



# Genotype to Phenotype

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## PTC Taster Lab



# Instructor's Guide Contents



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# At a glance

Examine the link between genotype and phenotype using yourself as an experimental subject!

Students will assess their ability to taste the chemical phenylthiocarbamide (PTC) and determine how that ability correlates with their genotype for a gene that encodes a bitter taste receptor protein.

## TECHNIQUES

Micropipetting  
DNA extraction  
PCR  
Restriction digest  
Gel electrophoresis

## TOPICS

Genotype to phenotype  
Inheritance  
Biotechnology

## LEVEL

General High School  
Advanced High School  
College

## WHAT YOU NEED

Micropipettes  
PCR thermal cycler  
Gel electrophoresis and visualization system

## AP CONNECTION

AP Biology units  
1.5 4.1-4.3, 5.3, 5.6, 6.1,  
6.7-6.8, 7.1-7.2, 7.4-7.5

Skills and Practices  
1A-C, 2A-D, 3D-E,  
4A-B, 5A-D, 6A-E

## Planning your time

SINGLE CLASS PERIOD: 3 hours

OR

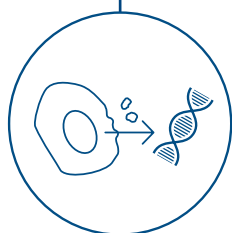
PERIOD 1: 45 MINUTES

Possible  
stopping  
point

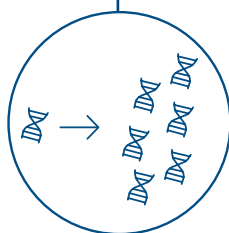
PERIOD 2: 45 MINUTES\*

After starting PCR samples may be left unattended. PCR products are stable at room temperature for at least one week.

Cheek cell  
DNA extraction



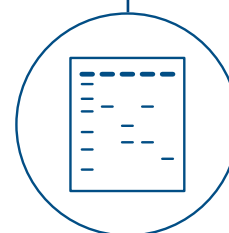
PCR



Restriction  
digest



Gel  
electrophoresis



\* Allow an additional 25 minutes if students will be preparing the gels in class.

## Additional supports



Help your students build proficiency in pipetting, PCR, and gel electrophoresis with additional instructional videos, worksheets, and activities available at:  
<https://www.minipcr.com/tutorials/>.

Taking it further — extension activities, page 35

- Quantifying PTC intensity
- Using the Hardy-Weinberg equation
- G protein-coupled receptors

For answers to the lab study questions and extensions, email [answers@minipcr.com](mailto:answers@minipcr.com). Please include the name of the lab, as well as your name, school, and title in the body of the email.



# Materials needed

## Supplied in kit (KT-1004-03)

Reagents and supplies	Amount provided in kit	Amount needed	Storage	Teacher's checklist
X-Tract™ DNA Extraction Buffer	1000 µl	50 µl per student	Freezer or room temperature	
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	
Fast DNA Ladder 2	150 µl	12 µl per group	Freezer	



# Materials needed (cont.)

## Sold separately in Learning Lab Companion Kit (KT-1510-01)

This lab requires reagents for running and visualizing DNA samples on a 2% agarose gel with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®). The Learning Lab Companion Kit provides enough electrophoresis reagents for 8 groups when using the blueGel™ electrophoresis system. Gels can also be prepared with agarose tabs or agarose powder. Refer to [www.minipcr.com/agarose-gel/](http://www.minipcr.com/agarose-gel/) for detailed instructions.

Reagents and supplies	Amount provided in kit	Amount needed	Storage	Teacher's checklist
All-in-one agarose tabs	8	One tab per agarose gel (2% agarose gel)	Room temp., protected from light	
TBE electrophoresis buffer <ul style="list-style-type: none"> <li>1X working solution</li> </ul>	Supplied as liquid concentrate or powder Sufficient to prepare 600 ml of 1X working solution	30 ml 1X solution per blueGel™ system	Room temp.	
PCR tubes (0.2 ml)	100	3 tubes per student		
Plastic tubes (1.7 ml)	50	4 tubes per group		



# Materials needed (cont.)

## Supplied by teacher

Available at miniPCR.com

Reagents and supplies	Amount needed	Teacher's checklist
<b>PCR thermal cycler:</b> e.g. miniPCR <sup>®</sup> machine	<b>1 per group</b> (can be shared between groups—you will need 1 tube for each student)	
<b>Horizontal gel electrophoresis apparatus:</b> e.g. blueGel <sup>™</sup> electrophoresis system	<b>1 per group</b> (can be shared between groups—reserve 1 lane for ladder and 1 lane for each student)	
<b>Blue light transilluminator</b> *Note: A blue light transilluminator is integrated in the blueGel <sup>™</sup> electrophoresis system	<b>1 per group</b> (can be shared between groups)	
<b>Micropipettes</b> <ul style="list-style-type: none"> <li>• 2-20 µl</li> <li>• 20-200 µl</li> <li>• 0.5-10 µl</li> </ul>	<b>1 per lab group</b> <b>1 for the teacher</b> (to dispense reagents) <b>1 for the teacher</b> (to dispense restriction enzyme)	
<b>Disposable micropipette tips</b>	<b>At least 7 <u>per student</u></b>	
<b>Flathead toothpicks for cheek swabs</b>	<b>1 toothpick <u>per student</u></b>	
<b>PTC taste paper strips</b>	<b>1 PTC and 1 control strip <u>per student</u></b>	
<b>Distilled water for making agarose gels and diluting TBE buffer</b>	<b>50 ml per gel</b>	
<b>Flask or beaker to dissolve agarose</b>		
<b>Microwave or hot plate to dissolve agarose</b>		
<b>Other supplies:</b> <ul style="list-style-type: none"> <li>• Disposable laboratory gloves</li> <li>• Protective eyewear</li> <li>• Permanent marker</li> <li>• Cup to dispose of tips</li> </ul>		



# Lab setup

The following activities can be carried out by the instructor ahead of class. Reagents are sufficient for 32 students. Except for the restriction enzyme, reagents are stable at room temperature for 24 hours, but should remain cold for short-term storage and frozen for long-term storage. The restriction enzyme should be stored in the freezer until use.



Gloves and protective eyewear should be worn for the entirety of this lab.

## Class period 1: DNA extraction and PCR

### A. Preparation for DNA extraction

- Provide each student with one flat-end toothpick.
- For each student, aliquot 50  $\mu$ l of X-Tract<sup>™</sup> DNA Extraction Buffer into a labeled 200  $\mu$ l PCR tube.

### Aliquot DNA extraction reagents for individual students

50  $\mu$ l X-Tract<sup>™</sup> DNA  
Extraction Buffer  
1 tube for each student



200  $\mu$ l  
PCR tube

### B. Preparation for PCR

- Thaw tubes containing 2X EZ PCR Master Mix and PTC Primer Mix by placing them on a rack or benchtop at room temperature.
- For each lab group, aliquot 15  $\mu$ l of 2X EZ PCR Master Mix per student into a labeled 1.7 ml tube. For example, a four student group would need 60  $\mu$ l of EZ PCR Master Mix.
- For each lab group, aliquot 15  $\mu$ l of PTC Primer Mix per student into a labeled 1.7 ml tube. For example, a four student group would need 60  $\mu$ l PTC Primer Mix.

### Aliquot PCR reagents for lab groups

EZ PCR Master Mix  
1 tube per lab group with  
15  $\mu$ l for each student



PTC Primer Mix  
1 tube per lab group with  
15  $\mu$ l for each student



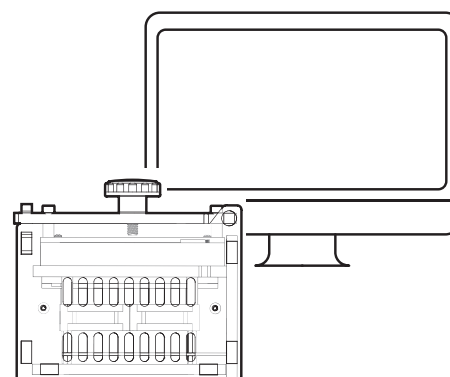
1.7 ml tubes



### C. Distribute supplies and reagents to lab groups

Check	At the start of this experiment, every lab group should have:	Amount per student	Amount for a 4 student group
	X-Tract™ DNA Extraction Buffer	50 $\mu$ l	n/a - aliquot for individual students in 200 $\mu$ l PCR tube
	2X EZ PCR Master Mix	15 $\mu$ l	60 $\mu$ l
	PTC Primer Mix	15 $\mu$ l	60 $\mu$ l
	Toothpick	1	4
	PCR tubes (200 $\mu$ l)	2	8
	Micropipette tips	At least 4	At least 16
	2-20 $\mu$ l micropipette		1
	Fine tipped permanent marker		1

- Ensure each lab group's bench is set up with a miniPCR® thermal cycler and power supply.
- Ensure the miniPCR® thermal cyclers that are going to be monitored throughout the PCR reaction are connected to a computer or compatible phone/tablet.
- Machines can be programmed ahead of time by the teacher or during class by the students.





## Class period 2: Restriction digest and gel electrophoresis

### A. Preparation for restriction digest and taste test

Important note: A 0.5-10  $\mu$ l micropipette is needed to add 1  $\mu$ l of restriction enzyme. Because pipetting small volumes is challenging, we recommend that the teacher perform this step.

Check	At the start of this experiment, every lab group should have:	Amount per student	Amount for a 4 student group
	PCR tubes (200 $\mu$ l)	1	4
	0.5-10 $\mu$ l micropipette		1 (can be shared by class)
	2-20 $\mu$ l micropipette		1
	Micropipette tips	At least 2	At least 8
	Fine tipped permanent marker		1
	PTC paper and control paper	1 each	4 each
	miniPCR <sup>®</sup> thermal cycler or other heat block		1 (can be shared between groups)
	blueGel <sup>™</sup> (or other electrophoresis apparatus)		1 (can be shared between groups)

### B. Preparation for gel electrophoresis

- Distribute 15  $\mu$ l Fast DNA Ladder 2 to each group.
- Have the banding pattern of the Fast DNA Ladder 2 handy (page 25) to help interpret the electrophoresis results.
- Prepare 1X TBE buffer.
  - TBE buffer is often provided as liquid concentrate or powder.
  - Follow manufacturer's instructions to prepare 1X TBE buffer solution.
  - Volume to prepare depends on method used to prepare gels; see "Important Note" below.
- Gels can be poured in advance of the class
  - This lab requires running and visualizing DNA samples on a 2% agarose gel with a fluorescent DNA stain (e.g., SeeGreen<sup>™</sup> or GelGreen<sup>®</sup>).
  - Pre-poured gels can be stored at ambient temperature, in a sealed container or wrapped in plastic wrap, and protected from light for up to three days.

### IMPORTANT NOTE: There are several ways to prepare agarose gels.

- Scan the QR code for detailed instructions on how to prepare agarose gels.
- Both written and video instructions are available.



[www.minipcr.com/agarose-gel/](http://www.minipcr.com/agarose-gel/)



# Student's Guide Contents

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# 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'

5'...ATATCATCCTGTGTTGCCTTCATC...3'

**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).

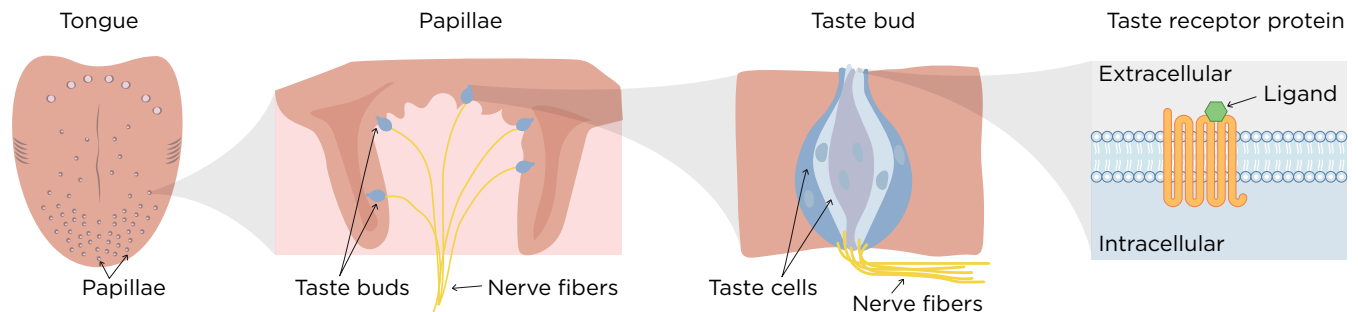
Today we will focus on SNPs in genes. While the term gene can be difficult to define, in this lab we refer to a *gene* as a stretch of DNA that contains the instructions for making a specific protein. Scientists use the term *allele* to describe different versions of a gene. Different alleles for the 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**hiocarbamide (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).



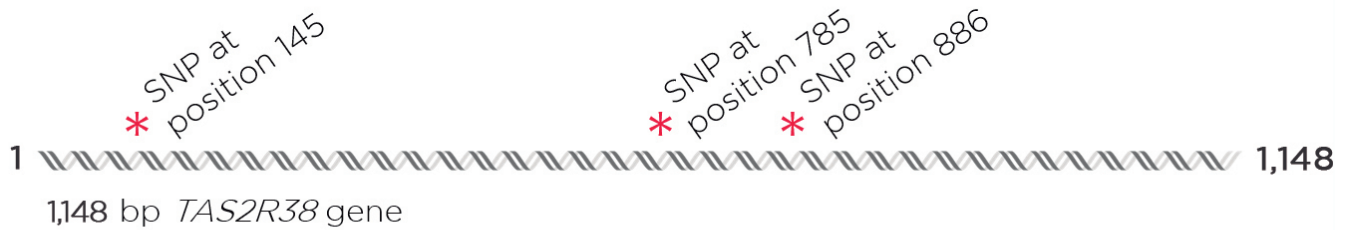
People detect taste through specialized cells found mostly on the tongue. On their surface, these taste receptor cells contain receptor proteins that bind various chemicals that enter the mouth. The receptor proteins set off a chemical relay within the cell, which then will send signals to the brain,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



<i>TAS2R38</i> gene position (bp)	TASTER ALLELE (T)		NON-TASTER ALLELE (t)	
	Codon	Amino acid	Codon	Amino acid
145	<u>C</u> CA	Pro	<u>G</u> CA	Ala
785	G <u>C</u> T	Ala	G <u>T</u> T	Val
886	G <u>T</u> C	Val	A <u>T</u> C	Ile

**Figure 3. Polymorphism in the *TAS2R38* gene.** The human *TAS2R38* 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**ssoleucine (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.

<i>TAS2R38</i> 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.

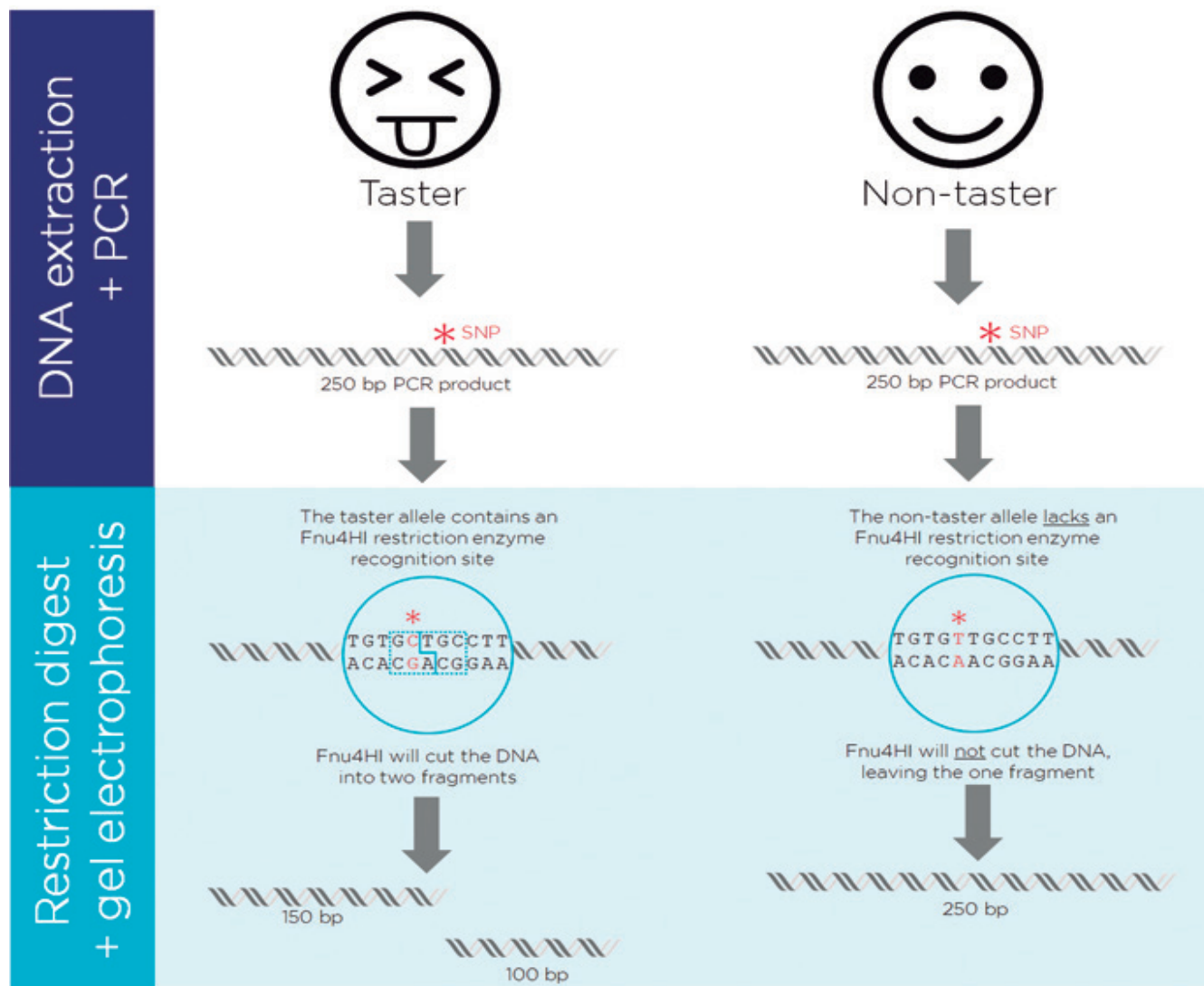


Figure 5. Overview of lab procedure.



# Laboratory guide



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

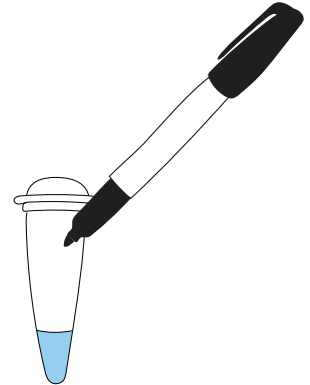
## DNA extraction

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

**1. You should receive a tube with 50  $\mu$ l extraction buffer in it.**

**Label the tube with your initials**

- Label tubes on the upper sidewall, as writing on the cap or lower sidewall may rub off.



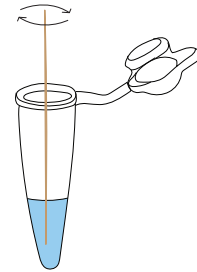
**2. Collect cheek cells**

- Gently scrape the inside of your cheek 3-4 times with a flat-end toothpick.
- It shouldn't hurt.

**3. Transfer cheek cells to tube with buffer**

- Dip the end of the toothpick with your cheek cells in the X-Tract™ buffer.
- Swirl the toothpick to dislodge the cells.
- Dispose of the toothpick.

Swirl toothpick in buffer



**4. Cap your tube**

**5. Incubate your tube for 10 minutes at 95°C**

- You can use a miniPCR<sup>®</sup> thermocycler in Heat Block mode, a water bath, or other heat block.
- Note: If using a heat block or water bath, weigh down the lids of your tubes so they don't pop open.

**6. Remove your tube from heat and immediately use DNA extract for PCR**

- Note: The DNA extract is stable for up to two hours, but the sooner you use it for PCR the better.



## PCR: setting up reactions

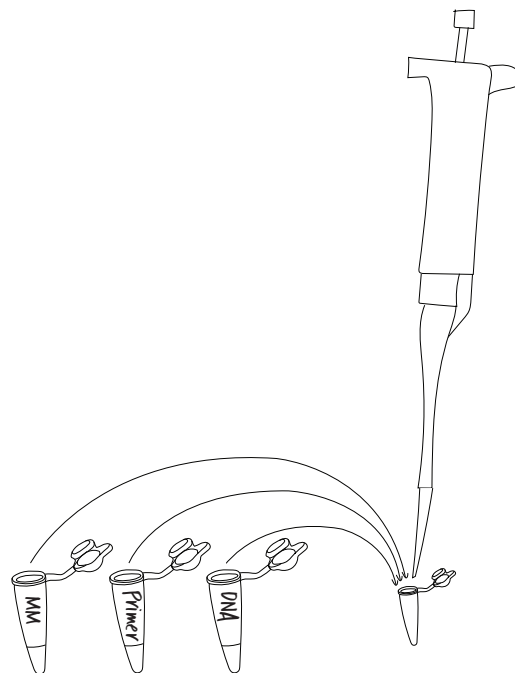
### 1. Label a PCR tube (200 µl tube) with your initials followed by "P" for PCR

- Note: label tubes on the upper sidewall, as writing on the cap or lower sidewall may rub off during PCR.

### 2. Add PCR reagents to your labeled PCR tube

- Change pipette tips between samples to prevent contamination.

Reagent	Volume
2X EZ PCR Master Mix™	12.5 µl
PTC Primer Mix	12.5 µl
Student DNA sample	3 µl
<b>TOTAL VOLUME</b>	<b>28 µl</b>



Note: EZ PCR Master Mix™ contains:

- Taq DNA polymerase
- dNTPs
- PCR buffer with Mg<sup>2+</sup>
- Gel loading dye

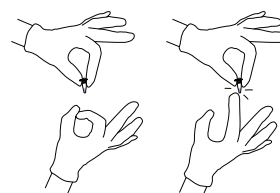
### 3. Cap the tube then flick to ensure the reagents are well mixed

### 4. Gently tap the tubes on your bench to ensure that the liquid collects at the bottom

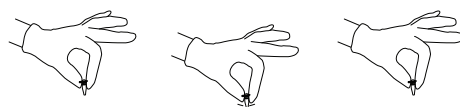
### 5. Place the tube inside the miniPCR® machine

- Close the miniPCR® lid and tighten it gently using the adjustment knob until you feel slight resistance.

Flick to mix



Tap to collect liquid at bottom





## PCR programming


-

These instructions are illustrated using miniPCR® software on a Windows PC. Software interfaces vary slightly by operating system. See the User's Guide for more details.

If using a different thermal cycler, PCR protocol parameters should remain the same (step 7).

### 1. Open the miniPCR® app and remain on the “Library” window

### 2. Connect your miniPCR® thermal cycler to your device using the supplied USB cable or via Bluetooth®

- Note: Bluetooth® is only available on certain models. To connect via Bluetooth®, select the  icon, located by “Devices” at the left of the desktop app or at the top of the mobile app.

### 3. Make sure your miniPCR® thermal cycler is plugged in and that the power switch is turned on

- Note: If your machine begins running a previously loaded protocol, you may stop it by clicking or tapping the “X” symbol in the top left box of the “Monitor” window.

### 4. While in the “Library” window, click the button to create a new protocol

- Button is located in the upper right hand corner of the window.

### 5. Select “PCR” from the drop-down menu

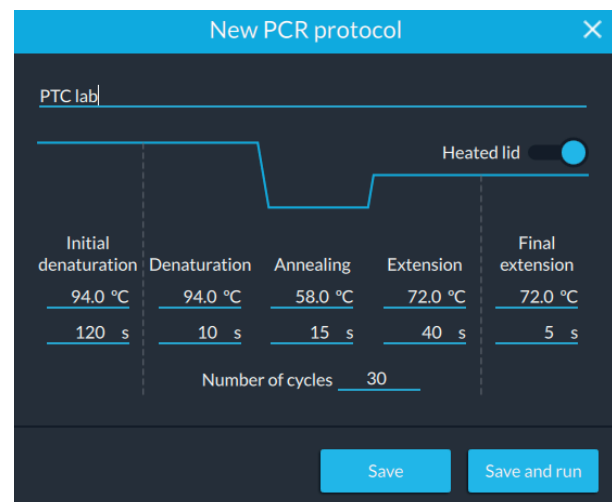
### 6. Enter a name for the protocol; for example:

- “PTC lab”

### 7. Enter the PCR protocol 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

Note: The “Heated lid” slider should be in the on position.



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

Heated lid ☒

Save Save and run

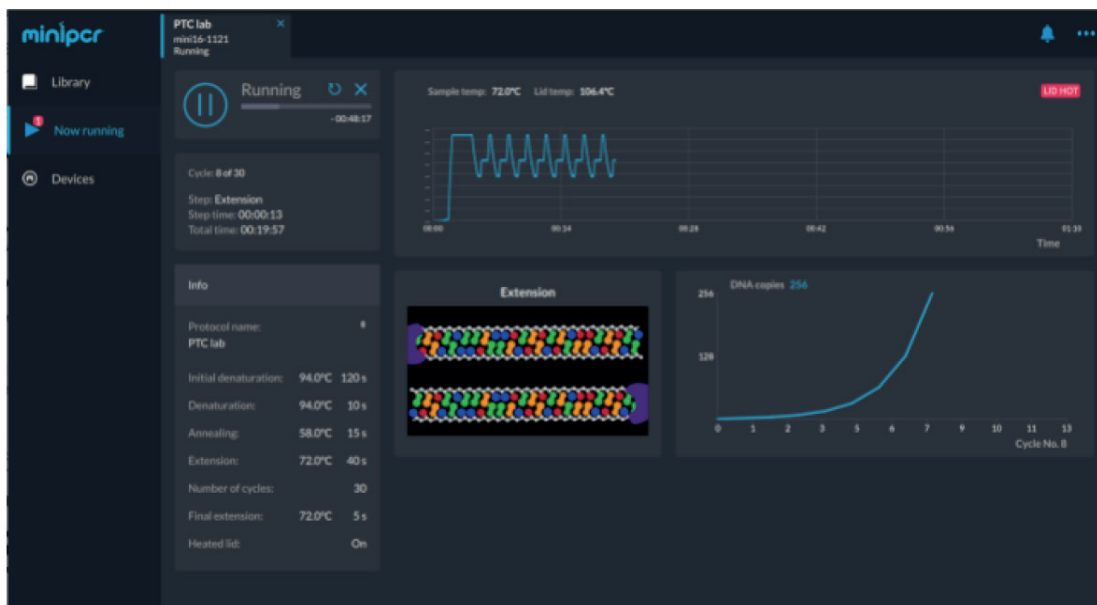


## 8. Click “Save and run” to start the protocol

- If connected to more than one machine, choose the serial number of the miniPCR® thermal cycler you are using. If asked “Do you want to stop the current protocol...?”, click “Yes”.
- The lights on the front of the miniPCR® thermal cycler will blink 3 times to indicate that the protocol has been loaded.
- Note: If needed, you may unplug the USB cable or disconnect Bluetooth® once the protocol has been loaded. Even if disconnected from your device, the protocol will continue to completion as normal.

## 9. Choose “Monitor” window

- The “Monitor” window can be selected on the left column in the desktop app and at the top in mobile app.
- If more than one miniPCR® thermal cycler is connected to the same device, choose which machine you would like to monitor using the tabs at the top of the window (desktop app) or bottom of the Library (mobile app).



The miniPCR® software allows each lab group to monitor the reaction parameters in real time.

## 10. When the PCR run has completed (approximately 70 min), app status will show “Finished” and the red, yellow, and green LEDs on your miniPCR® thermal cycler will light up and stay on



Be careful not to touch the metal lid which may still be hot

## 11. PCR product is stable at room temperature for several days. For longer term storage, move tubes to a fridge or freezer

- Tubes may remain inside the miniPCR® thermal cycler for several days following protocol completion.



## Set up restriction digest

### 1. Label a new PCR tube (200 $\mu$ l tube) with your initials and 'RE' for restriction digest

- Note: Label tubes on the upper sidewall, as writing on the cap or lower sidewall may rub off.

### 2. Transfer 14 $\mu$ l of your PCR reaction to your new tube

### 3. Use a 0.5-10 $\mu$ l micropipette to add 1 $\mu$ l restriction enzyme to your tube

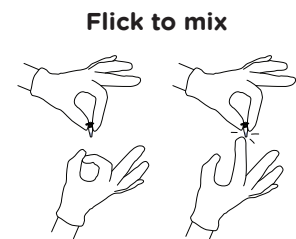
- Eject the restriction enzyme directly into the liquid already in your tube and pipette up and down several times to mix. Proper mixing is essential.
- Note: Your teacher might perform this step for you.

### 4. Cap the tube then flick to ensure the reagents are well mixed

### 5. Gently tap the tubes on your bench to ensure that the liquid volume collects at the bottom

### 6. Incubate at 37°C for 15 minutes

- You can use a miniPCR<sup>®</sup> thermocycler in Heat Block mode, or a water bath or dry block incubator.
- Note: The incubation can be extended overnight if needed based on your class schedule.



## PTC taste test (complete during restriction digest 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:

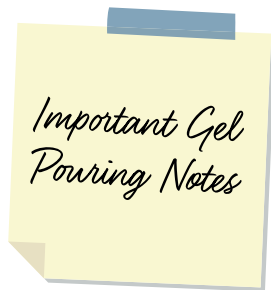
Reaction	Phenotype
The PTC paper tastes similar to the control paper	non-taster
The PTC paper tastes bitter	taster

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 36.



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

## Gel electrophoresis: Pouring gels (before or during class period)



Gels can be prepared up to three days ahead of time and stored at ambient temperature, covered in air-tight plastic wrap and protected from light.

You will need one lane for each student plus one lane for ladder per group. It is possible for groups to share a gel by using two combs.

These instructions are designed for use with the blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.

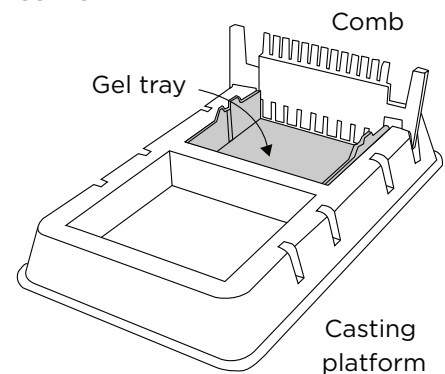
### 1. Prepare 1X TBE buffer (to be completed by teacher in advance)

- TBE buffer is often provided as liquid concentrate or powder.
- Follow manufacturer's instructions to prepare 1X TBE buffer solution.

### 2. Prepare a clean and dry casting platform with a gel tray and comb

- Place the clear gel tray in the white casting platform.
- Place a well-forming comb at the top of the gel tray.

### 3. Prepare a 2% agarose solution with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®) using the method indicated by your instructor



### IMPORTANT NOTE: There are several ways to prepare agarose gels.

- Scan the QR code for detailed instructions on how to prepare agarose gels.
- Both written and video instructions are available.



[www.minipcr.com/agarose-gel/](http://www.minipcr.com/agarose-gel/)

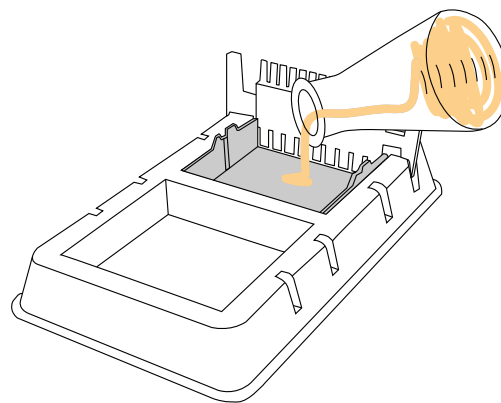


**4. Pour the agarose solution into the prepared casting platform with a gel tray and comb**

- The agarose solution should cover the bottom of the gel tray and the bottom 3 mm of the comb (roughly the bottom 1/3 of the comb).

**5. Allow gel to solidify completely and remove the comb by pulling firmly upwards**

- Gels will typically be ready in about 10 minutes.
- Gel is ready when cool and firm to the touch.





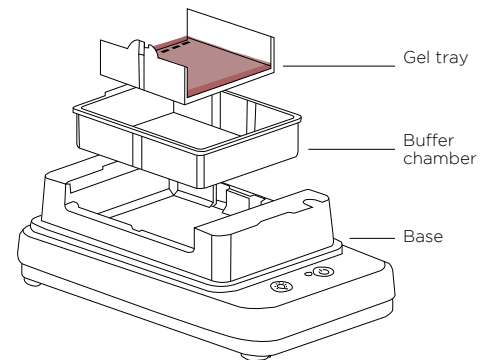
Protective gloves and eyewear should be worn for the entirety of this experiment.

## Gel electrophoresis: Running the gel

These instructions are designed for use with blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.

### 1. Place the gel tray containing your gel in the buffer chamber

- Ensure that the clear buffer chamber is inside the blueGel™ electrophoresis system.
- The wells of the gel should be on the same side as the negative electrode, away from the power button.



### 2. Add 30 ml of 1X TBE electrophoresis buffer

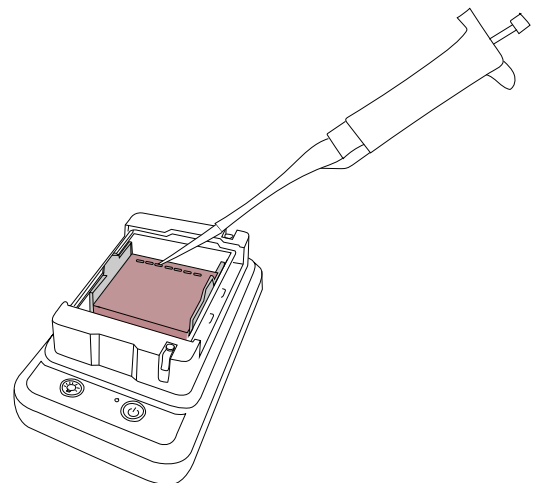
- The buffer should just cover the gel and wells.
- Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).

### 3. Load 10 µl Fast DNA Ladder 2 in the leftmost well

### 4. Load 15 µl of each student's restriction digest into subsequent wells

Note the order in which you loaded your group's samples below:

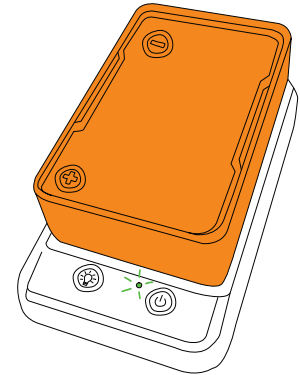
- **Well 1:** Ladder
- **Well 2:** \_\_\_\_\_ (student name)
- **Well 3:** \_\_\_\_\_ (student name)
- **Well 4:** \_\_\_\_\_ (student name)
- **Well 5:** \_\_\_\_\_ (student name)
- **Well 6:** \_\_\_\_\_ (student name)
- **Well 7:** \_\_\_\_\_ (student name)
- **Well 8:** \_\_\_\_\_ (student name)
- **Well 9:** \_\_\_\_\_ (student name)





### 5. Place the orange cover on the blueGel™ electrophoresis system

- To prevent fogging, make sure that ClearView™ spray has been evenly applied to the inside of the orange cover.
- Match the positive and negative electrode signs on the orange lid with the corresponding positive and negative signs on the blue base.
- The electrodes of the lid should be aligned with the metal leads on the base.
- The orange lid should sit flush with the blue base using little force.



### 6. Press the “Run” ⏻ button

- Check that the green light beside the power button remains illuminated.

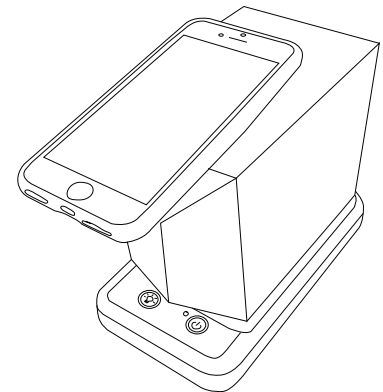
### 7. Conduct electrophoresis for 20-30 minutes

- Note: Check the progress of your samples every 10 minutes to monitor the migration of your DNA samples.
- Longer electrophoresis times will result in better separation of similar sized DNA fragments. However, if run too long, small DNA fragments can run off the end of the gel or lose fluorescence.

## Gel electrophoresis: Visualizing results

### 1. Press the “light bulb” 💡 button to turn on the blueGel™ transilluminator

- For best viewing, dim lights or use Fold-a-View™ photo documentation hood with a smartphone camera.
- Gels may be viewed at the end of the run or periodically throughout the run.
- If the image appears hazy, wipe off the inside of the orange cover and reapply ClearView™ spray.

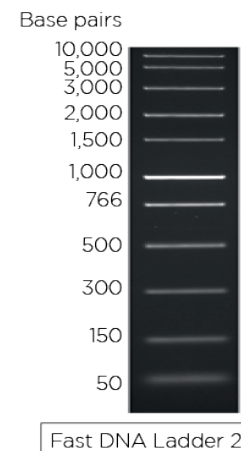


### 2. Ensure that there is sufficient DNA band resolution in the 50-300 bp range of the Fast DNA Ladder 2

- Run the gel longer if needed to increase resolution.

### 3. Document your results

- Compare the bands from the DNA samples to the ladder to obtain size estimates.
- Place Fold-a-View™ photo documentation hood on the blueGel™ electrophoresis system to take a picture with a smartphone or other digital camera.





# Pre-lab study 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 digest, 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

4. 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:

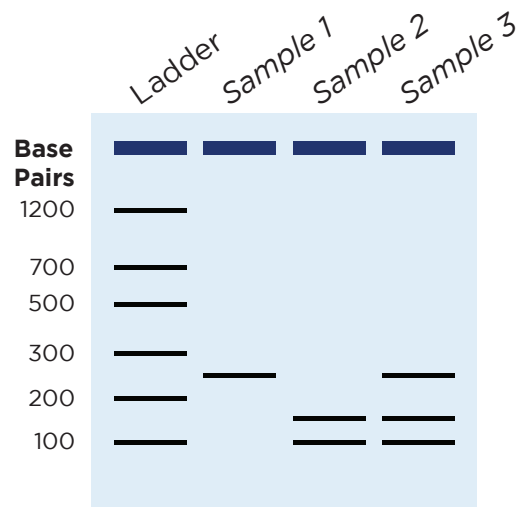
- b. Pretend the gel electrophoresis results to the right are from testing three individuals, including Fox and Noller. Use the information to answer the following questions:

Which sample(s) could have come from Fox:

Explain your reasoning:

Which sample(s) could have come from Noller:

Explain your reasoning:





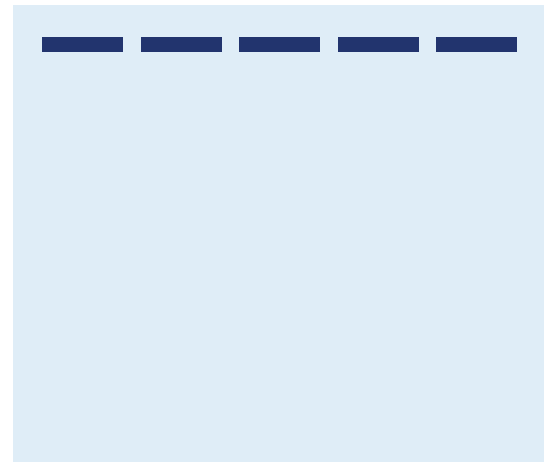
# Post-lab study questions

## Interpreting results

1. Based on the PTC taste test, what is your phenotype?

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

3. Use the image on the right to illustrate your gel electrophoresis results. There are five lanes on the gel: one for your ladder, and one for each member of your lab group. Be sure to label each lane with the source of the sample.
4. Next to each band, write approximately how long (in base pairs) you think the DNA in that band is. Use the image of the ladder from page 25 to help you.



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.

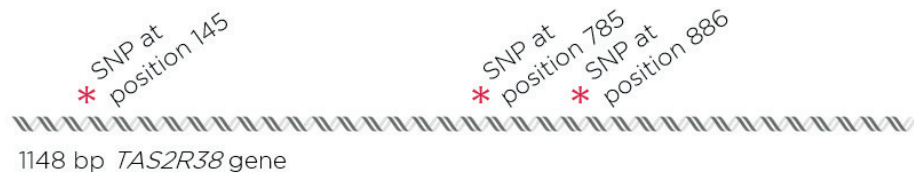
b. In your class, how closely does genotype match the expected phenotype?

Student	Phenotype (taster or non-taster)	Genotype (TT, Tt, tt)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
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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, 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.

1. 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.



2. 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.

3. 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. Use the rubric on the next page to help write your answers.

### 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
<b>CLAIM</b> 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.
<b>EVIDENCE</b> Data from the experiment that supports the claim. Data must be <u>relevant</u> and <u>sufficient</u> 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.
<b>REASONING</b> 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 Activities

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Quantifying PTC taste intensity

P.36

Using the Hardy-Weinberg equation

P.41

G protein-coupled receptors

P.48



## Extension: Quantifying PTC taste intensity

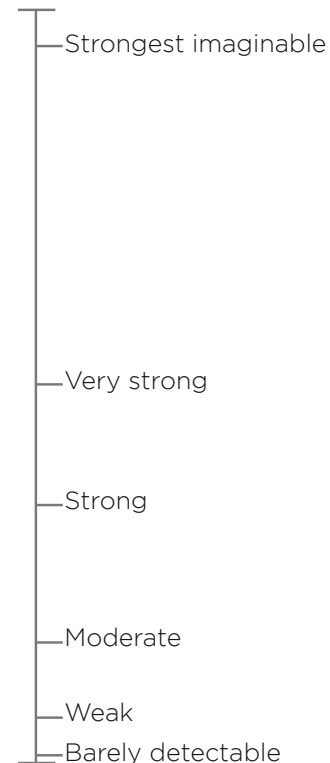
In this lab you scored your PTC tasting phenotype as either being a taster or a non-taster. However, some evidence from PTC taste tests in humans suggests that people who are heterozygous for the *TAS2R38* gene (Tt) may have an intermediate sensitivity to PTC (Bufe et al., 2005). In this activity, you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

<i>TAS2R38</i> 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.

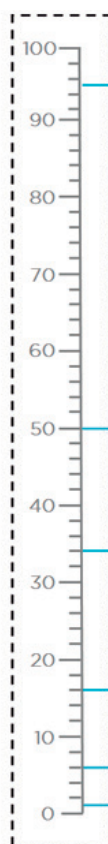


**Labeled magnitude scale.** Rate your perceived intensity of PTC taste by marking an "X" on the scale.



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 millimeters 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 perceived 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		
5		
6		
6		
7		
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11		
12		
13		
14		
15		
16		
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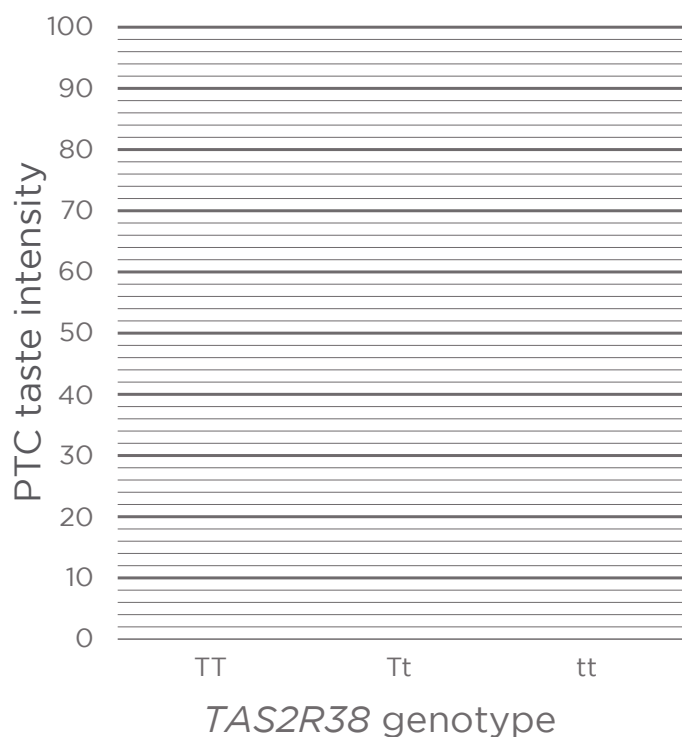
2. Calculate the average labeled magnitude scale score and standard error of the mean for each *TAS2R38* genotype:

<i>TAS2R38</i> 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  $\pm 1$  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

1. 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 genotypic frequencies with what would be predicted in a population that is not evolving and is in Hardy-Weinberg equilibrium.

To calculate the predicted genotypic 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 genotypic 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 observed 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 genotypic frequencies for a population that is not evolving:  $p^2 + 2pq + q^2 = 1$

$p^2$  = 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 genotypic 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: $\chi^2$ test

Use the  $\chi^2$  (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 $\chi^2$ for your class data by filling in the table below:

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

Table 5: Calculating $\chi^2$			
Genotype	Observed # of individuals (from Table 4)	Expected # of individuals (from Table 4)	$\frac{(\text{observed} - \text{expected})^2}{\text{expected}}$
TT			
Tt			
tt			
$\chi^2$ Value			$\Sigma =$

### 10. Use the $\chi^2$ value you calculated in Table 5 to determine whether the variation in the observed results could be due to chance. The $\chi^2$ distribution table is on the next page.

- a. Based on your  $\chi^2$  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  $\chi^2$  distribution table

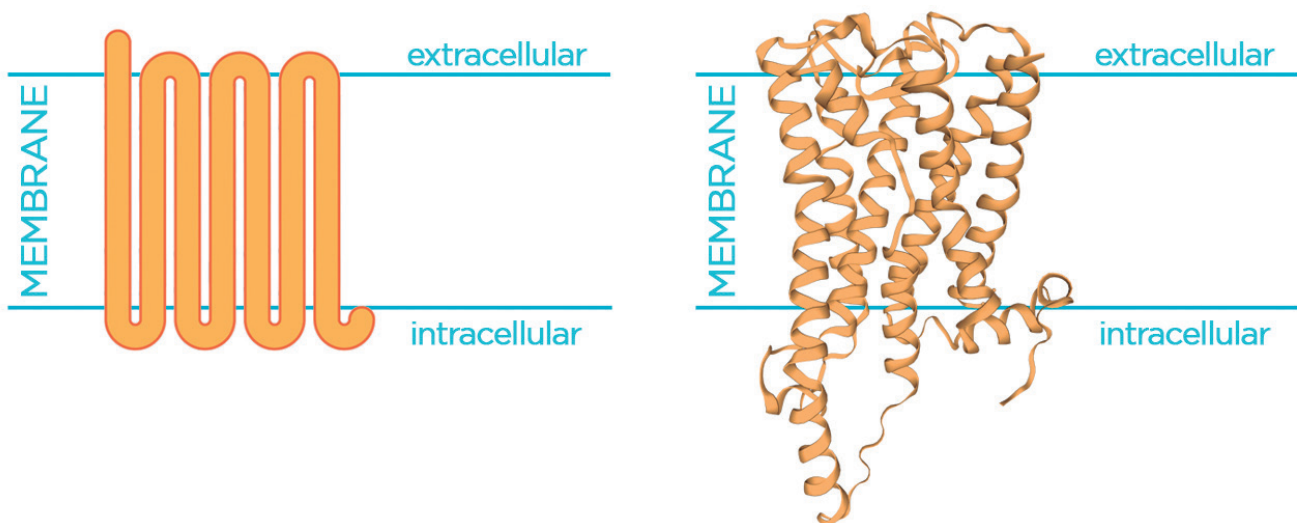
p value	Degrees of freedom							
	1	2	3	4	5	6	7	8
<b>0.05</b>	3.84	5.99	7.82	9.49	11.07	12.59	14.07	15.51
<b>0.01</b>	6.64	9.21	11.34	13.28	15.09	16.81	18.48	20.09



## 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).



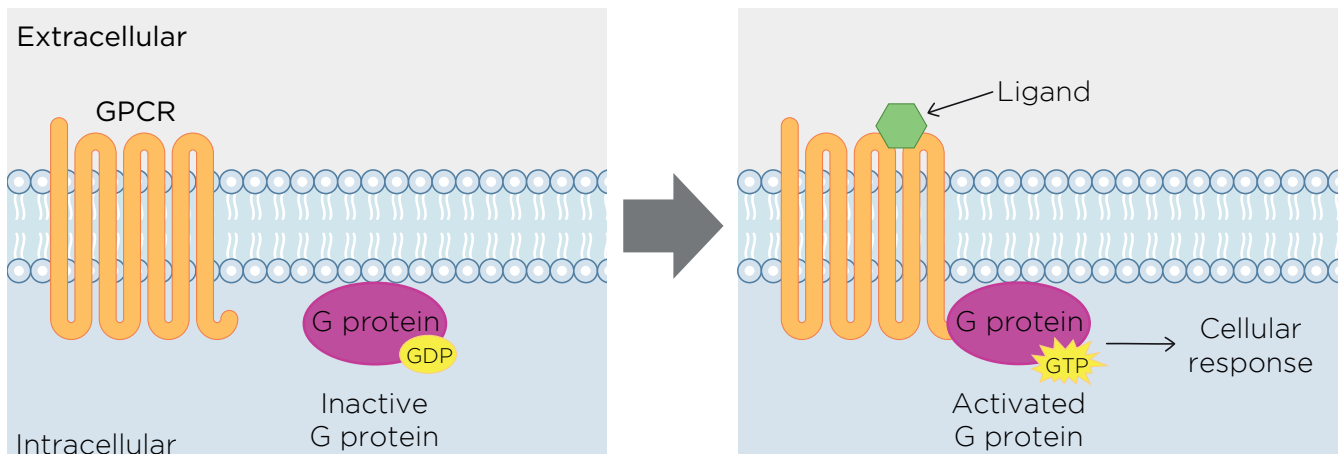
**Figure 1. GPCR structure.** All GPCRs have a similar structure with seven transmembrane domains. For simplicity, GPCRs are often depicted schematically with the seven transmembrane domains arranged in a row along the membrane (left). In reality, the seven transmembrane domains interact with each other 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.** GPCRs 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 neurotransmitters. This diversity 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.



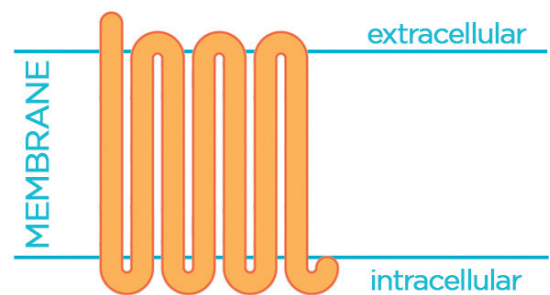
1. We know that the SNPs in the *TAS2R38* gene affect a person's ability to perceive the bitter chemical PTC. Based on what you know about GPCR mediated cellular signaling, which step(s) could be directly affected by the changes in the *TAS2R38* protein? Select all appropriate answers.
  - a. ligand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

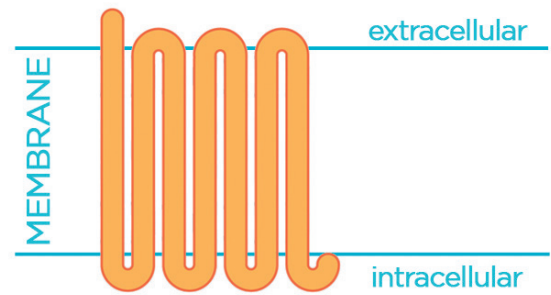
- a. If the different versions of the *TAS2R38* protein bound to ligands differently, roughly where would you expect the proteins to differ? Circle or otherwise clearly mark the general region(s) you would expect to be affected on the drawing to the right.



Explain your reasoning:

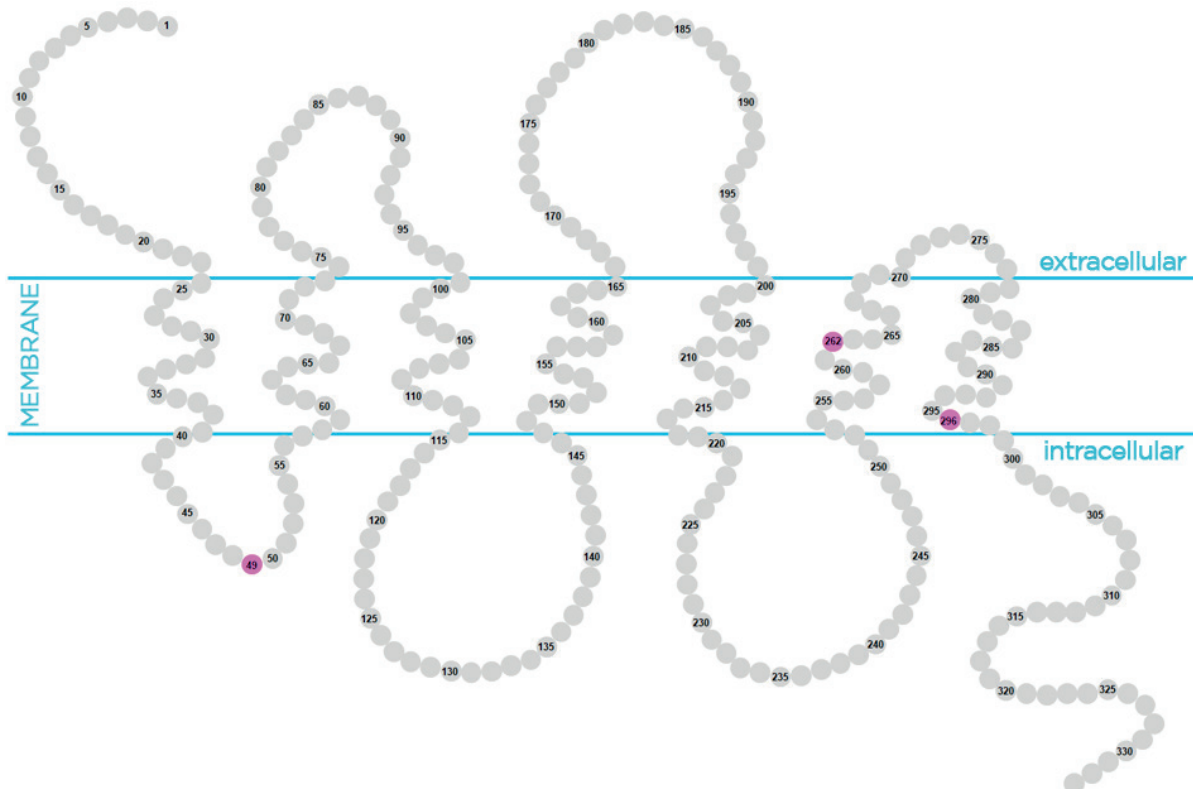


- b. If the different TAS2R38 proteins interacted with the G protein differently, roughly where on the protein would you expect the proteins to differ? Circle or otherwise clearly mark the general region(s) you would expect to be affected on the drawing to the right.



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's Guide

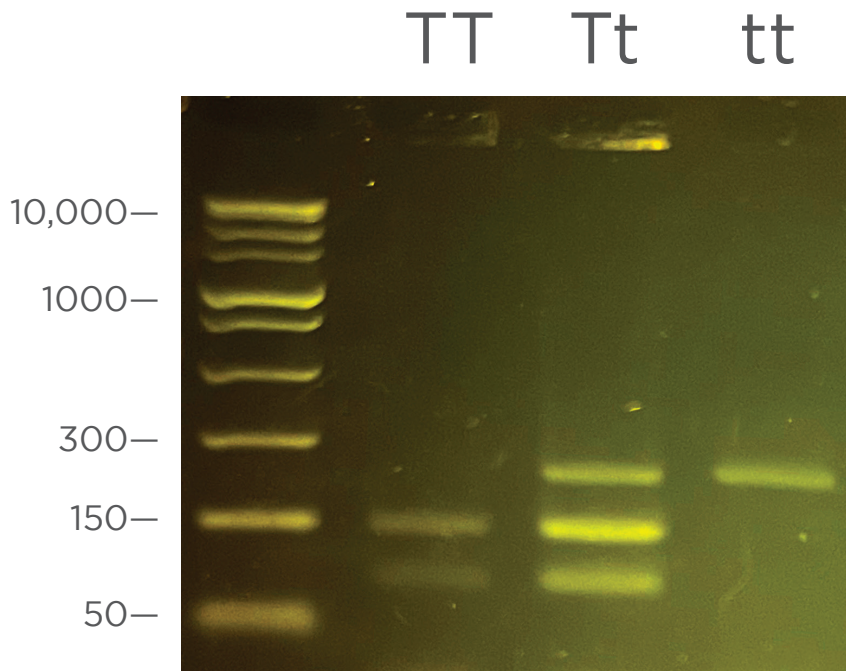
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# Expected results



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

- Before restriction digest, the PCR product for both the taster and non-taster alleles should be ~250 base pairs (bp).
- After the restriction digest:
  - 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 will typically display two bands: one at ~150 bp and one at ~100 bp.
  - A heterozygous taster will display three bands: one at ~250 bp, one at ~150 bp, and one at ~100 bp.
  - A non-taster will display only one band: ~250 bp.
- Note: You may observe bands smaller than 100 bp. These represent primer dimer caused by non-specific binding between excess primers.



# Troubleshooting

The most common source of error in this protocol is the DNA extraction. If some students don't see bands for their samples, it is likely that their DNA extractions failed. Repeat the DNA extraction and (1) have students rinse their mouths with water before collecting cheek cells, (2) ensure students only lightly scrape their cheeks as too much DNA can inhibit PCR, and (3) conduct PCR no later than 12 hours after DNA extraction.

Note: If you want to have your students collect their cheek cells at home, we recommend the following:

- Send each student home with one flathead toothpick and a 200  $\mu$ l tube with 50  $\mu$ l of extraction buffer.
- The morning of the lab, have students collect their cheek cells and transfer them to the tube with extraction buffer (perform steps 1-4 of the protocol on page 17).
- In class, resume the protocol at step 5 by heating samples for 10 minutes at 95°C.

# Notes on lab design

This lab serves as an introduction to the relationship between genotype and phenotype. Specifically, this examines 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.

- 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 31.
- *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).



# Differentiation

This lab serves as an introduction to the relationship between genotype and phenotype. With simple modifications, this activity can be used effectively in classes ranging from general high school through college.

**Introductory classes:** For introductory classes, focus on the link between genotype and phenotype. The standard introduction and review questions for this lab take this approach.

**Advanced classes:** For advanced classes, you can have a more nuanced discussion of the relationship between *TAS2R38* genotype and the PTC tasting phenotype using the background on haplotypes presented in the *advanced questions* on page 31. Additionally, evidence from PTC taste tests in humans suggests that heterozygous individuals have an intermediate sensitivity to PTC (Bufe *et al.*, 2005). Have your students score their perception of PTC bitterness to investigate this relationship using the *extension activity* on page 36. You can also use the *extension activities* to dive into population genetics and the Hardy-Weinberg equilibrium (page 41) or highlight the fact that TAS2R28 is a G protein-coupled receptor and discuss signal transduction in detail (page 48).



# Additional student supports

The following optional supports are to help provide additional scaffolding for students who need it.

At miniPCR bio™, we are committed to preparing students to be successful in the laboratory through high quality curriculum and training. We have created an extensive set of resources to help your students succeed in molecular biology techniques, all of which are available for free download at the miniPCR bio™ tutorials page of our website.

<https://www.minipcr.com/tutorials/>

Those activities most relevant to this lab are listed below.

**Micropipetting:** Video and 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.

# Extension activities

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

**Quantifying PTC taste intensity** (page 36): 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 41): 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 48): *TAS2R38* is a G protein-coupled receptor. Delve into GPCRs and signal transduction.



# Placement in unit

**Genetics:** This lab is well suited to demonstrate the link between genotype and phenotype, as well as to provide a way to examine the inheritance of a relatively simple trait.

**Cell communication:** This lab could be used to link the study of G protein-coupled receptors and signal transduction to an observable phenotype in humans.

**Biotechnology:** This lab can be used as a culminating activity to demonstrate mastery of micropipetting, PCR, restriction digests, and gel electrophoresis. For this approach, we recommend spending more time on some of the activities discussed in the *Additional student supports* section of this lab (page 58) before beginning the experiment.

# 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:

- Students will follow detailed experimental protocols
- Students will interpret data presented in a chart or table
- Students will make a claim based in scientific evidence
- Students will use reasoning to justify a scientific claim

## Molecular Biology Skills:

- Micropipetting
- Isolation of genomic DNA
- Principles and practice of PCR
- Restriction digests
- Preparation of agarose gels
- Agarose gel DNA electrophoresis
- Staining, visualization, and molecular weight analysis of DNA fragments



# Standards alignment

## Next Generation Science Standards

Students who demonstrate understanding can:

<b>HS-LS3-1.</b>	Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
<b>HS-LS3-3.</b>	Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.

Science and Engineering Practice	Disciplinary Core Ideas	Crosscutting Concepts
<ul style="list-style-type: none"> <li>• Asking Questions and Defining Problems</li> <li>• Developing and Using Models</li> <li>• Planning and Carrying Out Investigations</li> <li>• Analyzing and Interpreting Data</li> <li>• Using Mathematics and Computational Thinking</li> <li>• Constructing Explanations and Designing Solutions</li> <li>• Engaging in Argument from Evidence</li> <li>• Obtaining, Evaluating, and Communicating Information</li> </ul>	<p><b>LS1.A:</b> From Molecules to Organisms: Structures and Processes</p> <p><b>LS3.A:</b> Inheritance of Traits</p> <p><b>LS3.B:</b> Variation of Traits</p>	<ul style="list-style-type: none"> <li>• Patterns</li> <li>• Cause and Effect</li> <li>• Structure and Function</li> <li>• Interdependence of Science, Engineering, and Technology</li> </ul>

## Common Core ELA/Literacy Standards

RST.9-10.1	Cite specific textual evidence to support analysis of science and technical texts, attending to the precise details of explanations or descriptions.
RST.9-10.3	Follow precisely a complex multistep procedure when carrying out experiments, taking measurements, or performing technical tasks, attending to special cases or exceptions defined in the text.
RST.9-10.4	Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 9-10 texts and topics.
RST.9-10.5	Analyze the structure of the relationships among concepts in a text, including relationships among key terms (e.g., force, friction, reaction force, energy).
RST.9-10.9	Compare and contrast findings presented in a text to those from other sources (including their own experiments), noting when the findings support or contradict previous explanations or accounts.
WHST.9-10.1	Write arguments focused on discipline-specific content.
WHST.9-10.2	Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes.
WHST.9-10.9	Draw evidence from informational texts to support analysis, reflection, and research.

\* For simplicity, this activity has been aligned to high school NGSS and grades 9-10 Common Core standards.



# Ordering information

To order PTC Taster Lab kits, you can:



Call (781)-990-8PCR



email us at [orders@minipcr.com](mailto:orders@minipcr.com)



visit <https://www.minipcr.com>

**PTC Taster Lab (catalog no. KT-1004-03) contains the following reagents supplies:**

- 2X EZ PCR Master Mix, Load-Ready™
- PTC Primer Mix
- Restriction Enzyme Fnu4HI
- Fast DNA Ladder 2
- X-Tract™ DNA Extraction Buffer

Materials are sufficient for 32 students

All components should be kept frozen at -20°C for long-term storage

Reagents must be used within 12 months of shipment

**Other supplies needed:**

- PTC paper (available on Amazon)
- Agarose (electrophoresis grade)
- Fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®)
- Gel electrophoresis buffer (e.g., 1X TBE)
- Distilled or deionized water (to dilute TBE buffer concentrate)

**Note:** Agarose, DNA stain, and TBE buffer are available at [minipcr.com](https://www.minipcr.com) as part of the Learning Lab Companion Kit (KT-1510-01)



# About miniPCR bio Learning Labs™

This Learning Lab was developed by the miniPCR bio™ team in an effort to help more students understand concepts in molecular biology and to gain hands-on experience in real biology and biotechnology experimentation.

We believe, based on our direct involvement working in educational settings, that it is possible for these experiences to have a real impact in students' lives. Our goal is to increase everyone's love of DNA science, scientific inquiry, and STEM. We develop miniPCR bio Learning Labs™ to help achieve these goals, working closely with educators, students, academic researchers, and others committed to science education.

The guiding premise for this lab is that a two period PCR based experiment can recapitulate a real-life biotechnology application and provide the right balance between intellectual engagement, inquiry, and discussion.

Starting on a modest scale working with Massachusetts public schools, miniPCR bio Learning Labs™ have been well received, and their use is growing rapidly through academic and outreach collaborations across the world.