Introduction

We’ve all seen television shows like CSI where an analyst injects an instrument with some unknown fluid and moments later a printer prints results full of long chemical names only the most sophisticated organic chemist could love. Indeed, these are the contents of the finest prime-time television dramas, but they are never accurate representations of real-life instrumental chemistry. It is pretty accurate, however, when it comes to your senses – senses such as sight, sound, smell and taste. Your senses are the most efficient chemical and physical instruments ever developed on the face of the earth and you never have to wait for results. For example, your eyes are terrific spectrometers as they can determine wavelengths of light very accurately in the form of colors. Your nose can detect even the smallest concentration of discreet and even mixed molecules (e.g. esters smell floral, ethers smell fruity, and even molecules such as peutractivine smell like rotting flesh). Your ears are terrific instruments detecting different frequencies of transverse compression sound waves in the form of different pitches of sound. We can even feel electromagnetic waves with wavelengths longer of those able to be detected with our eyes – they are infrared waves and our skin feels them as heat!

All of these senses have been imparted to us through our genes, or traits afforded to us through our DNA and passed to us from our parents. Sometimes, the gene that codes for the protein responsible for the development of the sense (the development of cones in the retina of the eye, the number of cilia in the ear, the receptors for a particular taste in taste buds on your tongue, for example) are miscoded in our DNA and we may lose that particular sense. This miscoding is called either a mutation or a Single Nucleotide Polymorphism, or SNP (pronounced SNiP). By definition, if greater than 1% of the population possesses miscoded DNA for a particular allele it is officially referred to as a DNA SNP, if less than 1% of the population possesses the miscoded DNA it is called a DNA mutation.

We will be measuring the frequency of SNP occurrence in the class experimentally by determining which of us possess the SNP on the gene that determines our ability to taste bitter things. Afterwards, you can go home and ask your parents if they can taste bitter things and calculate the Mendelian probabilities to find if you are homozygous or heterozygous recessive for the gene, which is officially referred to as “T” for taste (hint: ask your parents if aspirin tastes bitter if they chew it)
Figure 1. An ideogram of chromosome 7

Typical graphical representations of chromosomes, called ideograms, are used by scientists to map gene loci, or areas of DNA that code for a protein or series. The gene we are measuring today is located at the q34 locus on chromosome 7 and is called TAS2R38. This gene contains the instructions to make the receptor protein on your tongue that allow you to taste bitter things. The ends of TAS2R38 are labeled with red and blue lines in figure 1. The TAS2R38 gene is 1144 base pairs in length; I have included the complete sequence of the gene at the end of this write-up.

If you possess an error in your copy of the TAS2R38 gene, the receptor protein will not be made correctly and the receptor will not function. The consequence of this, of course, is the inability to taste this particular molecule.

This is an SNP lab, so by definition we are not measuring a mutation, but rather a Single Nucleotide Polymorphism. Remember that an SNP is where a single base (nucleotide without its Ribose) is changed in the sequence that code for the functioning TAS2R38 gene. This is the actual part of the gene, with the correct base (cytosine) and the polymorphic base inserted (guanine).

**SNP:** CCTGATATCAGGATGGCTGCATGTCC[G]GAAGGACAAGCTGCCATCATCCCTA

**Correct:** CCTGATATCAGGATGGCTGCATGTCC[C]GAAGGACAAGCTGCCATCATCCCTA

We will harvest your DNA from your cheek cells, amplify the q34 locus on chromosome 7 using Polymerase Chain Reaction (PCR) until we have billions of copies of that sequence. How can we tell if we’re homozygous for the recessive p allele? Knowing the sequence of bases that confers the SNP, we can use a protein called a restriction enzyme to cut the sequence at exactly the place where the SNP occurs… Here’s the deal, it’s pretty cool.

We will have our PCR product - billions of copies of the q45 locus from chromosome 7. Each copy is exactly the same length. Cool. If our copy of q45 contains the SNP, we can use the restriction enzyme to restrict (cut) the DNA at the site of the SNP. The restriction enzyme recognizes the sequence **CCGG** (the polymorphism) and cuts DNA between the C and G. If we don’t have the SNP, our PCR product will remain uncut, but if we do possess the SNP, the enzyme will have cut our DNA and we will have two short fragments of q45 rather than one complete q45.

We still need to find a way to detect whether or not the restriction enzyme cut our DNA or not, so we will visualize it on a gel after we perform gel electrophoresis. Gel electrophoresis is a method to separate fragments of DNA based upon the sizes of the fragments. The number of base-pairs determines how far the fragment will **run** up the gel.
The DNA in the gel will then be stained with Ethidium bromide, a stain that sticks to DNA and also is fluorescent and then I will take a picture of it and post the picture on Orca.

Rs713598 SNPID

**Procedure:** Day 1.
1. Outside the lab, place about 10 mL of normal saline into your mouth and vigorously swish for 30 seconds or longer. Spit the saline back into the cup and enter the lab.
*** At this point you should have nitrile gloves on at all times. Remember to never touch a doorknob with gloves on. Be safe!
2. Pipette 1.5 mL of the saline into a 2.0 mL eppendorf tube and wait for Greg to help you with the centrifuge.
3. Spin the tube at 16,000 RCF for 1 minute to pelletize cells at the bottom of the tube.
4. Decant the supernatant spit into your cup, making sure to not disturb the pellet of cells at the bottom of the tube.
5. Close the lid to the tube and use a vortexer to resuspend your cheek cells in the residual saliva.
6. Obtain from Greg a 200 microliter Lysis Tube tube with Chelex in it. Label the side of the tube with your initials using a fine-tipped sharpie. Into this small PCR tube place 50 microliters of your cheek cell suspension.
7. Place the Labeled PCR tube in the thermalcycler up front, making sure to mark your position in the instrument on the provided sheet.

What is happening now is called lysis, or the breaking open of your cells. We need to break them open to get to your DNA. The Chelex is a very small bead that absorbs all the other stuff in the cell (like the membrane, weird proteins, and other junk we don’t want). We’ll be accomplishing lysis by holding the PCR tubes at 99°C (almost boiling) for 10 minutes.

1. When lysis has been complete, shake or rack your tube for 5-10 second to make sure the Chelex has been well mixed.
2. Have Greg help you spin the PCR tube at 16,000 RCF for 90 second.
3. Acquire a 200 microliter Reaction Tube from Greg containing upstream and downstream primers, Taq polymerase and dNTPs.
4. Label the Reaction Tube with your initials just as you did the Lysis Tube.
5. Into the Reaction Tube, place 5 microliters of template DNA into the Reaction Tube. Template DNA is dissolved in the solution in the Lysis Tube.
6. Place your Reaction Tube into the same well in the thermalcycler as your Lysis Tube.

When everyone’s Reaction Tube is in the thermalcycler, Greg will run the program that will amplify the q45 loci on your 7th chromosome; the one that contains the TAS2R38 gene. **This is the end of this lab period.** Tomorrow Greg will do the restriction digest to cut your DNA if you possess the SNP site and next Wednesday we will run the reaction
products out on a gel. **DO NOT THROW THIS LAB WRITE-UP AWAY!** You’ll need it next week!

Wenednesday 7/21

Now that you’ve isolated your DNA, amplified the TAS2R38 gene and Greg has performed a restriction digest on the DNA with the HaeIII enzyme, we are ready to run our products out on a gel.

Now, if you recall from lecture, all we did during PCR was amplify a single fragment of known length into millions of copies of itself. At the end of the PCR process, we should have a tube with millions of exact copies of that gene in solution. If, however, you possess the SNP, the restriction enzyme would have cut this strand of DNA into two shorter fragments. How do we sort this out? How do we find out if our DNA was digested (SNP) or undigested (no SNP)?

We do a thing called gel electrophoresis.

We measure fragments of DNA in terms of numbers of base pairs (bp). Gel electrophoresis is a technique that allows researchers to separate mixtures of fragment sizes into visible bands in a gel. Think about it: you’ll remember from lecture that DNA has a slight negative charge. Suppose we had a gel that was porous, but not too porous – just porous enough to let DNA fragments flow through it with a little bit of resistance. If we could get our mixture of DNA fragments into the mixture, we can use electricity to move them through the gel (the electrophoresis part). Remember that DNA has a negative charge and under an electric field is attracted to a positive electrode – this fact is what will make our fragment mixture move through the gel.

How does this separate DNA fragments by the size of those fragments? Remember that this gel is pretty porous, but not too porous. This means that the smaller the DNA fragment size, the faster and farther it will move in the gel – smaller fragments (100bp) can make their way through the small pores more easily. The larger the DNA fragment (10Kbp), the more difficult it is to move through the small pores. This fragment will run very slowly. This is how mixtures of DNA fragments are isolated and purified.

Between labs, Greg took a small portion of your PCR product from last week and digested it to see if the restriction enzyme would cut it at the SNP. The rest of the PCR product was placed back in the freezer. You will run both your PCR product (uncut) and the digest (cut). The PCR product represents the natural state of your TAS2R38 gene. The digest represents whether or not you possess the SNP. There are two possibilities for your gel with your “cut” digest – either you have a fragment of identical size as your uncut (no SNP) or you two fragments of a smaller size (possess the SNP). We can see the difference after we run the gel.
Lab procedure.

Practice loading wells.
How do we get our DNA stuff in the gel? There are small “wells” cut into the gels that you must “load” very, very, very carefully! We will practice a bunch before we do the real thing. There is “practice loading dye” to practice with. Pay attention during Greg’s demo.

The real deal – put on gloves!

1. You and your partner should find a gel with at least four lanes open. Greg might assign lanes if necessary. Write your name down on the lane assignment sheet next to the gel box to claim your space. YOU MUST ASSIGN YOURSELF 2 LANES LABELED WITH YOUR NAME and CUT and UN CUT.
2. Obtain your digested and undigested DNA and a small tube of REAL 6X loading dye.
3. Obtain two 200 microliter tubes and label one cut and the other uncut.
4. Into both tubes place 2 microliters of 6X loading dye.
5. Into the UN CUT tube you just labeled, place 10 microliters of the undigested PCR reaction from last week.
6. Into the CUT tube you just labeled, place 10 microliters of the digest DNA that Greg made for you.
7. Close both tubes and “rack” them. Greg will demonstrate.
8. Go to your gel box and identify your lanes (cut and uncut).
9. From the tube labeled UN CUT, remove 12 microliters and load the well you’ve chosen to be the uncut lane.
10. From the tube labeled CUT, remove 12 microliters and load the well you’ve chosen to be the cut lane.
11. When your gel is full (you may have up to 4 people per gel (8 wells total), Greg will load the ladder and turn on the electrophoresis. It will take approximately a half hour to run. Greg will either lecture during this time or continue to help other groups, but if your gel is running, you should be paying attention to it and not having social time.
12. The period is over after all the gels are run. Greg will stain the gels and image them. We will look at your results on Friday.

But you already know if you have the SNP or not, so I guess we will see how good your biology lab technique is when we see your results…
Complete sequence of TAS2R38 Homo Sapiens q45.7

aatatata acatctttcaaatcccaaga agttcccttcaataaacctcttg cagccaccacc
aagtgcctgca cccgtcagctc tgggaattta attttgtaaatgttgccctt
tcccagaaag tcttctacca tattttttta gcggttcggat gtcggttgagac
tatattagtt gcattgttgg cctttctccctc cagcttccat acgtggatgga
tgtgcttacca ttttgttttt gagcagcataacctc cagcagtaag cttcttctttgcctttg
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