

miniPCR Learning Lab™: PTC Taster Lab From Genotype to Phenotype

The DNA Times ^{Free}

Parents Genetically Engineer Children to Like Broccoli

A diagnostic clinic in California is developing an assay to test couples for sensitivity to bitter chemicals as the first step to genetically engineering their children to like broccoli

Boston, October 30th, 2037. Parents are tired of having to force feed their kids broccoli. Doctors are now offering the option of genetically engineering their unborn children to decrease their sensitivity to broccoli's bitter taste. After identifying the gene responsible for aversion to the bitter tasting compound found in broccoli, a new gene therapy is about to hit the market to correct the aversion in unborn children.



The Taqdale Clinic in California is developing a screening assay to test future parents to determine if they are candidates for gene therapy. The clinic is using phenylthiocarbamide (PTC), a bitter compound similar to that found in broccoli, that can only be tasted by people carrying specific variants of the *TAS2R38* gene. Student volunteers have been invited to help test the assay before its market release.

Woolly mammoth finally cloned at
Brooklyn Zoo p.5



Transgenic heart-shaped bananas
could solve world hunger p.7



miniPCR Learning Lab™: PTC Taster Lab From Genotype to Phenotype

Student's Guide Contents

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To taste or not to taste, that is the question. And the answer is surprisingly simple: a few nucleotides. In this lab, we will examine how single nucleotide polymorphisms (SNPs) can change our ability to perceive the world around us. The human sense of taste is composed of an intricate neurophysiological network, but it only takes small changes to one gene to change the way we taste...

1. Synopsis

Approximately 99.9% of the human genome is identical across individuals; the other 0.1% makes each one of us unique. The human genome contains about 3 billion base pairs but just a single nucleotide change can alter or abolish gene function and can result in a new phenotype. A *single nucleotide polymorphism*, or SNP (pronounced "snip"), is a common variant at a single position in a DNA sequence among individuals of the same species. SNPs are the most common form of variation in the human genome and account for much of our genetic and phenotypic diversity.

In this Learning Lab, students will explore SNPs associated with their own phenotypes. Students will assess their ability to taste the chemical phenylthiocarbamide (PTC) and determine how that ability correlates with their genotype at the *TAS2R38* locus, which encodes for a taste receptor expressed in gustatory papillae. There are two common alleles for the *TAS2R38* gene, a 'taster' allele and a 'non-taster' allele. The difference between these alleles results from the combination of just three SNPs, or three single-nucleotide changes. It is a goal of this lab to illustrate how very small genetic changes can have significant functional consequences.

Students will extract their own DNA from cheek cells, perform PCR (polymerase chain reaction) to amplify the *TAS2R38* gene, use a restriction enzyme on the PCR product to distinguish between *TAS2R38* alleles, and perform gel electrophoresis to visualize the results. This activity allows students to directly explore the genetic basis of a fundamental aspect of neurophysiology—our ability to taste.

- **Techniques utilized:** DNA extraction, PCR, restriction endonuclease digestion, gel electrophoresis, and DNA visualization
- **Time required:** Two 45-minute periods (see Section 6, *Planning your time*)
- **Reagents needed:** *miniPCR PTC Taster Lab* reagents kit (available at miniPCR.com), gel electrophoresis reagents (See sections 5 and 9)
- **Suggested skill level:** Familiarity with central dogma of molecular biology, molecular genetics, micropipetting techniques

2. Background and significance

The Molecular Physiology of Taste

G-protein-coupled receptors (GPCRs) are a large class of diverse and highly conserved proteins with seven transmembrane domains (Figure 1). Thousands of different human GPCRs have been identified. They play a role in a wide range of physiological processes, from hormonal communication to sensory perception. GPCRs are embedded in a cell's plasma membrane where they interact with G proteins that trigger signal transduction cascades inside the cell.

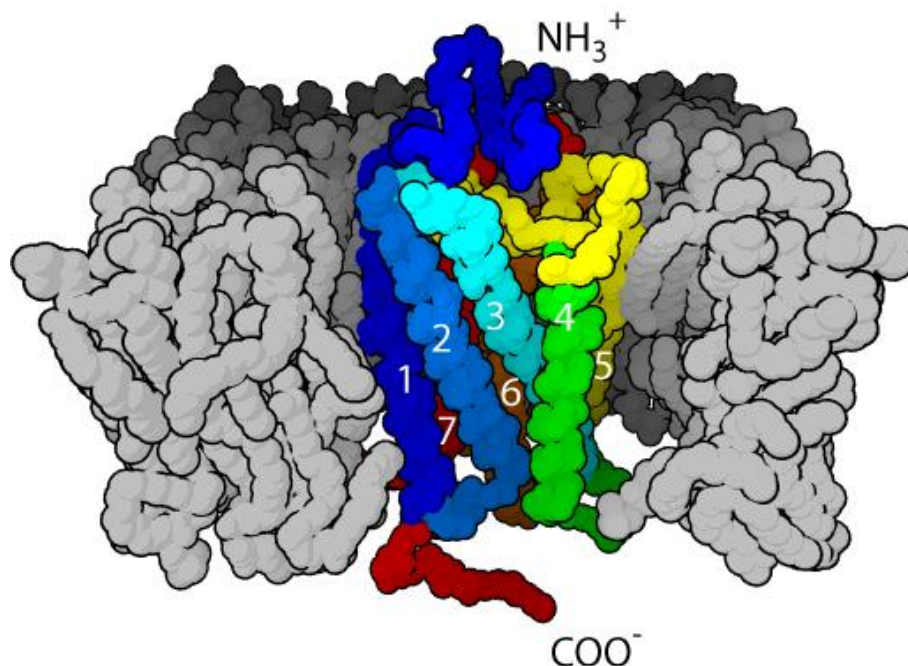


Figure 1. The seven-transmembrane structure of a G protein–coupled receptor, via [Wikipedia](#)

Our ability to taste is mediated by GPCRs. Taste receptor genes of the TAS1R and TAS2R families encode GPCRs expressed in taste receptor cells of the gustatory papillae in the tongue. Various taste receptors mediate specific taste modalities (bitter, sweet, savory, etc.). Humans have 43 different receptors for bitter taste within the TAS2R gene family. One of the best characterized taste receptors is encoded by the *TAS2R38* gene. This receptor binds ligands such as 6-n-propylthiouracil (PROP6) and phenylthiocarbamide (PTC), two chemicals similar to those found in bitter tasting vegetables. When ligands bind to the receptor they cause a conformational

change in the TAS2R38 receptor protein, which activates a signal transduction pathway that ultimately transmits a nerve impulse to the brain letting it know “that’s bitter”.

To taste or not to taste... determined by a single gene

The TAS2R38 receptor is one of the only taste receptors for which variations at the DNA level are known to influence taste perception. Single nucleotide polymorphisms (SNPs) in the *TAS2R38* gene have been associated with impaired bitter taste perception (for a broad genomic analysis of bitter taste perception see Roudnitzky et al., 2015¹). This is thought to be due to a deficit in signal transduction after the receptor binds to its ligands (such as the chemicals PROP6 and PTC). There are 3 SNPs commonly found on the *TAS2R38* gene that are correlated with an individual’s ability to taste PTC, resulting in two alleles often referred to as *PAV* and *AVI*, as detailed in Table 1.

Position (bp)	Position (amino acid)	Taster (<i>PAV</i>)		Non-Taster (<i>AVI</i>)	
		base	aa	base	aa
145	49	C	Pro	G	Ala
785	262	C	Ala	T	Val
886	296	G	Val	A	Ile

Table 1. Polymorphisms within the *TAS2R38* gene

The *PAV* allele is associated with high sensitivity to the bitter taste of PTC (‘Taster’ allele), while *AVI* is associated with little or no sensitivity (‘Non-taster’ allele). In human populations we find individuals that are homozygous for *PAV* (‘strong tasters’), homozygous for *AVI* (‘non-tasters’), or heterozygous (‘moderate tasters’).

Implications of SNPs in human health

There are roughly 10 million SNPs in the human genome, and most of them have no known effect in health or development. Yet many SNPs have been linked to other forms of phenotypic

¹ Roudnitzky N, Behrens M, Engel A, Kohl S, Thalmann S, Hübner S, Lossow K, Wooding SP, Meyerhof W. Receptor Polymorphism and Genomic Structure Interact to Shape Bitter Taste Perception. PLoS Genet. 2015 Sep 25;11(9):e1005530. doi:10.1371/journal.pgen.1005530. eCollection 2015 Sep. PubMed PMID: 26406243; PubMed Central PMCID: PMC4583475.

variation besides sensitivity to PTC, including SNP association with certain diseases. In some cases SNPs may directly influence physiology (e.g. by directly altering the coding sequence for a gene), and in other cases they simply serve as biological markers that are associated with genetic traits (e.g. when they occur in the vicinity of a gene). Once phenotypic associations of SNPs are known, scientists can examine changes in the surrounding DNA in an attempt to identify the gene or genes responsible for the trait. Hence some SNPs can help predict a patient's risk of developing a certain disease.

Some SNPs can also determine a patient's differential response to a drug. About 50% of medicines on the market target GPCRs, therefore a better understanding of GPCRs and their associated polymorphisms may serve as a gateway to personalized medicine. Understanding a patient's genotype at a specific pharmacological target can help predict their response to treatment, enabling healthcare providers to harness our growing understanding of SNPs to inform their practice.

Today's Lab

You will determine the genotype of SNP785 of your own *TAS2R38* genes, in which a **cytosine to thymine** change at the nucleotide level results in an **alanine to valine** change at the amino acid level. At the same time, you will test your ability to taste the bitter compound PTC and correlate it with your genotype, which will later be tested through molecular analysis.

You will obtain cheek cells by taking a swab of the inside of your mouth and will isolate your own genomic DNA by lysing the cells. Using your genomic DNA as template, you will amplify a 250 bp DNA region surrounding SNP785 of your *TAS2R38* gene using PCR. In order to distinguish between 'taster' and 'non-taster' alleles, you and your classmates will perform a restriction digest of the PCR products using a restriction endonuclease that can only recognize and cleave the 'taster' allele (Figure 2). The cleaved 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. The difference in size will be visualized using gel electrophoresis.

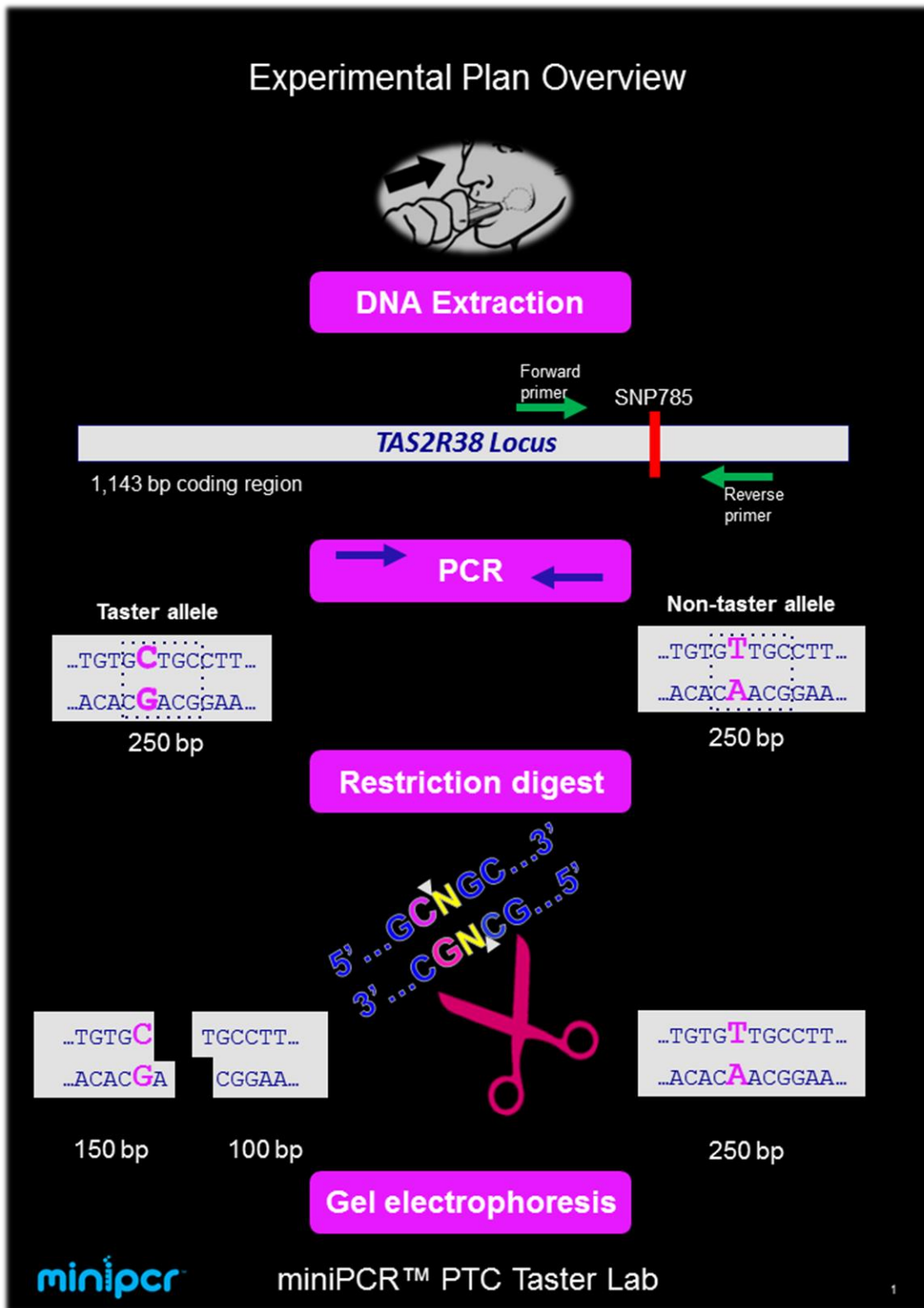


Figure 2. PTC Taster Lab overview

3. Laboratory guide

A. DNA Extraction

1. **Extraction Tube Preparation:**

- Dispense **1 PCR tube (200 μ L thin-walled PCR tube) per student**
- **Label tube on the side, not cap, of the tube** with the student's initials

2. **Add 50 μ L of X-Tract™ DNA Extraction Buffer** to each 200 μ L tube



NOTE: for maximum DNA extraction efficiency, please abstain from eating or chewing gum ~20 minutes prior to cheek cell collection

3. **Scrape the inside of your cheek** 5-6 times with a flat-end toothpick to collect cells at the end of the toothpick



Rub gently along cheek, it shouldn't hurt!

4. **Dip the toothpick in the 200 μ L tube** swirling it in X-Tract™ Buffer

- Swirl toothpick thoroughly in the buffer to release cells

5. **Tightly cap the tubes**

6. **Incubate the tube in miniPCR™ for 10 minutes at 95°C**

- Insert tubes and close the PCR machine lid and gently tighten the lid using the adjustment knob
- Use miniPCR™ Heat Block mode, or alternatively use a 95°C heat block or water bath. *If you use a heat block or water bath make sure to put pressure on the lids, otherwise the lids might pop open.*

7. **Remove tubes from heat** and let them rest in a tube rack at room temperature



We recommend using the DNA extract immediately for PCR

B. PCR set up

- PCR Tube Preparation:** Label 4 clean PCR tubes (200 μ L thin-walled tubes) per group
 - Student's initials on the side wall of the tube



Remember to change tips at each of the following steps!

- Add PCR reagents** to each tube
 - 12.5 μ L PTC Primer Mix
 - 12.5 μ L EZ PCR Master Mix

PCR Tube (Test Groups)	
PTC Primer Mix	12.5 μ L
2X EZ PCR Master Mix	12.5 μ L
Template DNA	3 μ L
	28 μ L

- Add DNA samples** to each new tube

- 3 μ L Student DNA extracts from the top of the solution
- Pipette up and down to mix directly into the PCR solution

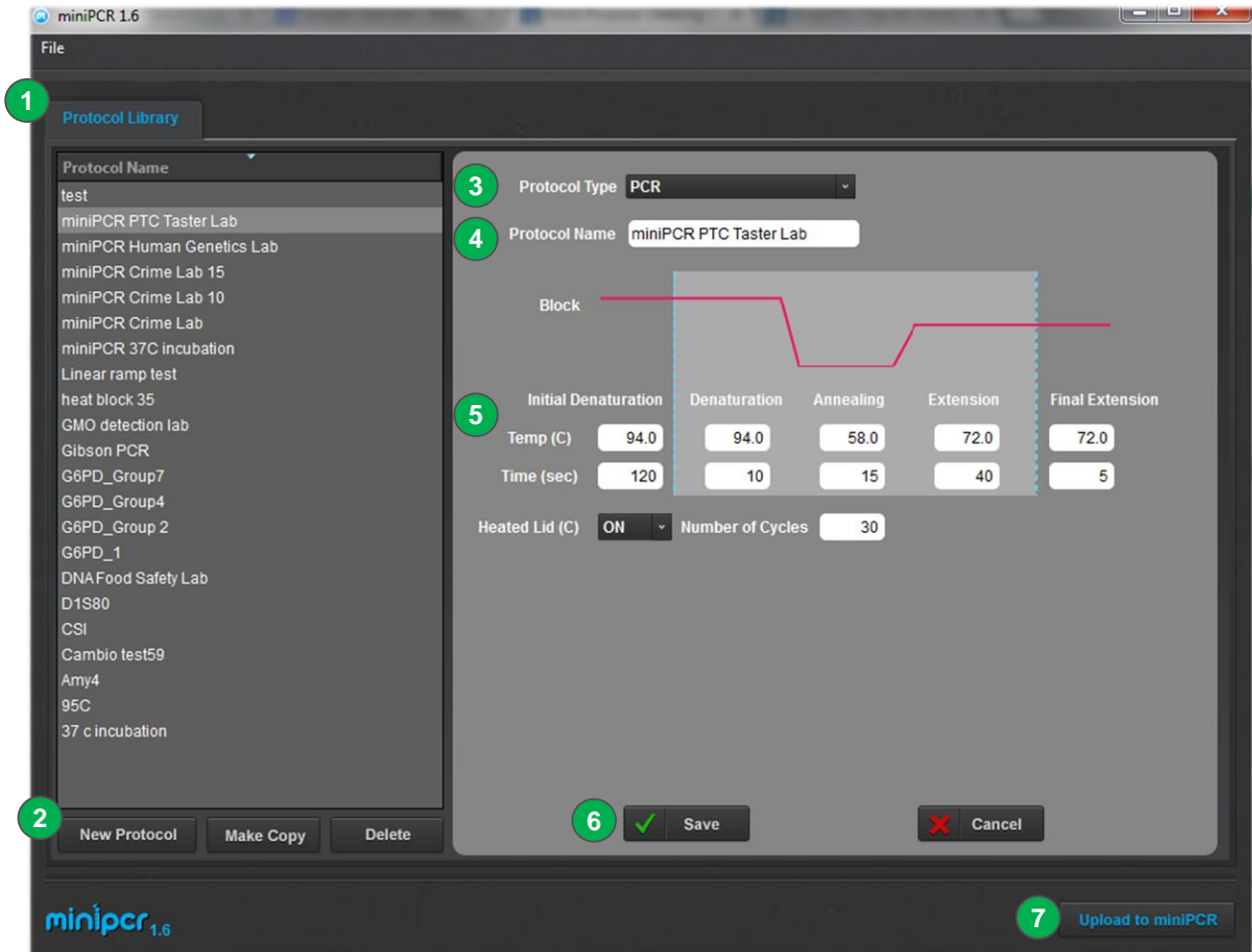


If there is a cloudy precipitate at the bottom of the tube *be careful not to transfer it to the PCR set up*

- Cap the tubes**
 - Make sure all the liquid volume collects at the bottom of the tube
 - If necessary, spin the tubes briefly using a microcentrifuge, or tap them on the benchtop
 - Press firmly on the tube caps to ensure a tight fit
- Place the tubes inside the miniPCR™ machine**
 - Close the PCR machine lid and gently tighten the lid

PCR programming and monitoring (illustrated using miniPCR™ software)

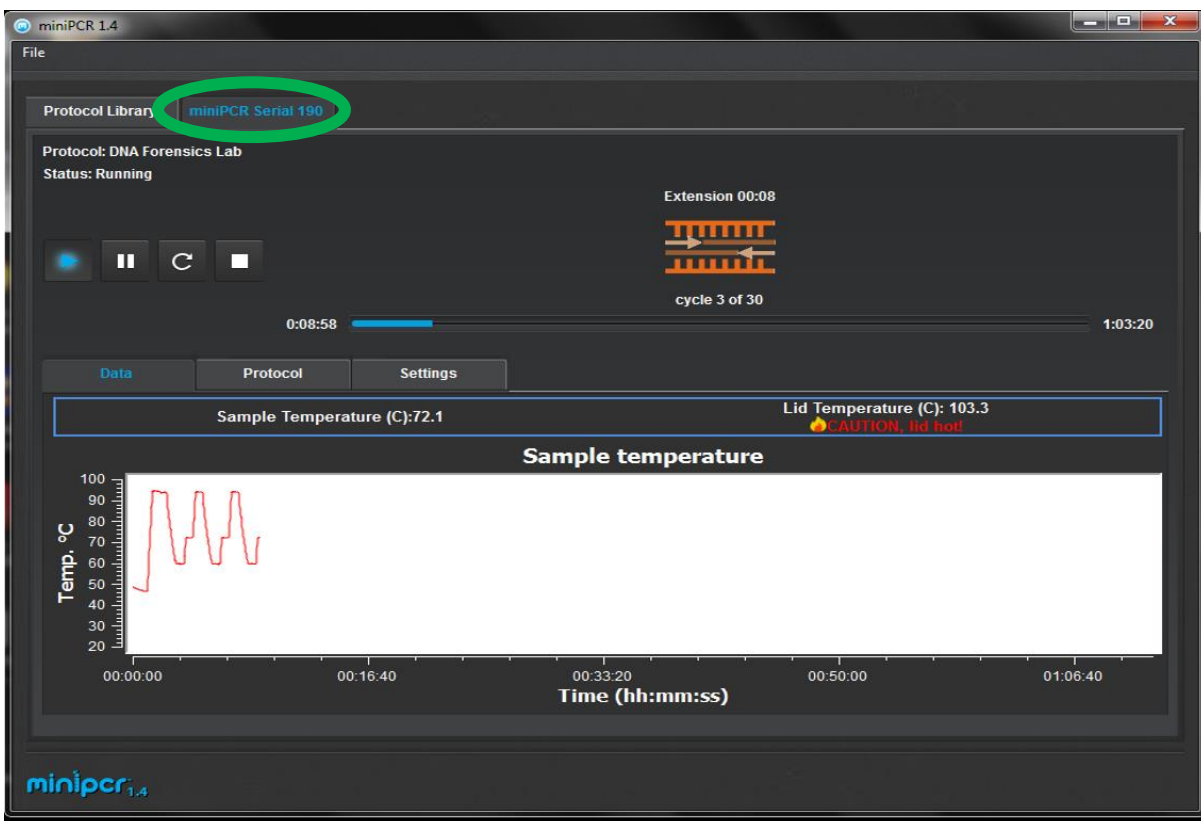
1. Open the miniPCR software app and remain on the "Protocol Library" tab



2. Click the "New Protocol" button on the lower left corner
3. Select the PCR "Protocol Type" from the top drop-down menu
4. Enter a name for the Protocol; for example "PTC Taster Lab – Group 1"
5. 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
- **Heated Lid** ON

6. Click **"Save"** to store the protocol
7. Click **"Upload to miniPCR"** (and select the name of your miniPCR machine in the pop-up dialogue window) to finish programming the thermal cycler.
8. Make sure that the **power switch in the back** of miniPCR is in the **ON** position
9. Click on **"miniPCR [machine name]"** tab to begin monitoring the PCR reaction (the miniPCR serial number can be found on the back of the miniPCR).



The miniPCR™ software allows each lab group to monitor the reaction parameters in real time, and to export the reaction data for analysis as a spreadsheet.

10. Once the PCR run is completed (approximately 90 min), the screen will display: **"Status: Completed"**. All LEDs on the miniPCR machine will light up.



You can now open the miniPCR lid and remove your tubes.
Be careful not to touch the metal lid which may still be hot

C. Restriction Digest and PTC Taste Test

1. **Digest Tube Preparation: Label 4 clean PCR tubes** (200 μ L thin-walled tubes) per group
 - Student's initials on the side wall, followed by "-R" (e.g. SK-R)
2. **Add 14 μ L of PCR Product** to the bottom of each Digest Tube
3. **Add 1 μ L of Restriction Enzyme** to each Digest Tube, directly into the PCR product.
 - Use a 0.5-10.0 μ L range micropipette



Pipette up and down to mix well. This mixing is critical for efficiency of the digest

	Volume
PCR Product	14 μ L
Restriction Enzyme	1 μ L
FINAL VOLUME	15 μL

4. **Cap the tubes**
 - Make sure all the liquid volume collects at the bottom of the tube
 - If necessary, spin the tubes briefly using a microcentrifuge
5. **Place the tubes inside the miniPCR machine**
 - Press firmly on the tube caps to ensure a tight fit
 - Close the PCR machine lid and gently tighten the lid
6. Incubate in the miniPCR machine **15 minutes at 37°C**
 - a. Open the miniPCR software app and remain on the "**Protocol Library**" tab
 - b. Click the "**New Protocol**" button on the lower left corner
 - c. Select the "**Heat Block**" **Protocol Type** from the top drop-down menu
 - d. Enter a name for the Protocol; for example "**Restriction Digest – Group 1**"
 - e. Enter the Heat Block parameters:
 - **Heat Block** 37°C, 15 minutes
 - f. Click "**Save**" to store the protocol
 - g. Click "**Upload to miniPCR**" (and select the name of your miniPCR machine in the dialogue window) to finish programming
 - h. Make sure that the **power switch in the back** of miniPCR is in the **ON** position
 - i. Click on "**miniPCR [machine name]**" tab to begin monitoring the incubation

7. ***PTC taste test (while Restriction digest is running)***

- a. Begin by asking students to taste “control” paper strips to establish a baseline, this can be any chemical-free paper (e.g. thin strips of standard printer paper)
- b. Pass out PTC paper strips to students, one per student
- c. Ask students to taste paper, and record their ability to taste the PTC chemical (i.e. *non taster, weak taster, strong taster*)
- d. Collect class data, if possible

Student Name	Assessed Phenotype	Predict Genotype	Actual Genotype
	Strong taster		
	Moderate taster		
	Non-taster		
		

- 8. Once the Restriction Digest is completed (15 min), open miniPCR lid and remove tubes
- 9. The restriction digest must be used immediately for Gel Electrophoresis



Because Fnu4HI enzyme cannot be heat inactivated, *we do not recommend storing the digest for use at a later date*, as it may result in sample degradation

D. Gel electrophoresis – Pouring agarose gels (Preparatory activity)

If this lab is going to be performed over two class periods, gels can be prepared up to one day ahead of the second period and stored in a refrigerator, covered in plastic wrap and protected from light.

1. Prepare a clean and dry agarose gel casting tray
 - Seal off the ends of the tray as indicated for your apparatus (not needed for blueGel™ users)
 - Place a well-forming comb at the top of the gel (5 lanes or more)
2. For each lab group, prepare a 2% agarose gel using electrophoresis buffer
 - Adjust volumes and weights according to the size of your gel tray
 - For example, add 1 g of agarose to 50 ml of electrophoresis buffer
 - **For blueGel™:** 0.3 gr of agarose in 20 ml of 1X TBE electrophoresis buffer
 - Mix reagents in glass flask or beaker and swirl to mix
 - If pouring more than one gel you can dissolve all the agarose at once (e.g. 4g in 200 ml for 4 gels)
3. Heat the mixture using a microwave or hot plate
 - Until agarose powder is dissolved and the solution becomes clear
 - Use caution, as the mix tends to bubble over the top and is very hot
4. Let the agarose solution cool for about 2-3 min at room temperature.
 - Swirl the flask intermittently
5. Add gel staining dye (e.g. GreenView Plus available at miniPCR.com)
 - Follow dye manufacturer instructions
 - Typically, 10 µL of staining dye per 100 mL of agarose solution
 - Note: We recommend the use of safe alternatives to ethidium bromide.
6. Pour the agarose solution into the gel-casting tray with comb
7. Allow gel to completely solidify (until firm to the touch) and remove the comb
 - Typically, 10-20 minutes
8. Place the gel into the electrophoresis chamber and cover it with 1X TBE electrophoresis buffer

Gel electrophoresis – Running the gel

1. Make sure the gel is completely submerged in electrophoresis buffer
 - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged)
 - Fill all reservoirs of the electrophoresis chamber and add just enough buffer to cover the gel and wells
 - **blueGel™ users:** Use no more than 25 ml of electrophoresis buffer
2. Load DNA samples onto the gel in the following sequence
 - **Lane 1:** 10μL DNA ladder
 - **Lane 2:** 12μL Restriction Digest from **Student Sample 1**
 - **Lane 3:** 12μL Restriction Digest from **Student Sample 2**
 - **Lane 4:** 12μL Restriction Digest from **Student Sample 3**
 - **Lane 5:** 12μL Restriction Digest from **Student Sample 4**

* You will need 5 Lanes per group (i.e. 1 Ladder + 4 Samples)

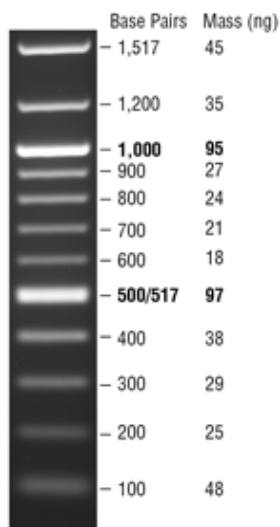
Note: there is **no need to add gel loading dye to your samples.**

The *miniPCR EZ PCR Master Mix* and *100 bp DNA Ladder Load-Ready™* come premixed with loading dye, and ready to load onto your gel!

3. Place the cover on the gel electrophoresis box
 - Ensure the electrode terminals fit snugly into place
4. Set the voltage at 100-130V and conduct electrophoresis for 15-20 minutes, or until the colored dye has progressed to at about half the length of the gel (if using blueGel™, simply press the “Run” button.)
 - Check that small bubbles are forming near the terminals in the box
 - Longer electrophoresis times will result in better size resolution
5. Once electrophoresis is completed, turn the power off and remove the gel from the box

E. Size determination and interpretation

1. Place the gel on the blue-light transilluminator
 - If using blueGel™ simply press the illuminator button
 - *If using UV light, cover any exposed skin and cover eyes with UV-protective goggles*
2. Verify the presence of PCR product
3. Ensure there is sufficient DNA band resolution in the 100-300 base pair (bp) range of the 100bp DNA ladder
 - Run the gel longer if needed to increase resolution
 - DNA ladder should look approximately as shown



100 bp DNA Ladder visualized by ethidium bromide staining on a 1.3% TAE agarose gel.

Source: New England Biolabs

4. Document the size of the PCR amplified DNA fragments by comparing the PCR products to the molecular weight reference marker (100 bp DNA ladder)
 - Capture an image with a smartphone camera or if available, use a Gel Documentation system

4. Study questions - Pre-lab

1. Genetics of PTC sensitivity

PTC, or **1.** _____, was discovered accidentally in a lab by the scientists Arthur L. Fox and C.R. Noller. One night in his lab Fox accidentally over-poured PTC and complained of the **2.** _____ taste in the air as the powder diffused around him. His colleague Noller, on the other hand, tasted **3.** _____. Intrigued by the vast differences in their tasting Fox and Noller went on to explore the phenomenon further and to find an **4.** _____ for why only one of them could taste PTC while the other could not. They then gathered hundreds of people and had them sample PTC. They found that about 25% of respondents said they did not taste anything. Through further experimentation they ultimately found that a single gene was responsible for the **5.** _____ in taste perception of PTC. We now know that polymorphisms in the *TAS2R38* receptor are associated with differences in bitter taste perception. This phenotypic difference is passed down from **6.** _____ to **7.** _____ in a Mendelian fashion. At the DNA level, the two most common **8.** _____ for PTC differ in three **9.** _____. Each of these nucleotide differences is known as a SNP, for **10.** _____ .

2. Mendelian inheritance of PTC sensitivity

Sensitivity to the bitter taste of PTC is passed along from parents to offspring in a Mendelian fashion. This pattern can be analyzed using a Punnett square. In the example below, we observe the four possible genotypes for the offspring of two parents for the “Taster Gene”, where “T” represents the dominant allele (Taster phenotype) and “t” represents the recessive allele (Non-Taster).

Suppose a heterozygous parent marries someone who is also heterozygous at the “Taster” locus.

- What genotypes would you expect in the progeny? What phenotypes?
- What type of cross is this? What allelic frequencies would you expect?

Punnett Square		Parent 1	
		Gametes	T
Parent 2	T		
	t		

3. Neurophysiology of taste

Taste receptors, or gustatory cells, are found in the **1.**_____ clustered in a group called a **2.**_____ _____. Gustatory papillae convey to the brain whether a food is salty or sweet, among other qualities. Humans have about **3.**_____ different receptors for bitter taste. These receptors are transmembrane proteins of the **4.**____-_____ coupled receptor family.

4. Predict your taster phenotype and genotype

- a. Predict whether or not you will exhibit the PTC taster phenotype.
- b. If you are a taster of PTC, what are your possible genotypes at the *TAS2R38* locus?
- c. In which ways can single nucleotide polymorphisms (SNPs) affect the function of a gene?
- d. What do we mean when we say a SNP is *associated* with a certain phenotypic trait?
- e. Why do you think the three SNPs in the *TAS2R38* gene are inherited together (haplotype)?

5. G-Protein Coupled Receptors (GPCRs)

- a. What is the subcellular localization of GPCRs?
- b. List 3 known functions of GPCRs.
- c. List 3 types of cells that might be rich in GPCRs.

Study Questions - During the Lab

1. DNA Extraction

- a. During DNA extraction we heated the cheek cells to 95°C. Why?
- b. Explain the role of the following components of a DNA extraction buffer:
 - **Detergent:**

 - **Chelating agent:**

 - **Basic (alkaline) ions:**

2. Polymerase Chain Reaction (PCR)

- c. What is happening to DNA molecules at each step of the PCR cycle?
 - **Denaturation:**

 - **Annealing:**

 - **Extension:**
- d. What is the purpose of the following PCR mix components?
 - **dNTPs:**

 - **Mg⁺²:**

 - **Taq DNA polymerase:**
- e. What temperature do you think is optimal for most enzymes?

- What feature of Taq DNA polymerase makes this enzyme uniquely suited for PCR?

- f. How many additional DNA molecules are there at the end of each PCR cycle? If the PCR consists of 30 cycles, how much more DNA will there be after the PCR completes?

- g. Which of the following is NOT a characteristic of PCR primers?
 - A. Short synthetic oligonucleotide

 - B. Typically 18-25 bases in length

 - C. Double stranded DNA

 - D. Unique homology to the DNA template

 - E. Sequence with ~50% G:C content

3. Restriction Digest

- a. Shown below is a partial sequence of your expected PCR product for the taster *TAS2R38* allele. Draw a box around the Fnu4H1 restriction enzyme recognition site. Draw lines to illustrate where you expect the restriction enzyme will cut. How would the non-taster allele differ?

```

5' C T G C T T C T T T G T G A T A T C A T C C T G T G C T G C C T T C A T C T C T G T G C C C C T A C T 3'
3' G A C G A A G A A A C A C T A T A G T A G G A C A C G A C G G A A G T A G A G A C A C G G G G A T G A 5'
  
```

- b. What type of chemical bond is the Restriction Enzyme hydrolyzing (breaking)?

- c. Why is this enzyme called a *restriction endonuclease*? How is it different from an *exonuclease*?

- d. Why do we keep enzymes on ice when not in use? What does ice do to enzymatic activity?

- e. After the restriction digest, what is the expected size of the DNA fragments for the "Taster" allele?

Study questions - Post Lab

- a. After tasting PTC paper, what did you expect your Taster genotype to be?
- b. Did this match your experimental results?
- c. What percentage of your class could taste PTC? What percentage couldn't? Did the experimental results match these distributions?
- d. What would be the expected allelic frequencies in a T/t x T/t cross? (Taster/non-Taster)
- e. Speculate what adaptive advantage might exist for animals able to taste PTC
- f. Speculate what adaptive implications might exist for humans able or unable to taste PTC
- g. Do you think the allelic frequencies of Taster and non-Taster vary across populations? Why?
- h. Why might someone with a heterozygous genotype find that they are unable to taste PTC?