Using A SNP to Predict the Ability to Taste PTC	Student Name:	
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Using a SNP to predict the ability to taste PTC (beads)

A single nucleotide polymorphism (SNP) is a single nucleotide difference between two alleles. An allele is one of two or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome. Depending on the nucleotide substitution, the SNP may or may not change the amino acid coded for.

Our sense of taste depends upon the binding of chemicals to receptors in the taste buds of the mouth. The chemical phenylthiocarbamide (PTC) is perceived as bitter by some individuals. The gene for tasting PTC is called TAS2R38, and is located on chromosome 7. The two most common alleles are called AVI and PAV (named for the variations in their amino acid sequences). These are the two alleles we will investigate in this experiment.

The PAV allele is the ancestral allele, as it is shared by chimpanzees, lowland gorillas and orangutans, as well as by some monkeys.

People with two copies of the PAV allele will find the PTC test paper to taste distinctly bitter. People with one copy of the PAV and one of the AVI allele normally find the taste bitter, but will not have as strong a reaction. People with two copies of the AVI allele will be nontasters.

In the North American white population, approximately 20% are homozygous tasters, 50% are heterozygous, and 30% are nontasters.

There are three locations in the gene that vary.

Nucleotide position	Change in nucleotide (nontaster→taster)	Change in codon	Change in amino acid
145	G → C	GCA → CCA	Ala → Pro
785	T →C	GTT → GCT	Val → Ala
886	A → G	ATC → GTC	Val → Ile

Chelex is made of resin beads that act as chelators. They preferentially bind heavy metal ions, including copper, iron and transition metals, over monovalent cations such as sodium and potassium. The boiling of human cells in the presence of Chelex is an established method of isolating DNA for use in PCR (Walsh et al 1991).

Restriction endonucleases (restriction enzymes or RE) are named for the organism from which they came. For example, EcoRI comes from *E. coli*. Most REs that we use function best at 37° C, the optimal temperature for growth of the bacteria the enzyme comes from. The buffer in which the sample and enzyme are suspended must provide the proper ionic and pH environment for the enzymatic activity. Each RE will cut any type of DNA at a specific site within the 4 to 10 base pair (nucleotide) recognition sequence. The purpose of an RE in a bacterium is to protect against foreign DNA, such as that from bacteriophage (bacterial viruses).

The HaeIII enzyme will only cut the PCR product of those who have the taster allele.

Polymerase chain reaction (PCR) is a cell-free method of DNA replication. The reaction uses a DNA polymerase from a thermophilic bacterium, such as *Thermus aquaticus (Taq)*, whose enzymes are not denatured by near-boiling temperatures. In addition to polymerase, the reaction requires template DNA (the DNA you want to make copies from), nucleotides (dNTPs: A, T, G, C), a pair of primers that flank the region of DNA you wish to amplify, and a reaction buffer that contains the necessary ions for proper polymerase activity. The reaction is subjected to repeated temperature cycles; usually about 30 - 34 cycles are used. Denaturation of the template DNA occurs at 95° C, which unzips the DNA double helix by breaking the hydrogen bonds between the two strands. Priming or annealing is done at 50° to 65° C to allow the primers to base pair with the single stranded template DNA. Extension occurs at 72° C, which is the optimal temperature for *Taq* DNA polymerase activity.

DNA gel electrophoresis uses a gel containing agarose (extra-pure agar), which forms a molecular sieve. The gel also contains a salt solution, and is bathed in a salt solution (a buffer). The gel is placed in a tank with electrodes that will apply electricity to the solution. The ions from the salts allow effective conduction through the bath and gel. Since DNA is negatively charged, it will migrate through the gel toward the positive pole. Smaller pieces of DNA will migrate faster, since their small size allows them to move more easily through the molecular sieve formed by the gel. The progress of the DNA movement through the gel can be estimated by including a dye with the DNA sample.

I. DNA isolation using saline mouthwash

Reagents & Supplies

10 mL 0.9% saline	Permanent marker	Micropipetors (100-1000 µL & 5-50 µL or 10-100 µL)
100 µL 10% Chelex in water	Micropipette tips	Microcentrifuge tube rack
Paper cup	1.5 mL microcentrifuge tubes	Microcentrifuge
	0.2 mL tube	Thermalcycler or waterbath
		Vortexer

1. **Label a 1.5 mL microcentrifuge tube**, a **0.2 mL tube** and a **paper cup** with your initials or assigned number.
2. **Pour 10 mL of saline** into your mouth. Vigorously swish for 30 seconds.
3. Spit the saline into your paper cup.
4. Swirl the cup gently to mix in any cells that have settled to the bottom of the cup.
5. Use a micropipet with tip to **transfer 1000 µL** of solution from the cup to the 1.5 mL tube.
6. Centrifuge at full speed for **90 seconds.** (Balance the centrifuge!)
7. Pour off the supernatant into your cup, being careful not to disturb the pellet.
8. Resuspend the pellet in the liquid that remains in the tube (50-100 µL), using a micropipet set to **30 µL** or by vortexing. Be careful to minimize bubbles.

9. Obtain a **0.2 mL tube containing 100 µL Chelex solution** (or pipet 100 µL Chelex into a 200 µL PCR tube). Add **30 µL** of your cell suspension to the Chelex.
10. Place your tube in the thermalcycler along with those of the other students. Set the thermalcycler to **99 ˚C for 10 minutes**. This lyses the cells, releasing the DNA.
11. **Shake the tube vigorously** (or vortex) for 5 seconds.
12. Place your tube in a balanced microcentrifuge. (You may need to use adapters.) **Centrifuge for 90 seconds** at maximum speed to pellet the Chelex and cell debris.
13. **If you are proceeding directly to Part II, pipet 2.5 µL of the supernatant containing your DNA directly into the 0.2 mL PCR tube that contains the PCR bead and continue following the directions in Part II. PCR.** (If you are not continuing to PCR immediately, place a fresh tip on your micropipetor and transfer 30 µL of the supernatant to a labeled 1.5 mL tube. Be careful to avoid the pellet. Put your DNA sample on ice or in a -20 ˚C freezer until you are ready to continue with Part II.)

Part II. PCR

Cheek cell DNA from Part I	0.2 mL tube with "Ready-to-Go" PCR bead	Micropipettors (1-10 µL & 10-100 µL or 5-50 µL)
PCR master mix*	Pipet tips (yellow & red box)	PCR tube rack
*PTC Primer/loading dye mix may be substituted	Indelible marker	Thermalcycler

1. Label a **0.2 mL PCR** tube containing the PCR bead on the top and side, as instructed during the lab.
2. Add the following to your PCR tube. Avoid touching the PCR bead with the pipet tip.

22.5 µL of PTC PCR primer (master) mix	**2.5 µL** of your cheek cell DNA from part I

Store your sample on ice until everyone is ready to run the PCR.

3. Put your tube in the thermal cycler.

4. We will run the following PCR program:
   Hot start denaturation 2 min 95˚C
   35 cycles of:
   Denaturation 30 sec. 95˚C
   Annealing 45 sec 64˚C
   Extension 45 sec. 72˚C
   Final extension (termination) 3 min. 72˚C
   Hold at 4˚C infinitely

5. Store the samples at -20˚C or on ice until ready for **Part III**.

Part III: Restriction Digest of PCR products with *Hae III*

200 µL PCR tubes	Microcentrifuge tube rack	37 ˚C thermal cycler	PCR product from Part II
Pipet Tips	Micropipet (1-10 & 10-100 or 5-50 µL)	*Hae III* restriction enzyme	PCR Tube rack with ice

1. Label **a 200 µL PCR tube** as instructed in lab.
2. **Transfer 10 µL of your PCR product from Part II** to this tube. Add **1 µL *Hae III*** restriction enzyme. Pipet the enzyme carefully, **as it is in a viscous fluid**. **Pay close attention to the micro-pipetter as you only need 1 µL of Hae III.**
3. Place this tube in the thermal cycler set at **37 ˚C for one hour**.
4. Keep the rest of your PCR reaction frozen or on ice while doing the digest.
5. Store your sample at 4°C infinitely or on ice or at -20 ˚C or in the lab freezer until ready for Part IV.

Part IV: DNA gel electrophoresis

Undigested PCR product from Part II	Pipet tips	Micropipetors (1-10 & 5-50 or 10-100 µL)
PCR product digested with *Hae III* from Part III	Ethidium bromide 1%	Electrophoresis apparatus
TAE or TBE buffer (1X TBE buffer prep: 50 mL concentrate from PTC-PCR kit + H_2O, qs 1 L – this is enough for 6 gels and 3 electrophoresis tanks)		Power supply
		Loading buffer (dye)

1. Prepare two **2.25% agarose gels**. Place the following in an Erlenmeyer flask (125 or 250 mL):
   27 mL dH2O
   3 mL 1X TBE (Tris-Borate-EDTA)
   0.675 g agarose

To prepare more than two gels, simply double, triple, quadruple, ad nauseum, the ingredients.

Gel Prep Method 1:

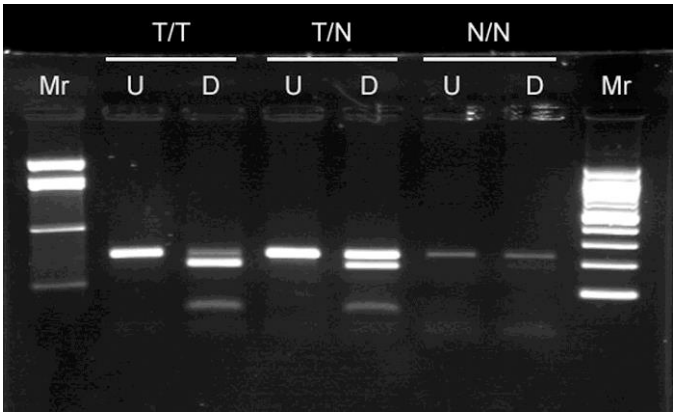
Cover with parafilm or plastic wrap with a small hole punched in it. Prepare a “hot pad” by folding two paper towels. Microwave for about 30 sec, to bring to a boil, swirl. You should not see any crystals of agarose. If crystals are still visible, microwave some more. Place in 55˚ C water bath until ready to pour. (NOTE: your instructor may prepare the gel to this point for you.)

Gel Prep Method 2:

Place the agarose in a 125 mL Erlenmeyer flask with the water and buffer (tap water can be used – remember, though, that it will accelerate the electrophoresis by a few minutes) on a hot plate and heat to a clear mixture (no observable crystals and no obvious boiling). Stir

periodically with a glass stirring rod. (Alternatively, a Bunsen burner can be used, providing you use great care to not "over-boil" the melting agarose.)

1. Place the dams on your gel tray. Add the appropriate comb. **Add 3 µL 1% ethidium bromide (EtBr) to your gel solution** (wear gloves! Ethidium bromide is carcinogenic). Swirl gently to mix, without introducing bubbles. Discard tips and other items that have touched EtBr into the proper waste container.
2. Pour the agarose solution into your gel tray. If there are any bubbles in your gel, use a pipet tip to move them to the edge of the gel.
3. *(If there is time, practice gel loading while your gel solidifies. Mix 90 µL water with 10 µL gel loading buffer (dye). Use the "practice" gel prepared by your instructor to practice loading a gel. Make sure the practice gel is under a thin layer of water. Pipet 15 µL of your practice mix into a well. Place the tip of the pipet just at the top of the well, and eject slowly letting the solution fall into the well. Avoid depressing the plunger all the way to the 2nd stop, to avoid creation of an air bubble which could bounce some of your sample out of the well. Repeat as needed until you feel confident.)*
4. *(If there was no dye in your PCR mix, add 2 µL 10x loading buffer (dye) to each of your samples (digested and undigested PCR products).)*
5. When your gel has solidified (it will look cloudy), carefully remove the comb by pulling it straight up. Place the gel in the gel rig. Cover with enough running buffer (1X TBE) to cover the gel and wells completely, but not deeply (about 1-2 mm depth over the gels). **Add 6 µL of EtBr to the running buffer.**
6. Plan the loading of the gel along with the other students who will be sharing it with you. If you put the lanes at the "top", the wells are numbered starting with 1 on the left. Include a well for the DNA size marker, as directed by your instructor.
7. Load the gel. Work carefully, but not slowly. **Add 10 µL of undigested PCR product from Part II**, and **10 µL of digested PCR product from Part III** to the appropriate wells. (Add 10 to 20 µL of DNA size marker, as (if) directed by your instructor.)
8. Place the lid on the gel rig, and turn on the power supply to 150 mV and run it for 30-45 minutes, until the dye has traveled about ¾ the length of the gel.
9. Turn off the power supply. Remove lid from gel box. Carefully remove gel from box, and slide gel into a Ziploc sandwich bag.
10. The lab instructor or professor will instruct you as to safe disposal of used chemicals and supplies. Running buffer should be transferred to an appropriate bottle using a funnel. **DO NOT pour running buffer down the drain!**
11. View gel using UV transilluminator and use a camera to take a picture of it.
12. Analyze the gel bands image example at right) by observing the number of bands in your samples. A tt (N/N) non-taster (homozygous recessive) shows a single band (221 bp) in the "D" sample identical to the band in the "U"



sample. A TT (T/T) taster (homozygous dominant) shows a band in the "U" sample that is closer to the origin, and one or two band in the "D" sample that are located a little farther from the origin than the "U" sample (176 & 45 bp). The 45 bp fragment can be difficult to see. Tt T/N) tasters (heterozygous) show one band in the U sample, and two or three bands in the "D" sample, one of which is at the same location as the "U" sample (221, 176 and 45 bp). In short, a single band indicates homozygosity, while the presence of two bands indicates heterozygosity.

13. Obtain PTC and control test papers, and determine whether you taste PTC.

Optional: Analyze the Gel Bands by measuring the distance each marker band traveled from the well (in mm) and plotting that against the log of the base pairs (bp) for each band of the marker, as described below. Then measure the distance each sample band traveled from the well. Use your graph to determine the size (in bp) of each sample band.

Determining the Sizes (Molecular Weights) of DNA Bands:

Determine the molecular weights are. You can **determine the molecular weights of the bands in the gel by comparing them to the DNA ladder**, **which has bands of known molecular weight.** Your instructor will give you information on the DNA ladder used. This is done by creating a **standard curve of the DNA ladder bands** as follows:

1. Number the bands of the DNA size marker or ladder (such as Lambda HindIII digest), starting with 1 as the band closest to the well. Measure **in mm from the front edge of the well to the leading edge of the band for each band in the ladder.** (The leading edge is the sharp line in the front, not the smear.) The small bp bands may be too faint to see on our gel. Record your numbers in Excel.

2. The distance that a band will migrate is proportional to the **log value** of its molecular weight. This piece of information is what allows us to create a standard curve of the bands in the DNA ladder. Set up your Excel spreadsheet as follows. You will plot columns B and D.

	A	B	C	D
1	**Band Number**	**Distance (mm)**	**Molecular Weight (bp)**	**Log Molecular Weight (bp)**
2	1			=LOG(C2)
3	2			=LOG(C3)
4	3			=LOG(C4)

3. Create an **XY scatter** graph in Excel which plots the distance in mm migrated on the X axis (column B) and the log molecular weight in base pairs (bp) on the Y axis (column D). Highlight the Distance and Log Molecular Weight columns. Then choose **Insert ➔ Chart ➔ XY scatter plot ➔ Next ➔ Finish**. Now right click on one of the data points on your graph and select **"Add trendline."** Choose **"linear."** This will create the best straight line possible through the data points. Add minor gridlines: **chart tools→layout→gridlines→minor**. Add axis titles: **chart tools→layout→axis titles.** If you need to extend the axes in order to extend the trendline: **chart tools→layout→format**

axis→axis options→change the min/max from auto to fixed (type in the min/max numbers you want).

4. To estimate the size of a band from one of the other digests, measure the distance that band traveled. Locate that distance on the X-axis of your standard curve. From that position on the X-axis, draw a pencil mark to the standard line, and then follow the graph line over to the Y-axis. This is the approximate **log size** of your unknown DNA band. **To determine the approximate size (i.e. to get rid of the log value), take the inverse log (=10^(cell)) of the value on the Y-axis**.

References:

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Optional Questions:

1. Did you taste PTC?

2. What alleles did the experiment show you have? Are you homozygous (dominant or recessive) or heterozygous?

3. Explain your taste response using the data from the PCR bands (connect genotype and phenotype)

4. What percent of the class had each allele?

5. What percent of the class had each genotype?

6. How do the class percentages compare to the percentages for the US population?