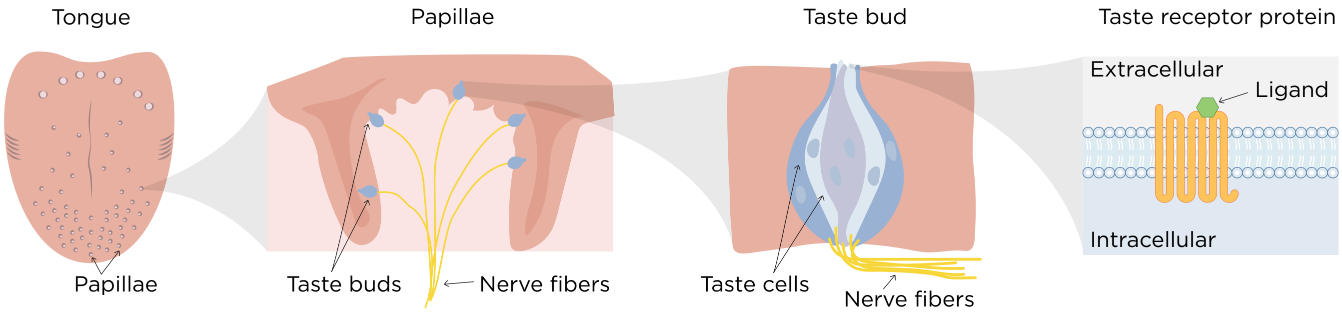


Figure 2. Detection of taste. The tongue is covered in bumps called papillae, many of which contain taste buds. A taste bud is a cluster of specialized taste cells, the tips of which contain receptors that detect various chemicals that enter the mouth. Information collected by taste receptors leads to a signal being sent to the brain where the perception of taste occurs. While there are several types of taste receptors, many are G protein-coupled receptors, including the receptors that detect sweet, umami, and bitter tastes.

Humans can detect several different tastes, including bitter, sweet, umami, salty, and sour. Different families of taste receptors cells specialize in conveying a particular tastes, and contain taste receptor proteins that detect compounds with similar taste properties. For example, bitter taste is mediated by cells that express receptor proteins that bind to bitter molecules.

There are 25 known bitter taste receptors in humans, each responsible for binding to a different molecule or class of molecules. All 25 of bitter taste receptors are a type of protein called a G protein-coupled receptor (GPCR). A wide array of compounds are perceived as bitter, and all bitter compounds are detected by GPCR taste receptors located at the tip of a taste cell. We will focus on one specific bitter taste receptor: TAS2R38. The TAS2R38 protein detects several bitter compounds, including the chemical PTC.

TAS2R38 gene position (bp)	TASTER ALLELE (T)		NON-TASTER ALLELE (t)	
	Codon	Amino acid	Codon	Amino acid
145	**C**CA	Pro	**G**CA	Ala
785	G**C**T	Ala	G**T**T	Val
886	**G**TC	Val	**A**TC	Ile

Figure 3. Polymorphism in the *TAS2R38* gene. The human *TASR38* gene is 1,148 base pairs (bp) long. Three nucleotide positions commonly vary in people: positions 145, 758 and 886. Two alleles are commonly observed in people. The most frequent 'taster' allele has a C at position 145, a C at position 785, and a G at position 886, coding for the amino acids **p**roline, **a**lanine, **v**aline (PAV). The most frequent 'non-taster' allele has a G at position 145, a T at position 785, and an A at position 886, coding for the amino acids **a**lanine, **v**aline, **i**soleucine (AVI). The 'taster allele" encodes a version of the TAS2R38 protein that can detect PTC, while the 'non-taster' allele encodes a version of the TAS2R38 protein that cannot detect PTC.

There are two common alleles of the *TAS2R38* gene that vary by three SNPs (Figure 3). The 'taster' allele (abbreviated T) confers the dominant ability to detect the bitter chemical PTC, while the recessive 'non-taster' allele (abbreviated t) correlates with the inability to detect PTC. Because the 'taster' allele is dominant, typically people with TT or Tt *TAS2R38* genotypes can detect PTC, while people with the tt genotype cannot detect PTC (Figure 4). Scientists don't know for sure how the different SNPs affect the function of the TAS2R38 protein. It may be that the differences in the protein affect how different chemicals bind to the protein, or it may be that the differences affect the protein's ability to send a signal.

TAS2R38 genotype	PTC tasting phenotype
TT	Taster
Tt	Taster
tt	Non-taster

Figure 4. *TAS2R38* genotype specifies PTC tasting phenotype. The ability to taste PTC is dominant, so people who are either homozygous dominant (TT) or heterozygous (Tt) for *TAS2R38* gene can taste PTC, while people homozygous recessive (tt) for the *TAS2R38* gene cannot taste PTC.

The complex relationship between genotype and phenotype

A core principle in genetics is that an organism's phenotype is determined by its genotype. In other words, the traits you have depend on your DNA. But this is often an oversimplification. Most phenotypes are influenced by a complex combination of both genetics and the environment. For example, human height is influenced by many genes, but it is also highly dependent on environmental factors such as diet.

In this lab, you will examine your *TAS2R38* genotype and link it to your PTC tasting phenotype. This is a rare example of a taste phenotype that is known to be linked to specific genotypes in a single gene. We know people experience many foods and tastes differently, but PTC is one of the few examples for which we can link those differences to underlying genetics.

The correlation between *TAS2R38* genotype and an individual's PTC tasting phenotype is not perfect, but nearly all individuals with the tt genotype report that they cannot taste PTC, whereas nearly all people with the TT genotype report that they perceive PTC as bitter. There is more phenotypic variation in heterozygous individuals. Depending on the human population being studied, *TAS2R38* genotypes correlate with the ability to taste PTC in 55-85% of cases (Kim et al., 2003).

Finally, it is important to remember that the *TAS2R38* genotypes that correspond to the PTC taster and non-taster phenotypes differ at three locations in the *TAS2R38* gene. While it is estimated that these differences are inherited together about 93% of the time, as in Figure 3, in some cases they are inherited in different combinations (see the *advanced questions* on page 31). You will only determine your genotype for one of the three SNPs in the *TAS2R38* gene. Scientists aren't sure how much each SNP affects the final phenotype. How might this affect the interpretation of your results?

Today’s lab

Today you will determine your *TAS2R38* genotype and examine if there is a correlation with your PTC tasting phenotype.

Nearly every cell in your body contains your entire genome, so you can isolate DNA to test from almost any cell. In this lab, you will isolate DNA from cheek cells. Then, you will determine your *TAS2R38* genotype using a method known as PCR-RFLP (polymerase chain reaction - restriction fragment length polymorphism). This technique starts by using PCR (polymerase chain reaction). PCR is a method used to make many copies of a specific DNA sequence. In this lab, you will copy a 250 bp segment of the *TAS2R38* gene that spans the SNP at position 785 in the *TAS2R38* gene.

To differentiate between the two *TAS2R38* alleles, you will use a restriction enzyme. Restriction enzymes recognize specific, short DNA sequences (typically 4-8 base pairs long) and cut the DNA there. The restriction enzyme you will use is called Fnu4HI and only cuts the DNA sequence in the ‘taster’ allele. The cut DNA will result in two fragments of approximately 150 bp and 100 bp, while DNA corresponding to the ‘non-taster’ allele will remain intact at ~250 bp. Finally, you will use gel electrophoresis to separate and visualize the DNA to determine if it was cut.

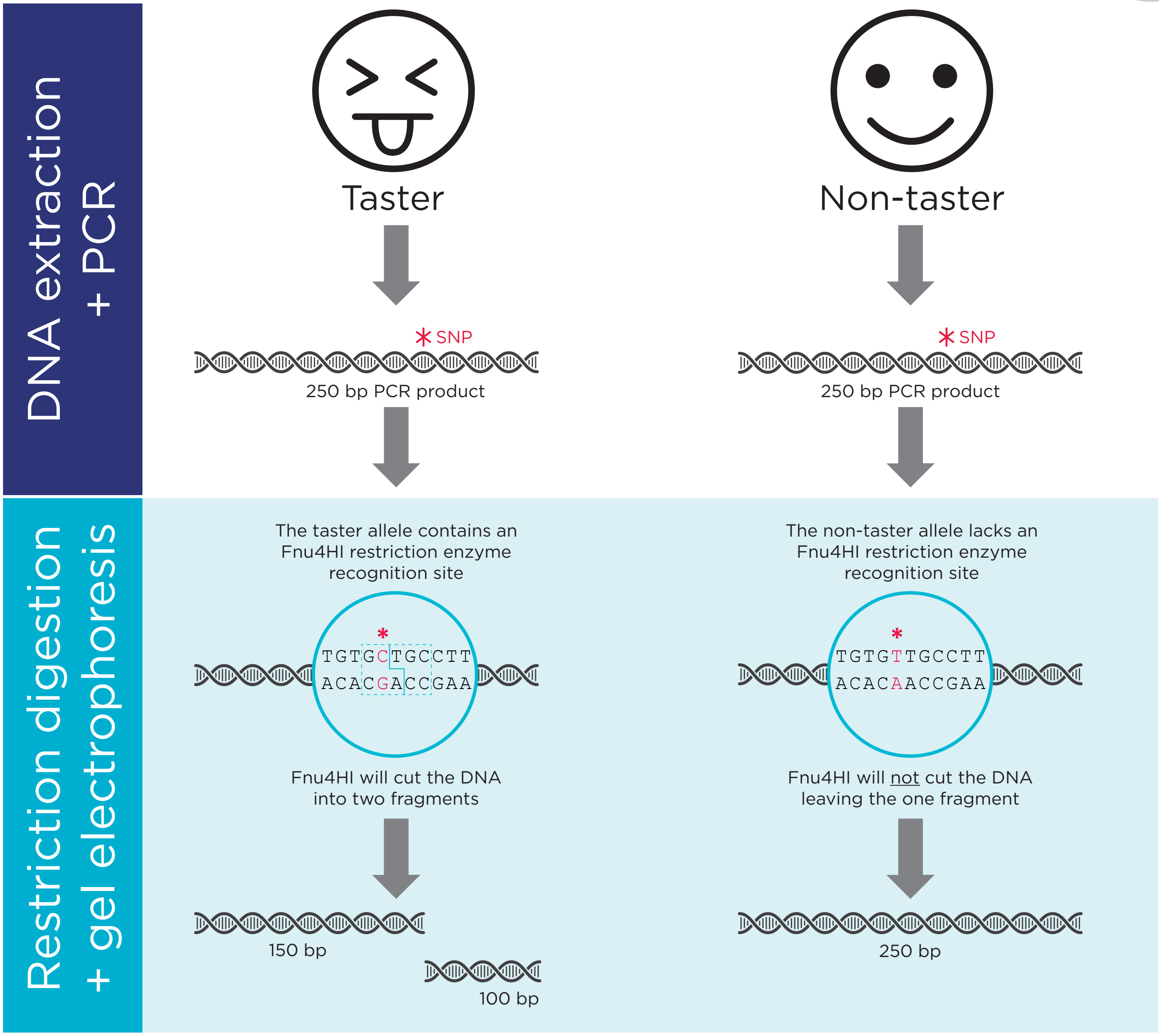


Figure 5. Overview of lab procedure.

Student lab protocol

DNA extraction

! Protective gloves and eyewear should be worn for the entirety of this experiment.

For best results, don’t eat or chew gum for ~20 minutes prior to cheek cell collection.

1. Each group member should receive a tube containing extraction buffer (tube E). Label the tube with your initials. Write on the upper sidewall of the tube.
2. Collect cheek cells by gently scraping the inside of your cheek 3-4 times with a flat-end toothpick. It should not hurt.
3. Dip the end of the toothpick with your cheek cells into the extraction buffer in your individual tube. Swirl the toothpick to dislodge the cells, then dispose of the toothpick.
4. Close the cap on the tube. When it is closed correctly, you should feel the cap “click” into place.
5. Incubate your tube for 10 min at 95 °C. You can use a miniPCR thermocycler in Heat Block mode, a water bath, or another heat block.
6. Remove your tube from heat and proceed to setting up the PCR.

Optional stopping point: The DNA extract can be stored in the refrigerator for up to eight days before proceeding to PCR.

Set up PCR samples

1. Label a new 0.2 ml PCR tube with your initials followed by “P” for PCR. Write on the upper sidewall of the tube.
2. Add PCR reagents to the labeled tube according to the table below. To prevent contamination, use a new tip for each addition.

Master Mix (Tube M)	12.5 µl
Primer Mix (Tube P)	12.5 µl
Student DNA sample from previous step	3 µl
Total volume	28 µl

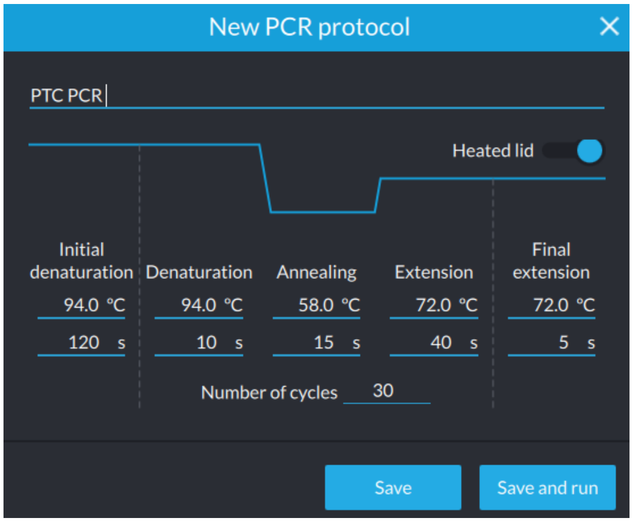
3. Close the cap on the tube. When it is closed correctly, you should feel the cap “click” into place.
4. Flick the tube to mix the contents. If available, a vortex mixer can be used.
5. Make sure all the liquid is at the bottom of the tube. If there is liquid stuck on the sides of the tube, shake it down with a flick of the wrist or a brief spin in a microcentrifuge.
6. Proceed immediately to the next section of the protocol.

Run PCR

- Program your thermal cycler with the following parameters:

Initial denaturation	94°C, 120 sec
Denaturation	94°C, 10 sec
Annealing	58°C, 15 sec
Extension	72°C, 40 sec
Number of cycles	30
Final extension	72°C, 5 sec

- The PCR takes approximately 70 min when using a miniPCR® thermal cycler.



PTC PCR

Initial denaturation	Denaturation	Annealing	Extension	Final extension
94.0 °C	94.0 °C	58.0 °C	72.0 °C	72.0 °C
120 s	10 s	15 s	40 s	5 s

Number of cycles 30

- Optional stopping point: PCR product is stable at room temperature for several days. For longer term storage, move tubes to the freezer.

Detailed instructions for using a miniPCR thermal cycler

https://links.minipcr.com/minipcrRUN

Restriction digestion

1. Label a new 0.2 ml PCR tube with your initials followed by “R” for restriction digestion. Write on the upper sidewall of the tube.
2. Transfer 14 µl of your PCR product to your new “R” tube.
3. Add 2 µl diluted restriction enzyme directly into the liquid already in your tube. Pipette up and down several times to mix. Proper mixing is essential. Note: your teacher may perform this step for you.
4. Close the cap on the tube. When it is closed correctly, you should feel the cap “click” into place.
5. Make sure all the liquid is at the bottom of the tube. If there is liquid stuck on the sides of the tube, shake it down with a flick of the wrist or a brief spin in a microcentrifuge.
6. Incubate at 37 °C for at least 15 minutes and up to overnight. You can use a miniPCR thermocycler in Heat Block mode, a water bath, or another heat block.

   Optional stopping point: After the 37 °C incubation, the samples can be stored in the freezer before proceeding to gel electrophoresis.

PTC taste test (complete during restriction digestion incubation)

1. Place the control paper strip on your tongue. This will give you a baseline of what plain paper tastes like.
2. Place the PTC paper strip on your tongue and rate your reaction using the guidelines below:

Reaction	Phenotype
The PTC paper tastes similar to the control paper	non-taster
The PTC paper tastes bitter	taster

Note: Your teacher might also ask you to rate the PTC taste intensity using a semi-quantitative scale. Instructions can be found in the Extension activity on page 37.

Gel electrophoresis

Protective gloves and eyewear should be worn for the entirety of this experiment.

1. Place the prepared gel into the electrophoresis chamber.

2. Add enough electrophoresis buffer to fill the chamber and just cover the gel.
   - You will need 30 ml of TBE buffer for a blueGel™ or Bandit™ electrophoresis system. Do not overfill the chamber.
   - If using another electrophoresis system, refer to the manufacturer's instructions for the recommended buffer type and volume.

3. Use a micropipette to load samples in the following order. To prevent contamination, use a new tip for each sample. Note the order in which you loaded your group's samples below:
   - Well 1: 10 µl Fast DNA Ladder 2
   - Well 2: 10 µl Student name ______________________________
   - Well 3: 10 µl Student name ______________________________
   - Well 4: 10 µl Student name ______________________________
   - Well 5: 10 µl Student name ______________________________

Detailed operating instructions for miniPCR electrophoresis systems

blueGel

https://links.minipcr.com/blueGelRun

Bandit

https://links.minipcr.com/BanditViewit

4. Run the gel for 20-30 minutes.
    - The blueGel™ and Bandit™ electrophoresis systems run at a fixed voltage.
    - If using another gel electrophoresis system, set the voltage in the 70-90 V range.

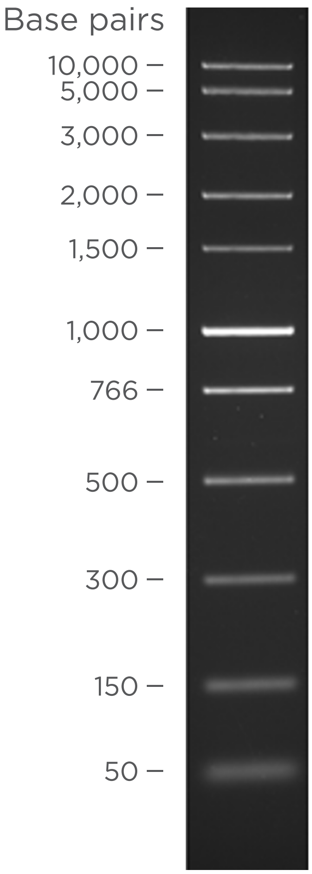
5. To visualize the DNA samples, turn on the blue light in your electrophoresis system, or move the gel to a transilluminator.

6. If needed, continue to run the gel until there is sufficient separation between the 50-300 bp bands in the ladder to interpret the results.

7. If desired, take a photo to document the gel electrophoresis results.

8. Compare the bands from the DNA samples to the DNA ladder to obtain size estimates.

Fast DNA Ladder 2



Pre-lab questions

Review

1. What is a SNP?

2. What is PTC?

3. What taste receptor protein binds PTC?

4. Describe the genetic differences between the two common alleles of the *TAS2R38* gene.

5. Is the ability to taste PTC considered dominant or recessive?

6. Fill in the table below using the following words and phrases: TT, tt, Tt, taster, non-taster

Phenotype	Possible genotype(s)

7. There are several steps to determine your *TAS2R38* genotype. Put the following experimental steps in chronological order: gel electrophoresis, DNA extraction, restriction digestion, PCR.

8. Explain why the Fnu4HI restriction enzyme acts differently upon the taster and non-taster alleles of the *TAS2R38* gene.

9. Summarize the gel electrophoresis results you expect to see for each *TAS2R38* genotype:

Genotype	**Expected band size(s) after digest**
TT	
Tt	
tt	

Critical thinking

10. In 1931, a scientist named Arthur Fox made the chemical PTC. Some of the PTC powder got into the air, and Fox's coworker C.R. Noller complained that it tasted bitter. Fox hadn't noticed, and even when he deliberately tasted the powder he didn't perceive any bitterness.

    a. Based on this information, list all the possible genotypes for Fox and Noller.

    Fox's genotype:
    Explain your reasoning:

    Noller's genotype:
    Explain your reasoning:

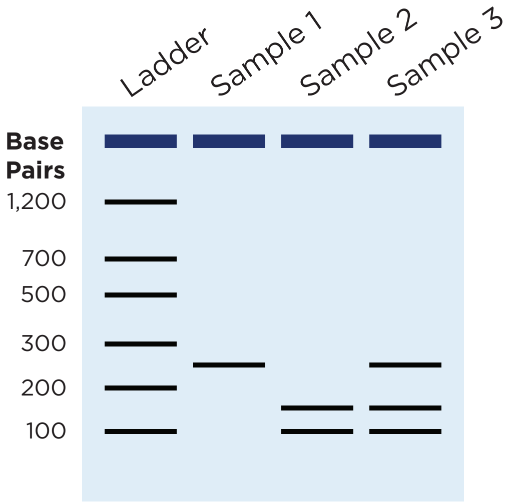
    b. Pretend the gel electrophoresis results to the right are from testing three individuals, including Fox and Noller. Use the information to answer the following questions:

    Which sample(s) could have come from Fox:

    Explain your reasoning:

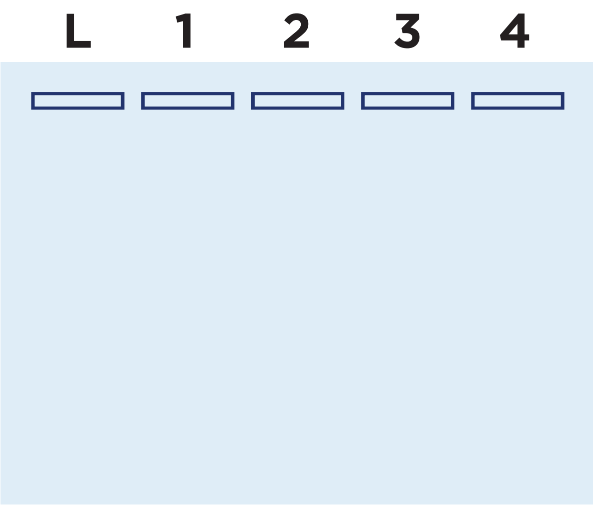
    Which sample(s) could have come from Noller:

    Explain your reasoning:



Post-lab questions

Interpreting results



1. Use the image on the right to draw what your gel looks like. For each sample, draw the bands that you see on your actual gel.

2. Label the bands with an approximate size (in base pairs). Use the image of the ladder from page 26 to help you.

3. Use your gel electrophoresis results to complete the table below.
   a. Use checkmarks to record the gel electrophoresis results in the first two rows of the table.
   b. Record each person's genotype and predicted phenotype.

	Student 1	Student 2	Student 3	Student 4
Non-taster allele (t) (250 bp)				
Taster allele (T) (150 + 100 bp)				
***TAS2R38* genotype**				
Predicted phenotype				

Critical thinking

4. Based on your phenotype, what did you expect your *TAS2R38* genotype might be? Explain your reasoning.

5. Based on the gel electrophoresis results, what is your *TAS2R38* genotype? Explain how you can tell.

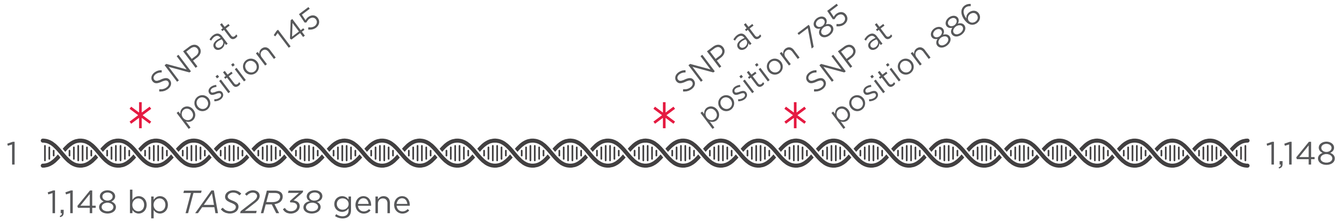
6. Based on your ability to taste PTC, do you have the *TAS2R38* genotype you expected? Explain your reasoning.

7. When examining the relationship between genotype and phenotype, it is helpful to test many individuals.
    a. Record your class data in the table below.
    b. In your class, how closely does genotype match the expected phenotype?

Student	Phenotype (taster or non-taster)	Genotype (TT, Tt, tt)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
31		
32		

Advanced questions

The three SNPs in the *TAS2R38* gene tend to be inherited together, such that two alleles (PAV and AVI) account for 93.5% of genotypes observed in humans (Figure 6). This is an example of a haplotype, a set of SNPs or other DNA variations that tend to be inherited together. Use the information in Figure 6 to answer the advanced questions below.



Allele	Frequency
PAV (Pro, Ala, Val)	50.8%
AVI (Ala, Val, Ile)	42.7%
AAI	3.4 %
AAV	2.5 %
AVV	0.3 %
PAI	0.2 %
PVI	0.1 %
PVV	0.1 %

Figure 6. *TAS2R38* alleles. The *TAS2R38* has three SNPs, which encode different amino acids at three locations in the TAS2R38 protein. This lab focused on the two common *TAS2R38* alleles, PAV and AVI, which together account for 93.5% of genotypes in humans. There are examples of haplotypes, a set of DNA variations that tend to be inherited together. The other combinations of three SNPs in the *TAS2R28* gene are much less common, but are observed at low levels in the overall human population; however, the frequencies vary across different groups depending on ancestry (Risso *et al.*, 2016).

8. Can you explain why certain DNA variations tend to be inherited in sets like in the *TAS2R38* gene? Use relevant vocabulary if you are able.

9. Imagine if a person discovered that they were a PAV, AAV heterozygote. Their biological parents are both PAV, AVI heterozygotes. Is that possible? Is it likely? Explain your reasoning.

10. Imagine if a person discovered that they were a PAV, AAI heterozygote. Their biological parents are both PAV, AVI heterozygotes. Is that possible? Is it likely? Explain your reasoning.

CER table

Fill in the table based on your results from the lab. Refer to the rubric on the next page.

Question:

Based only on your TAS2R38 genotype, would you predict that you are able to taste PTC?

Claim

Make a clear statement that answers the above question.

Evidence

Provide data from the lab that supports your claim.

Reasoning

Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.

Score	4	3	2	1
CLAIM A statement that answers the original question/ problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is highly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

Rubric score	3	4	5	6	7	8	9	10	11	12
Equivalent	55	60	65	70	75	80	85	90	95	100

Extension: Quantifying PTC taste intensity

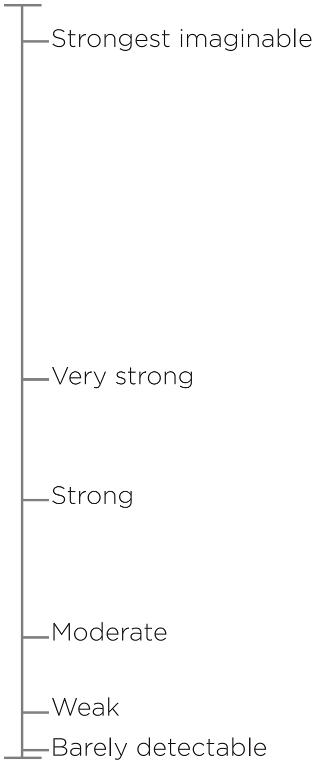
In this lab you scored your PTC tasting phenotype as either being a taster or a non-taster. However, some evidence from PTC taste tests in humans suggests that people who are heterozygous for the *TAS2R38* gene (Tt) may have an intermediate sensitivity to PTC (Bufe *et al.,* 2005). In this activity, you will score your perception of PTC bitterness to further investigate the relationship between *TAS2R38* genotype and PTC taste intensity.

TAS2R38 genotype	PTC tasting phenotype
TT	Strong taster
Tt	Intermediate taster
tt	Non-taster

Scientists can use something called a *labeled magnitude* scale to quantify the intensity of tastes. Subjects rate the intensity of a taste on a quasi-logarithmic scale of verbal descriptions. The physical location of the subject's rating is then matched with a numerical value. These data are subjective.

Data collection

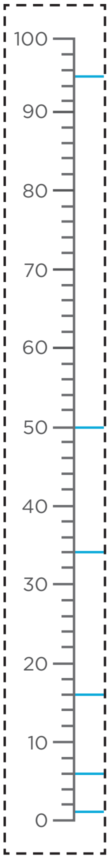
1. Place the control paper strip on your tongue. This will give you a baseline of what plain paper tastes like.
2. Place the PTC paper strip on your tongue and rate the intensity of the taste by physically marking an "X" on the labeled magnitude scale to the right.



Labeled magnitude scale. Rate your perceived intensity of PTC taste by marking an "X" on the scale.

3. Assign yourself a numeric score. The scale on the previous page where you marked your perceived taste intensity should be exactly 10 cm. Use a ruler to measure how many <u>millimeters</u> the X you drew is from the bottom of the scale. If your lab is not printed full size on 8.5'' x 11'' paper, or if you don't have a ruler, use the ruler below.

   Record your score here:



Labeled magnitude scale ruler. Cut out along the dashed lines, then place side-by-side with the scale where you marked your preceived taste intensity. This will allow you to convert the position on the scale into a number.

Data analysis

1. Compile your class data in the table below:

Student	Genotype (TT, Tt, tt)	Labeled magnitude score
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
31		
32		

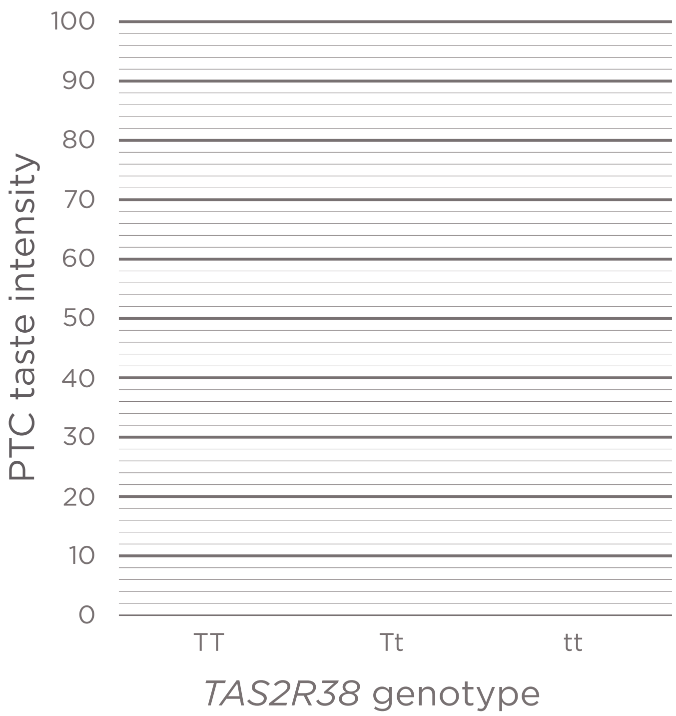
2. Calculate the average labeled magnitude scale score and standard error of the mean for each *TAS2R38* genotype:

***TAS2R38* genotype**	**Average labeled magnitude score**	**Standard error of mean**
TT		
Tt		
tt		

$$s = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$$

$$SE_{\bar{x}} = \frac{s}{\sqrt{n}}$$

3. Graph your class data. Include error bars that indicate ± 1 SEM.



4. Does your class data support the hypothesis that people who are heterozygous for the *TAS2R38* gene have an intermediate sensitivity to PTC? Explain your reasoning. What caveats apply to your analysis?

Critical thinking

5. Using a labeled magnitude scale to rate taste intensity is subjective, so it can be difficult to compare data from different individuals. Propose an additional experimental step that you could use to better compare data from different people.

Extension: Using the Hardy-Weinberg equation

After completing this lab, you know your own *TAS2R38* genotype and PTC tasting phenotype. While scientists are often interested in the genotypes of individuals, it is also important to consider the genetic composition of entire populations. In this activity, you will investigate the distribution of *TAS2R38* genotypes in your class.

1. Based on your class genotype data, calculate the observed genotype frequencies.

   Count the number of TT, Tt, and tt individuals in your class, then divide by the total number of individuals to get the observed genotype frequencies:

Table 1: Observed genotype frequencies

Genotype	Observed # of individuals	Observed genotype frequency
TT		
Tt		
tt		
total # of individuals in population		

Hardy-Weinberg equilibrium

In a population that is not evolving, the prevalence of different alleles within a population are expected to remain the same over time. A population in this state is referred to as being in *Hardy-Weinberg equilibrium.*

The Hardy-Weinberg equation describes the genotype frequencies you would expect to see in a population that is in Hardy-Weinberg equilibrium. This can be useful because one way to assess whether a population is evolving is to compare actual genotype frequencies with what would be predicted in a population that is not evolving and is in Hardy-Weinberg equilibrium.

To calculate the predicted genotype frequencies using the Hardy-Weinberg equation, first you need to calculate the allelic frequencies. Allelic frequencies tell you how common a particular allele is in the population regardless of the genotype frequencies. To calculate the allelic frequency, the total number of each allele in the population is counted. For a typical autosomal gene with two alleles in diploid organisms, this means that any homozygote will have two copies of an allele, while a heterozygote will have one copy of each allele. The total number of alleles in a population is therefore equal to two times the number of individuals in the population

In its simplest form, the Hardy-Weinberg equation describes a gene with two alleles. The convention is to use the symbols p and q to represent the frequencies of each allele.

2. Based on your class data, calculate the <u>observed</u> allele frequencies. Count the number of T and t alleles in your class, then divide by the total number of alleles to get p and q:

Table 2: Observed allele frequencies

Allele	**# of alleles in population**	**Allele frequency**
T		p =
t		q =
total # of alleles in population		

Hardy-Weinberg equilibrium

The Hardy-Weinberg equation predicts the genotype frequencies for a population that is not evolving: $\mathbf{p^2 + 2pq + q^2 = 1}$

p^2 = the genotype frequency for individuals homozygous for the p allele

$2pq$ = the genotype frequency for heterozygous individuals

q^2 = the genotype frequency for individuals homozygous for the q allele

3. Use the Hardy-Weinberg equation to determine if your class population is in Hardy-Weinberg equilibrium.

    a. First, use allele frequencies that you calculated for your class in Table 2 to determine the expected genotype frequencies for a population in Hardy-Weinberg equilibrium. Show your work below, then compile your answers into Table 3 below.

TT expected genotype frequency:

Tt expected genotype frequency:

tt expected genotype frequency:

Table 3: Expected number of individuals for each genotype

Genotype	Expected genotype frequency (calculated above in question 3a)	Expected # of individuals (calculated below in question 3b)
TT		
Tt		
tt		

b. Second, use the expected genotype frequencies that you calculated in the previous step to determine the expected number of individuals for each genotype in your class.

Multiply each genotype frequency by the total number of individuals in your class. Show your work below, then compile your answers into Table 3 on the previous page.

TT expected number of individuals:

Tt expected number of individuals:

tt expected number of individuals:

4. Compile your results from questions 1 and 3 in the table below:

Table 4: Comparing observed vs. expected

Genotype	**Observed # of individuals (from Table 1)**	**Expected # of individuals (from Table 3)**
TT		
Tt		
tt		

5. How similar are the observed and expected values?

6. With only this information, how confident do you feel in saying whether or not your class is in Hardy-Weinberg equilibrium?

Critical thinking

7. For a population to be in Hardy-Weinberg equilibrium, it is assumed that five criteria are met:

   - The population size is infinite (or very large).
   - There is no net migration into or out of the population.
   - There is no mutation at the locus being tested.
   - Mating in the population is random.
   - There is no natural selection on the alleles being tested.

   Based on these assumptions, would you expect your data to reveal a population in Hardy-Weinberg equilibrium? Explain why or why not.

AP connection: χ^2 test

Use the χ^2 (Chi-squared) test to determine if your class's divergence from the expected genotypic ratios is due to chance, or represents statistically significant variation that suggests that the population is not in Hardy-Weinberg equilibrium.

8. State the null hypothesis (H_0).

9. Calculate the χ^2 for your class data by filling in the table below:

$$x^2 = \sum \frac{(o-e)^2}{e}$$

Table 5: Calculating χ^2

Genotype	**Observed # of individuals (from Table 4)**	**Expected # of individuals (from Table 4)**	$\frac{(\text{observed - expected})^2}{\text{expected}}$
TT			
Tt			
tt			
		χ^2 Value	**Σ=**

10. Use the χ^2 value you calculated in Table 5 to determine whether the variation in the observed results could be due to chance. The χ^2 distribution table is on the next page.
    a. Based on your χ^2 value, do you reject or fail to reject the null hypothesis? Explain your reasoning.
    b. Explain what your statistical results mean in common language.

The X^2 distribution table

p value	Degrees of freedom							
	1	2	3	4	5	6	7	8
0.05	3.84	5.99	7.82	9.49	11.07	12.59	14.07	15.51
0.01	6.64	9.21	11.34	13.28	15.09	16.81	18.48	20.09

Extension: G protein-coupled receptors

The TAS2R38 protein is a G protein-coupled receptor (GPCR). GPCRs are a large class of membrane proteins that broadly function to transmit information from the outside of the cell to the inside of the cell. When a GPCR binds with its ligand on the extracellular side of the cell, it triggers a signal transduction cascade inside the cell that eventually results in responses like changes in gene expression. In this way, information can be transferred across the cell membrane without any actual molecules crossing the membrane.

All GPCRs have a very similar structure with seven transmembrane domains. While they are often depicted with the seven transmembrane domains arranged in a row along the membrane (Figure 1, left), the true structure is more of a cylinder formed from interactions between the transmembrane domains within the membrane (Figure 1, right).

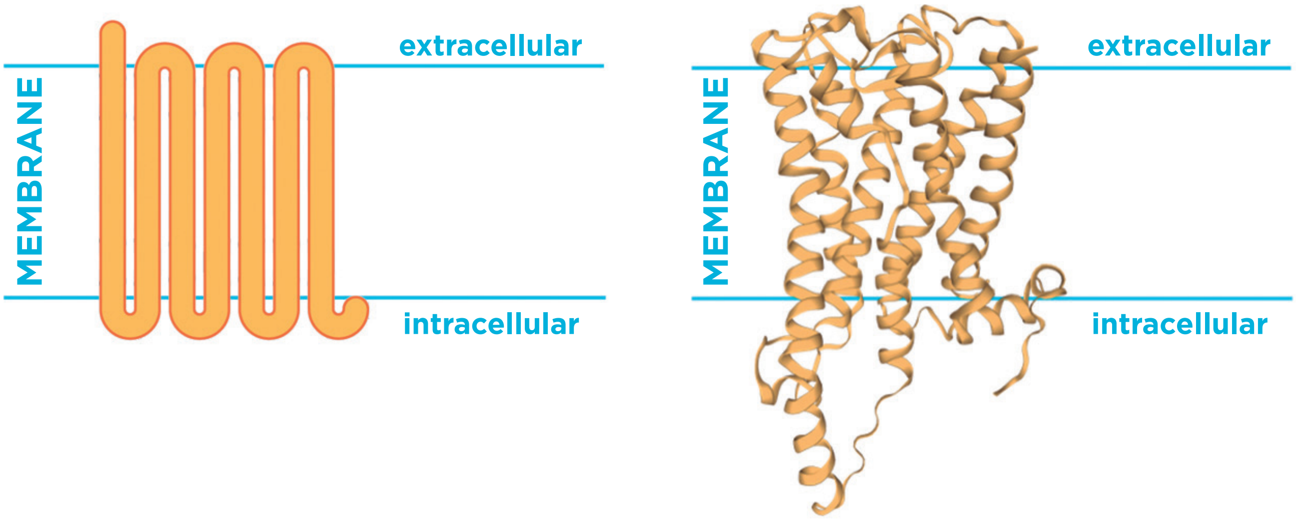


Figure 1. GPCR structure. All GPCRs have a similar structure with seven transmembrane domains. For simplicity, GPCRs are often depicted schematically with the seven transmembrane domains arranged in a row along the membrane (left). In reality, the seven transmembrane domains interact with each other to form a somewhat cylindrical 3D shape, as illustrated by the ribbon diagram (right).

Ribbon model of TAS2R38 protein made using Swiss-Model: Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer F.T., de Beer, T.A.P., Rempfer, C., Bordoli, L., Lepore, R., Schwede, T., SWISS-MODEL: Homology modelling of protein structures and complexes, *Nucleic Acids Res.* 46, W295-w303 (2018).

Different GPCRs can be activated by an incredibly diverse array of ligands including small chemicals, lipids, proteins, sugars, and even light. Depending on the ligand, the binding site could be on the extracellular loops that extend off the cell membrane, or fairly deep within the cylinder composed of the transmembrane domains. Preliminary research suggests that the ligand binding site for bitter taste receptors is in the transmembrane region (Behrens and Meyerhof, 2013).

For a GPCR to transmit a signal (Figure 2), it must first be stimulated from outside the cell, usually through the binding of a ligand. When a GPCR binds its ligand, the receptor undergoes a conformational change that triggers the activation of a G protein. The G protein is a protein located on the intracellular side of the membrane and is responsible for initiating the signalling cascade within the cell. The cascade begins when a guanosine diphosphate (GDP) molecule bound to the G protein is replaced with a higher energy guanosine triphosphate (GTP) molecule. The activated G protein then uncouples from the GPCR and moves along the cell membrane, eventually activating other proteins, which transmit the signal inside the cell via second messengers. Depending on the receptor and the type of cell, the cellular response could be anything from a change in gene expression to the initiation of a nerve impulse.

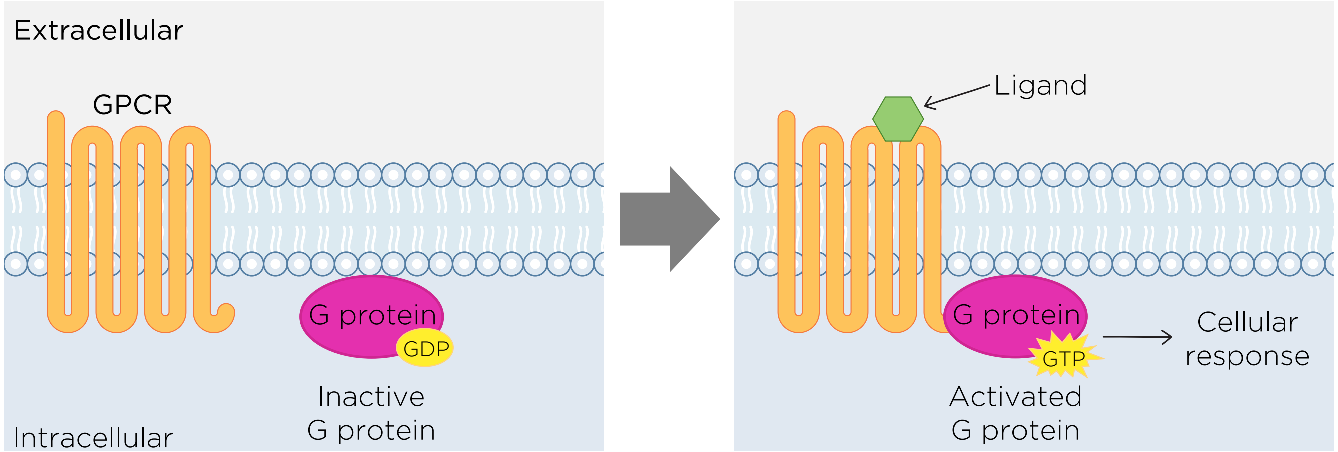


Figure 2. GPCR-mediated signal transduction. GCPRs span the cell membrane and transmit information from outside the cell. When a GPCR binds to its ligand, It triggers the activation of G protein when a GDP is replaced by GTP. The activated G protein then moves along the cell membrane to activate other proteins and transmit a signal inside the cell.

GPCRS make your body work!

G protein-coupled receptor (GPCRs) serve as a bridge between the extracellular and intracellular environments. GPCRs are responsible for detecting most external stimuli and mediate our senses of vision, smell, and taste. GPCRs also receive signals from other cells in the body, for example, messages sent via hormones or neurotransmistters. This divesity of function is possible because humans have more than 800 known GPCRs, and GPCRs are activated by a mindblowing array of ligands. Chemicals, peptides, lipids, sugars, and even photons of light are all detected by different GPCRs. The importance of GPCRs in human biology is highlighted by the fact that around half of all known drugs act on GPCR pathways.

1. We know that the SNPs in the *TAS2R38* gene affect a person's ability to perceive the bitter chemical PTC. Based on what you know about GPCR mediated cellular signaling, which step(s) could be directly affected by the changes in the *TAS2R38* protein? Select all appropriate answers.
   a. ligand binding
   b. signal transduction
   c. cellular response

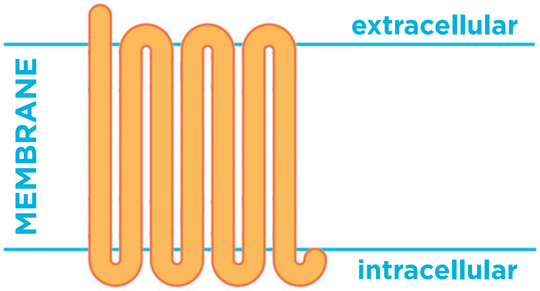
   Explain your reasoning:

2. Scientists do not know for sure how the SNPs in the *TAS2R38* gene affect TAS2R38 protein function.

   In the following questions, you will use what is known about GPCR signaling to make predictions about the TAS2R38 protein.

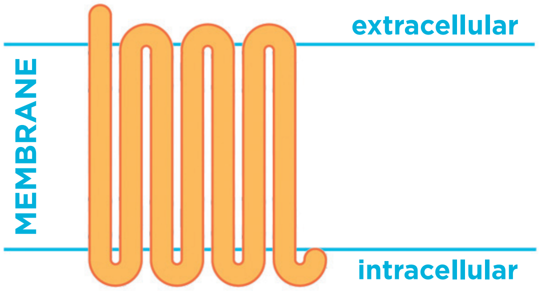
   a. If the different versions of the TAS2R38 protein bound to ligands differently, roughly where would you expect the proteins to differ? Circle or otherwise clearly mark the general region(s) you would expect to be affected on the drawing to the right.

      Explain your reasoning:



b. If the different TAS2R38 proteins interacted with the G protein differently, roughly where on the protein would you expect the proteins to differ? Circle or otherwise clearly mark the general region(s) you would expect to be affected on the drawing to the right.

Explain your reasoning:



3. In the following drawing, the 333 amino acids that make up the TAS2R38 protein are drawn where they are predicted to be in relation to the cell membrane. The three amino acids that vary between tasters and non-tasters are highlighted in pink.

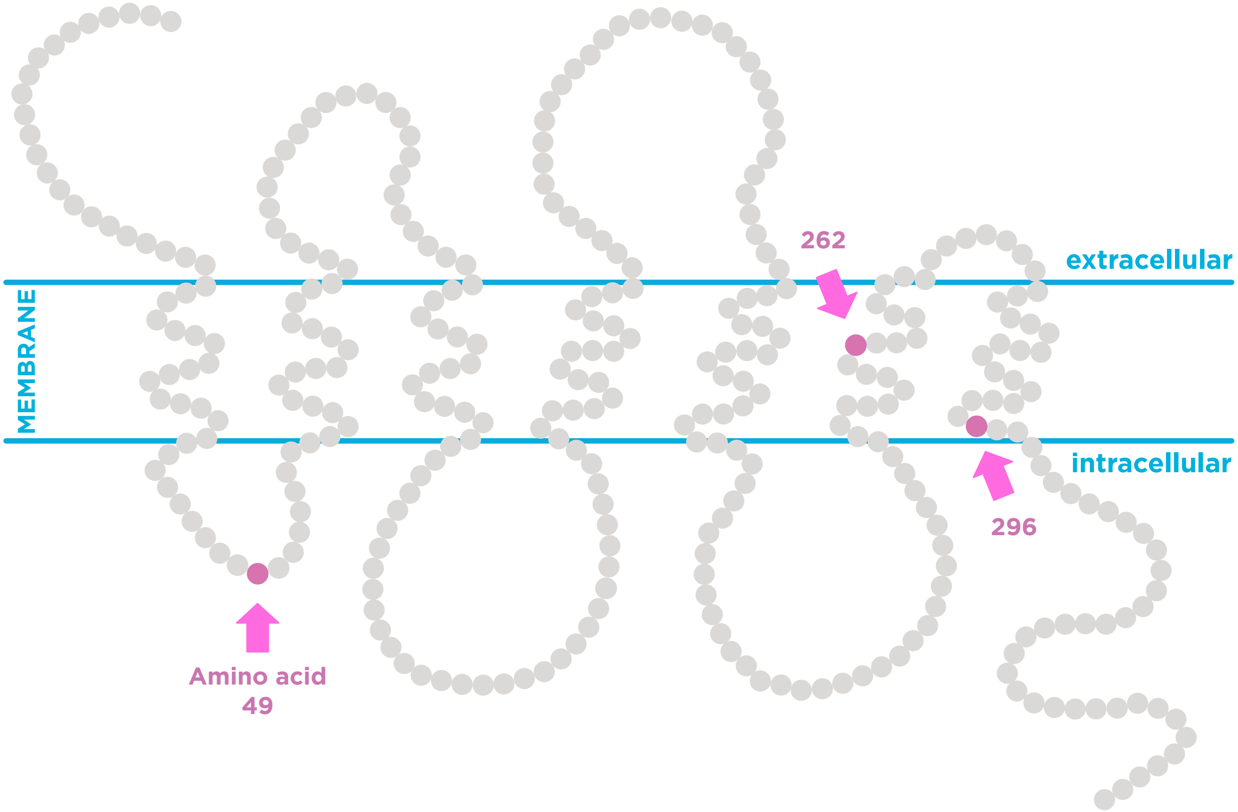


Figure 3. TAS2R38 protein. This snakeplot shows the predicted structure of the TAS2R38 GPCR. Each ball represents an amino acid, and the amino acids are numbered starting at the N-terminus. The three amino acids that vary in PTC taster and non-taster alleles are shown in pink (amino acids 49, 262, and 296).

a. By looking just at this diagram, would you predict that ligand binding or signal transduction is more likely to underlie the inability to taste PTC? Explain your reasoning.

b. Assume amino acid 49 was the only difference between the taster and non-taster forms of the TAS2R38 protein. In this case, would you predict that changes to ligand binding or signal transduction is more likely to underlie the ability to taste PTC? How confident would you be in your prediction? Explain your reasoning.

c. Assume amino acid 262 was the only difference between the taster and non-taster forms of the TAS2R38 protein. In this case, would you predict that changes to ligand binding or signal transduction are more likely to underlie the inability to taste PTC? How confident would you be in your prediction? Explain your reasoning.

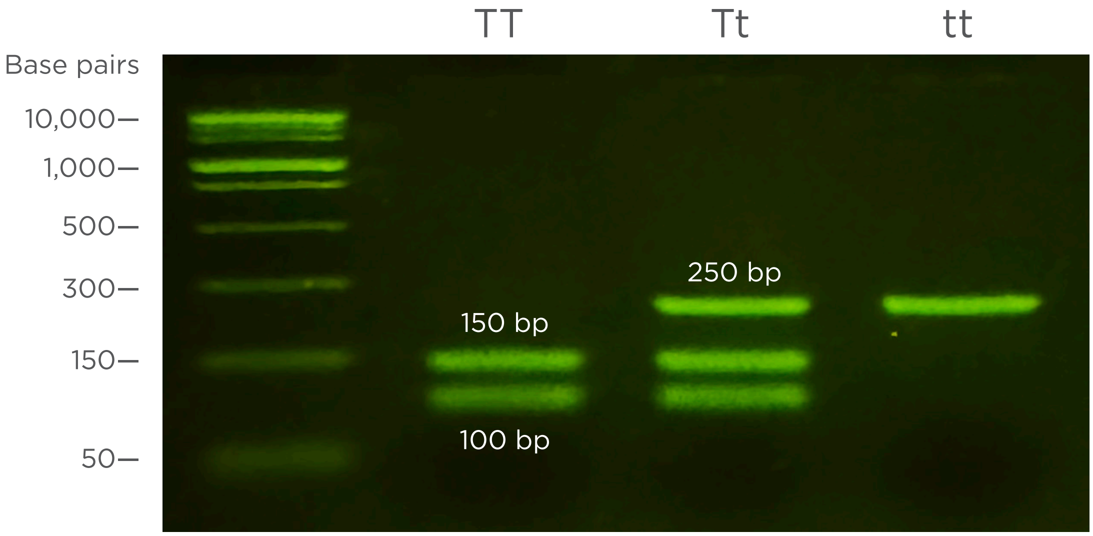
d. Assume amino acid 296 was the only difference between the taster and non-taster forms of the TAS2R38 protein. In this case, would you predict that changes to ligand binding or signal transduction are more likely to underlie the inability to taste PTC? How confident would you be in your prediction? Explain your reasoning.

e. Scientists don't know for sure whether the difference in perception between tasters and non-tasters is due to differences in ligand binding or in signal transduction. Some experimental evidence suggests that amino acid 262 has a particularly strong effect on PTC tasting (Bufe *et al.*, 2005), but scientists still don't know exactly what role amino acid 262 plays in the protein.

- Refer back to your answer in part c where you predicted whether you think amino acid 262 is more likely to affect ligand binding or signal transduction.
- Does knowing that amino acid 262 may have the greatest effect on tasting ability make you feel more or less confident predicting whether the differences between the *TAS2R38* alleles are more likely to be caused by differences in ligand binding or signal transduction? Explain your reasoning.

Instructor guide

Expected results



This image represents results obtained after a 30 minute run using a blueGel™. Note that is is likely that the top of the Fast DNA Ladder 2 will not fully resolve with a short gel run.

- Before restriction digestion, the PCR product for both the taster and non-taster alleles should be ~250 base pairs (bp).

- After the restriction digestion:
  - The non-taster allele won't be cut and will remain ~250 bp.
  - The taster allele will be cut into two separate fragments: one ~150 bp fragment and one ~100 bp band.

- This yields three patterns of bands on the gel for the three possible *TAS2R38* genotypes:
  - A homozygous taster (TT) will typically display two bands: one at ~150 bp and one at ~100 bp.
  - A heterozygous taster (Tt) will display three bands: one at ~250 bp, one at ~150 bp, and one at ~100 bp.
  - A non-taster (tt) will display only one band: ~250 bp.

- You can also watch a video explaining the interpretation of PTC results. Visit https://links.minipcr.com/ptc_results.

For technical support, contact **support@minipcr.com**

For answers to the student questions, email **answers@minipcr.com**
Please include in the body of the email:
- The name of the lab
- Your name, school, and job title

Unexpected results and troubleshooting

If fluorescent DNA bands are faint or entirely absent from one or more student sample lanes, the following may have occurred:

- Too much tissue: The most common source of error in this protocol is the DNA extraction. Repeat the DNA extraction, and ensure students only lightly scrape their cheeks, as too much DNA can inhibit PCR.

- Suboptimal PCR amplification: Pipetting errors during PCR setup can lead to suboptimal amplification for individual student samples.

- Failure to load the DNA samples on the gel: Loading DNA samples for gel electrophoresis takes a little practice. The bands will appear faint if students do not successfully deposit the full sample volume into the well. Refer to https://www.minipcr.com/how-to-load-a-gel-electrophoresis/ for gel loading tips.

If fluorescent DNA bands are not visible on the gel, even for the DNA ladder, the following may have occurred:

- Failure to use a fluorescent DNA stain: This lab requires agarose gels made with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®). DNA stains that reveal DNA with a visible blue compound are less sensitive and are not compatible with this lab kit.

- Incorrect visualization conditions: Fluorescent DNA stains (*e.g.,* SeeGreen™ or GelGreen®) must be viewed using a blue light or UV transilluminator. The blueGel system has an integrated blue light transilluminator. For DNA visualization, ensure that you have turned on the blueGel's blue light by pressing the light bulb button.

- Samples were run off the gel: If you run the gel too long, DNA samples may migrate off the gel. Monitor progress by occasionally checking the DNA samples under a transilluminator or tracking the loading dye, which is visible to the eye. Stop the run before the colored loading dye reaches the end of the gel.

- Reagents were stored improperly and/or are expired: The lab kit can be stored in the freezer for up to twelve months after receipt. Storage under different conditions or in excess of this guidance may impair performance.

A high rate of genotype-phenotype mismatches such that PTC tasters obtain a tt genotype suggests that the restriction digestion was incomplete. The following may have occurred:

- Restriction enzyme was stored improperly and/or is expired: The restriction enzyme can be stored in the freezer for up to twelve months after receipt. Storage under different conditions or in excess of this guidance may impair performance.

- Insufficient restriction enzyme added to the PCR product: To set up the restriction digestion, you must add 2 µl of the diluted restriction enzyme to 14 µl of the PCR product. Students often struggle to pipette such small volumes, so we recommend the teacher complete this step.

- Insufficient restriction enzyme digestion incubation: While a 15-minute incubation at 37 °C should be sufficient for the restriction enzyme to cut the DNA, allowing the reaction to run overnight at 37 °C gives more time for complete digestion.

For tips on picture-perfect gels, see https://www.minipcr.com/gel-electrophoresis-troubleshooting/.

For additional technical support, contact support@minipcr.com.

Notes on lab design

This lab serves as an introduction to the relationship between genotype and phenotype, specifically the *TAS2R38* gene and the ability to taste the chemical PTC. We believe this approach provides the right balance between intellectual engagement, inquiry, and accessibility. The design of this lab has simplified certain elements to achieve these goals. Some of these elements include:

- Two *TAS2R38* haplotypes that vary at three locations account for more than 90% of *TAS2R38* genotypes. The analysis in this lab uses one of the underlying SNPs to examine this 90%. Focusing on one SNP instead of all three simplifies the interpretation and discussion of the results. For more information, refer to the advanced questions on page 33.

- *TAS2R38* genotype does not correlate 100% with PTC tasting phenotype. Depending on the human population being studied, the *TAS2R38* genotype correlates with the ability to taste PTC in 55-85% of cases (Kim et al., 2003).

Citations

Behrens, M., and Meyerhof, W. (2013). Bitter taste receptor research comes of age: From characterization to modulation of TAS2Rs. Seminars in Cell & Developmental Biology 24, 215–221. https://doi.org/10.1016/j.semcdb.2012.08.006.

Bufe, B., Breslin, P.A.S., Kuhn, C., Reed, D.R., Tharp, C.D., Slack, J.P., Kim, U.-K., Drayna, D., and Meyerhof, W., (2005). The Molecular Basis of Individual Differences in Phenylthiocarbamide and Propylthiouracil Bitterness Perception. Current Biology 15, 322–327. https://doi.org/10.1016/j.cub.2005.01.047.

Kim, U., Jorgenson, E., Coon, H., Leppert, M., Risch, N, and Drayna, D. (2003). Positional Cloning of the Human Quantitative Trait Locus Underlying Taste Sensitivity to Phenylthiocarbamide. Science *299*, 1221–1225. https://doi.org/10.1126/science.108019.

Risso, D.S., Mezzavilla, M., Pagani, L., Robino, A., Morini, G., Tofanelli, S., Carrai, M., Campa, D., Barale, R., Caradonna, F., et al. (2016). Global diversity in the TAS2R38 bitter taste receptor: revisiting a classic evolutionary PROPosal. Sci Rep *6*, 25506. https://doi.org/10.1038/srep25506.

Additional student supports

E-worksheets: The student questions accompanying this lab are available for download here as editable text documents you can customize and upload to your LMS. E-worksheets can also be accessed from the Curriculum Downloads tab a https://www.minipcr.com/product/minipcr-genotype-to-phenotype-ptc-taster-lab/.

miniPCR tutorials: Access an extensive set of free resources to help your students succeed in molecular biology techniques. Visit https://www.minipcr.com/tutorials/. The resources most relevant to this lab are listed below.

- **Micropipetting:** Video, worksheet, and hands-on activity resources to train students in the basic use of a micropipette.
- **PCR:** Video and worksheet activity instructing students on the fundamentals and practice of PCR.
- **Gel electrophoresis:** Video and worksheet activity instructing students on the fundamentals and practice of agarose gel electrophoresis.

Video explanation of experimental results: This short video summarizes the PTC experimental design and walks students through how to interpret their gel electrophoresis results. Access at https://links.minipcr.com/ptc_results.

Digital lab companion and lab simulation: Visit https://digital.minipcr.com/ to explore interactive tools for experiment-based learning with or without the hands-on PTC Taster Lab.

PTC Taster Lab Control Bands (KT-1004-04): Ready-to-load controls allow students to compare their own genotyping results to known reference bands directly.

Extension activities

The following optional extension activities are provided for students to explore topics more deeply.

Quantifying PTC taste intensity (page 37): In this lab, students score their PTC tasting phenotype as either being a taster or a non-taster. However, some evidence from PTC taste tests in humans suggests that people who are heterozygous for the *TAS2R38* gene (Tt) may have an intermediate sensitivity to PTC (Bufe *et al.,* 2005). Use one of the methods used by scientists to have students score their perception of PTC bitterness and further investigate the relationship between *TAS2R38* genotype and PTC taste intensity.

Using the Hardy-Weinberg equation (page 42): Use the genotype data from your class to see if your class represents a population in Hardy-Weinberg equilibrium and test for divergence using a Chi-squared test.

G-protein coupled receptors (page 49): TAS2R38 is a G protein-coupled receptor. Delve into GPCRs and signal transduction.

Learning goals and skills developed

Student Learning Goals:

- Correlate genotype and phenotype
- Apply rules of probability to genetic analysis
- Evaluate whether a population is in Hardy-Weinberg equilibrium
- Predict the effect of mutations on the function of G protein-coupled receptors

Scientific Inquiry Skills:

- Identify or pose a testable question
- Follow detailed experimental protocols
- Create tables or graphs to present their results
- Interpret data presented in a chart or table
- Make a claim based in scientific evidence
- Use reasoning to justify a scientific claim

Molecular Biology Skills:

- Micropipetting
- DNA extraction
- PCR
- Restriction digestion
- Agarose gel electrophoresis

Standards alignment

The standards alignment document for this activity is available for download here. This document can also be accessed from the Curriculum Downloads tab at https://www.minipcr.com/product/minipcr-genotype-to-phenotype-ptc-taster-lab/.

This activity is aligned to the following standards:
- Next Generation Science Standards: High School Life Science
- Advanced Placement Biology
- Texas Essential Knowledge and Skills: Biology
- Texas Essential Knowledge and Skills: Biotechnology
- Biotechnician Assistant Credentialing Exam
- Common Core ELA/Literacy Standards (9-10)

For additional information on alignment to state standards, please contact support@minipcr.com.