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

A single nucleotide polymorphism is a single nucleotide difference between two alleles. 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 at 7q34. 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. Diseases associated with TAS2R38 include dental caries and nicotine dependence [1].

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.

I. DNA isolation using saline mouthwash

Reagents & supplies

10 mL 0.9% saline (0.9 g/100 mL H_2O)	Permanent marker	Micropipetors (100-1000 µL & 5-50 µL or 10-100 µL)
100 µL 10% Chelex in water	Micropipette tips (blue & yellow box)	Microcentrifuge tube rack
Paper cup	1.5 mL microcentrifuge tubes	Microcentrifuge
Vortexer	200 µL PCR tube	Thermalcycler or waterbath

1. Label a 1.5 mL microcentrifuge tube, a 200 µL PCR 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 micropipet with tip to transfer 1000 µL of solution from the cup to the 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, using a micropipet set to 30 µL. Be careful to minimize bubbles.
9. Add 100 µL Chelex solution to the PCR tube FIRST. Add 30 µL of your cell suspension.

10. Place your tube in the thermal cycler along with those of the other students. Set the thermal cycler 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. Use the second rotor. Centrifuge for 90 seconds at maximum speed to pellet the Chelex and cell debris.
13. Using a fresh tip on your micropipetor, transfer 30 µL of the supernatant to a labeled 200 µL PCR tube. Be careful to avoid the pellet. FOR LARGE GROUPS: Put your DNA sample on ice or in a -20 ˚C freezer until you are ready to continue with Part II. FOR SMALL GROUPS: Go straight to Part II.

Part II. PCR

Cheek cell DNA from part I	0.2 mL microcentrifuge tube	Micropipettors (1-10 µL & 10-100 µL or 5-50 µL)
PTC Primer/loading dye mix	Pipet tips	PCR tube rack with ice
Ready-To-Go PCR beads (already in 200 µL tube)	Indelible marker	Thermal cycler

1. Label a 0.2 mL PCR tube with Ready-To-Go PCR beads.

2. Add the following to your PCR tube

22.5 µL of the above PCR PTC primer/loading dye mix to the tube. Allow the bead to dissolve (with a good shake) for a minute or so – avoid sticking the pipet tip into the bead!
2.5 µL of your cheek DNA mix from Part I. Avoid sticking the pipet tip into the bead.

   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 if time is available to go directly on to Part III.

Part III: restriction digest of PCR products with *Hae III*

200 μL PCR tubes	Microcentrifuge tube rack
Pipet Tips	Micropipet (1-10 & 10-100 or 5-50 μL)
Hae III restriction enzyme	37 ˚C water bath
PCR product	

1. Label a 200 μL PCR tube.
2. Transfer 10 μL of your PCR product to this tube. Add 1 μL *Hae III* restriction enzyme. Pipet the enzyme carefully, as it is in a viscous fluid.
3. Place this tube in the rack in the 37 ˚C Thermal Cycler for one hour.
4. Keep the rest of your PCR reaction frozen or on ice while doing the digest.
5. Store your sample on ice or at -20 ˚C until ready for part IV.

Part IV: DNA gel electrophoresis

Undigested PCR product	Pipet tips (yellow & red boxes)	Micropipetors (1-10 & 5-50 or 10-100 μL)
PCR product digested with *Hae III*	Ethidium bromide	Electrophoresis apparatus
TBE buffer (50 mL concentrate + H_2O, qs 1 L – this is enough for 6 gels and 3 electrophoresis tanks)	pBR322/BstNI MW marker solution	Power supply
Loading buffer (dye)		

1. Prepare a 2% agarose gel. Place the following in an Erlenmeyer flask (125 or 250 mL) (this is enough to make 2 gels for the current electrophoresis tanks/apparati in 201 ASP):

    27 mL dH2O
    3 mL 10x TAE (Tris-Acetate-EDTA) or TBE (Tris-Borate-EDTA)
    0.6 g agarose – tap this out … NO metal spatulas!

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.

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.

2. Place the dams in the ends of your gel tray[s]. Add the appropriate comb.
3. NOTE: 1% Ethidium bromide, 3 ul in the agarose and 6 ul in the running buffer assists in visualization of bands. The same can NOT be said for 30 ul and 60 ul, respectively of the 1 ug/mL sold in the PTC kit by Carolina – not strong enough, even if the gels are soaked in the ethidium bromide for 30 minutes.
4. Pour approximately half of 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. You'll have a good gel if about a third of the gel (or so) is over the comb teeth.
5. When your gel has solidified (it will look cloudy), remove the dams and carefully remove the comb by pulling it straight up. Place the gel in the gel rig, matching the notch to the tab on the rig. Cover with enough running buffer (1x TBE) to cover the gel and wells completely, but not deeply – ca 1-2 mm coverage.
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" or the "bottom", the wells are numbered starting with 1 on the left. Include a well for the DNA size marker (20 μL pBR322/BstNI) at the far right (last well in the gel).
7. Load the gel. Work carefully, but not slowly. Add 10 μL of undigested ("U") PCR product, and 16 μL of digested ("D") PCR product to the appropriate wells. Add 20 μL of DNA size marker, as directed by your instructor (see above).
8. Place the lid on the gel rig, connect the electrodes to the power supply and turn on the power supply to 150 mV and run it for 25-30 minutes for 2 gels per apparatus (and when using tap water to dilute the buffer), until the dye has traveled about ½-¾ the length of the gel.
9. Turn off the power supply. Remove lid from gel box. Carefully remove gel from box and slide into the ethidium bromide staining tray. Stain the gel for 30 minutes and rinse with tap water. Slide the rinsed gel into a Ziploc sandwich bag. Dispose of all ethidium bromide contaminated items as directed by your lab instructor.
10. View gel using the UV transilluminator (wear your goggles) and use a camera to take a picture of it.
11. Analyze the gel bands by observing the number of bands in your samples: a tt non-taster (homozygous recessive) shows a single band in the "D" sample identical to the band in the "U" sample; a TT taster (homozygous dominant) shows a band in the "U" sample that is closer to the origin and one band in the "D" sample that is located a little farther away from the origin than the band in the "U" sample. Tt tasters (heterozygous) shows one band in the "U" sample and 2 very intense bands in the "D" sample, the first of which is identical in location to the band in the "U" sample. Single bands, BTW, indicate homozygousity; double bands indicate heterozygousity.

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

Questions:

Did you taste PTC?

Explain your taste response using the band data from the electrophoresis, i.e., connect genotype with phenotype.

Are you homozygous or heterozygous?

References:

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"Tasting Phylthiocarbamide (PTC): A New Integrative Genetics Lab with an Old Flavor. *BioOne*