Activity: Isolation of Eukaryotic DNA from Cheek Cells

WARNING: Students who feel ill or have a chronic illness should inform the instructor that they feel ill this day and should not take part.

- All Students should handle their own tubes and saliva.
- All fluids shall be bleached.
- All solid waste goes into the red container.
- All surfaces will be wiped down with 70% ethanol
- Use gloves

1. **RINSE** your mouth vigorously for 60 seconds using 3 ml Gatorade. **EXPEL** the solution into cup.
2. **TRANSFER** 1.3 ml of solution into 2X 1.5ml tubes.
    - Use the P1000 and pipette 650µl twice into each tube → 1.3ml in each tube
3. Bleach each cup/tube used that contains any saliva
4. **CENTRIFUGE** the cell suspension for 2 min. at full speed to pellet the cells.
5. Set micropipet to 30µl. Resuspend cells in the remaining fluid by pipetting up and down and combine tubes
6. Add 100µl of Chelex® beads (make sure to suspend the beads)
7. Place tubes on heat block at 99°C for 10 minutes
8. **CENTRIFUGE** the cell suspension for 2 min. at full speed to pellet the beads.
9. Transfer 100µl to fresh labeled tubes and store in freezer.

Exercise: PCR reactions using PCR beads

1. Obtain a strip of PCR beads
2. Add 22µl of primer mixture (appropriate primer for each experiment)
3. Add 3µl of DNA
4. Place in PCR machine and use appropriate programmed

Exercise: PCR reactions using 2X mixture

1. Obtain PCR strip tubes
2. Distribute 12.5µl 2X PCR mixture to appropriate number of tubes
3. Add 9µl of primer mixture (appropriate primer for each experiment)
4. Add 3µl of DNA
5. Place in PCR machine and use appropriate programmed

Activity: Genetics leaves a bad taste in my mouth... or not

Some of our personal preferences arise from the way we were brought up. Culture plays a role in our likes and dislikes. Likewise, our experiences play a role in how we respond to certain stimuli. Another major factor that plays a role into our preferences comes wired in our genome. The DNA in our cells is the instruction manual for who we are. We are programmed to seek out things of a nutritive values in order to acquire raw materials like carbohydrates, proteins and lipids. In our search for nutritive compounds we have learned to avoid things that don't taste good. Bitter things have a tendency to be associated with toxic compounds in nature. When eating a food item for the first time, molecules hit our tongue and stimulate multiple sensations: sweet, sour, salty, savory and bitter. Attributed to these multiple taste types are a diverse family of receptors that bind to the molecules that result in our perception of these sensations. Something bitter might make us learn to avoid this food item in the future.

One type of bitter receptor senses the presence of a chemical called phenylthiocarbamide (**PTC**). This chemical chemically resembles toxic compounds found in plants but is non-toxic. The ability to taste PTC is comes from the gene called *TAS2R38*. This gene encodes a protein that on our tongues that communicates the bitterness of this chemical. There are two common alleles of this gene with at least five more uncommon variants. Within the two common forms, a single nucleotide polymorphism (**SNP**) is responsible for changing one amino acid in the receptor. It's this difference of one amino acid that results in the ability of the receptor to either respond or not respond to PTC. We inherit one copy of the gene from our father and one copy from our mother. Based on how our parents gametes were formed and what alleles we received during the fertilization event determines how we respond to this chemical. Because we each have 2 copies of this gene, we can utilize simple Mendelian genetics to understand which allele is dominant or recessive.

- Place a piece of “Control” paper on the tongue and indicate if there is a taste
- Place a piece of “PTC” paper on the tongue and indicate if there is a taste and the taste severity
- Fill out the table for the class to identify how many non-tasters, tasters or super-tasters there are.
- Indicate if you believe the trait is dominant or recessive (ability to taste or not taste)
- Assign a descriptor allele for the dominant (a capital letter) or the recessive (a lowercase letter) and draw a Punnet square for the F_2 generation of 2 Heterozygous parents.
- Compare the class tally of tasters and non-tasters in the class and discuss with your instructor if there is a clear dominance of this trait.

Table *.* PTC Tasting Tally

	Number	% Total
(Dominant or Recessive) **PTC Tasters**		
(Dominant or Recessive) **PTC Non-tasters**		
Total		

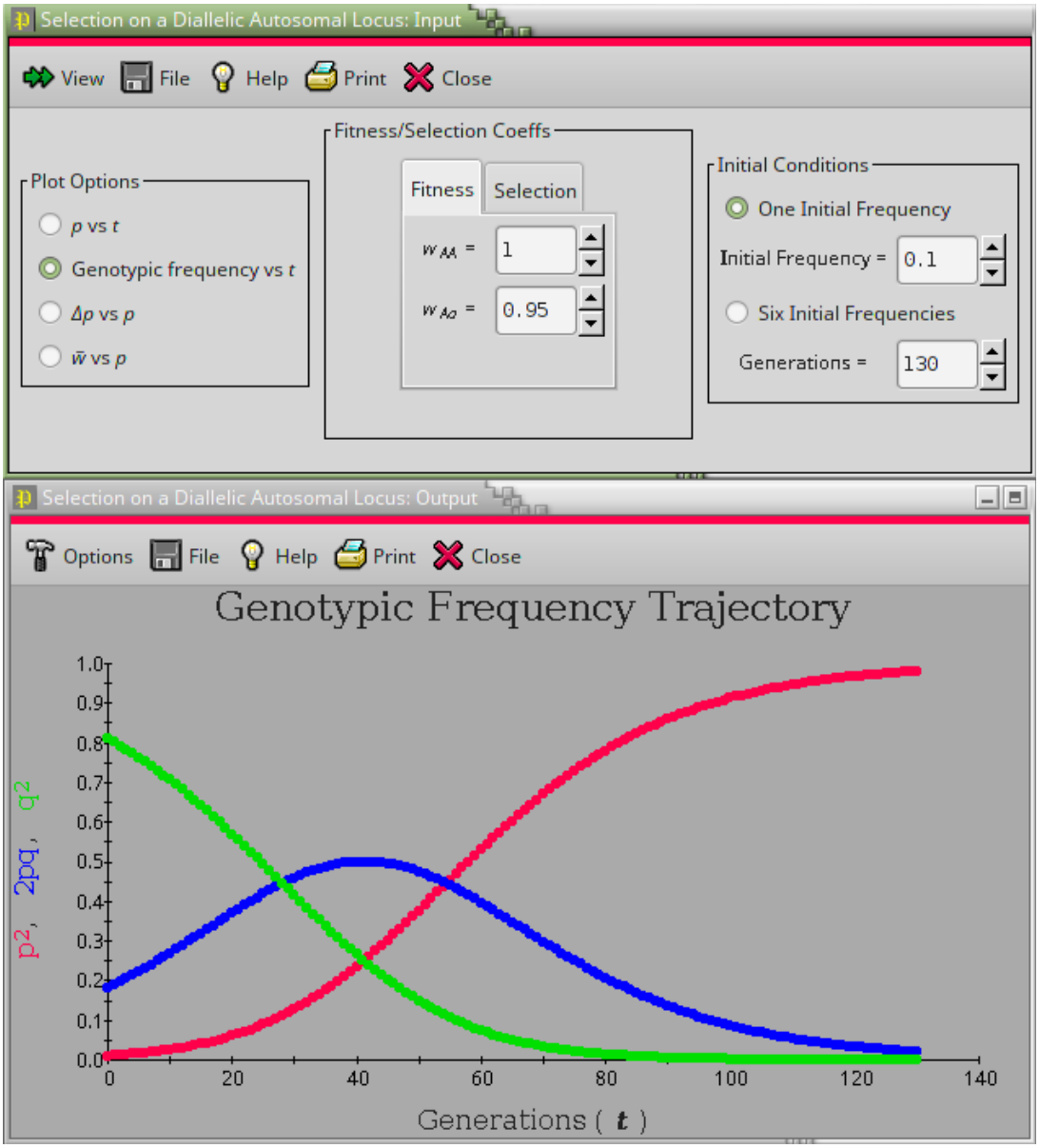
Questions:

1. How do you explain the presence of those who can't taste PTC, those who can taste it and those who really can't stand the taste of it?
2. This chemical is non-toxic and doesn't exist in nature. Do you think there is a selective pressure that confers an advantage to those who do taste it?

Activity: Modeling Hardy-Weinberg Principle

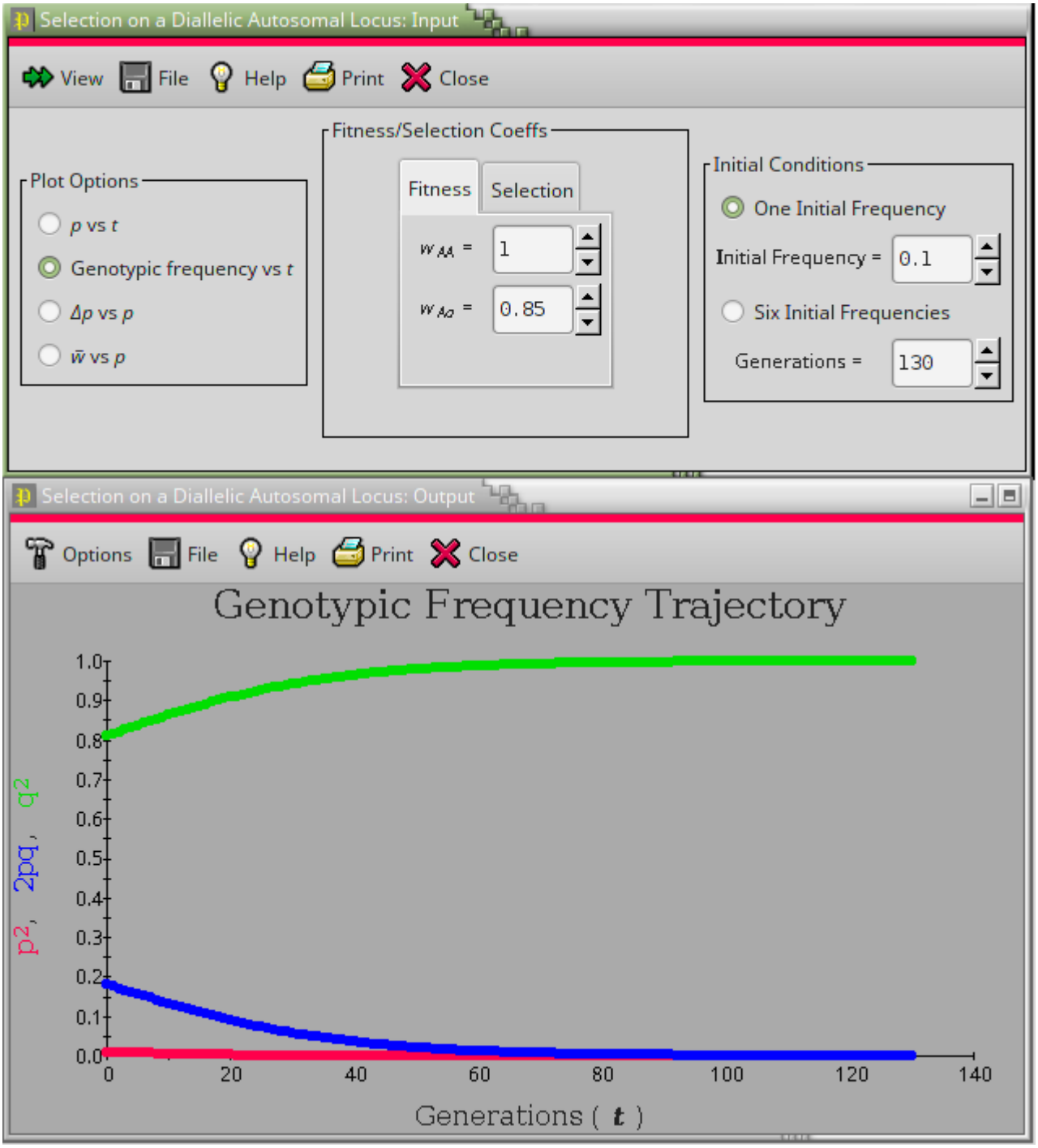
Hardy-Weinberg principle refers to the states of alleles and the genoytpic frequencies in a population between generations when there is an absence of external evolutionary or selective pressures. In a simple 2 allele system, each allele has a frequency . The frequencies for the alleles are traditionally referred to as *p* and *q*. We would describe the frequencies of the allele *A* as *p* and the frequency of allele *a* as *q*. Therefore, we would express *f*(AA) $=p^2$ and *f*(aa) $=q^2$. The heterozygous condition would be expressed as *f*(Aa) = 2pq. This can describe the general population by the quadratic equation $p^2 + 2pq + q^2 = 1$, where 1 indicates 100% of the population.

- Using the class data from above, indicate if the frequencies of alleles follow traditional Hardy-Weinberg equilibrium.
  - If the class is small, the instructor will supplement numbers from other classes
- Use Populus to model the frequencies of these alleles over many generations using the model called: Selection on a Diallelic Locus



In the case of a selective pressure, a fitness coefficient (*w*) can be introduced. A research article http://www.jci.org/articles/view/64240 has shown that the Tas2R38 receptor aids in the immune response against Pseudomonas. Imagine a situation where there is an epidemic of antibiotic resistant Pseudomonas. This would show that the dominant allele will have a selective advantage.

- Modify the fitness of the genotypes and describe the effects this would have over many successive generations.



Activity: Coding Bitterness

DNA is simply a storage vessel of genetic information. It sits in the nucleus and must be called upon through a process of **transcription** where an enzyme called **RNA Polymerase** “reads aloud” the stored information into a molecule called messenger RNA (**mRNA**). Since DNA is double-stranded in a reverse-complementary fashion, we automatically know the sequence of the second strand by knowing the first. The mRNA is made through complimentary base-pairing to the **template strand**, which is the reverse complement of the coding strand. The **coding strand** is the strand that reads identical in sequence to the mRNA with the exceptions of T's being replaced by U's. This coding strand is later decoded by the **ribosomes** with the help of transfer RNA's (**tRNA**'s) that act as a decoder of the information and protein assembler in a process called **translation**. The ribosome scans along the mRNA and recognizes nucleotides in batches of 3 . These batches of 3 can be translated into an amino acid and is known as a **codon**. Since there are 4 types of bases and they are read as groups of 3, there are 4^3 (or 64) combinations of these codons. However, there are only 20 amino acids used to build proteins. This indicates that there is room for redundancy. Three of these codons tell the ribosome to stop, like a period in a sentence. These are called **stop codons**. There is one special codon that performs double duty: ATG. The codon (ATG) that encodes the amino acid Methionine also acts as a **start codon** that tells the ribosome where to start reading from. Like nucleic acids, proteins have a polarity and are synthesized in an amino to carboxyl direction. We abbreviate this by terming the beginning of the protein sequence, N-terminal, and the ending of the sequence as the C-terminal.

The full coding sequence of *TAS2R38* is 1,002 bases (334 amino acids) long. A segment of the gene is shown below where the SNP (in red) occurs. Variant 1 is the version of the gene that encodes for the ability to taste PTC. Variant 2 is the version of the gene that is unable to bind to PTC. This SNP mutation is called a **missense** mutation because it changes the amino acid. Some mutations cause the insertion of a premature stop codon. This **nonsense mutation** results in a truncated protein and can be disastrous to the function. We already know that the simple substitution of one nucleotide translates to a change in one amino acid and determines the ability to taste PTC. Imagine if a large group of amino acids from the protein was missing.

With template strand (“Complement”) information:

1. Write the sequence of the coding strand.
2. Write the sequence of the mRNA
3. Use the Genetic Code Chart to translate the amino acid sequence

Variant 1

Coding Strand : 5'-
Complement : 3'-TTC TCC GTC CGT GAC TCG-5'
mRNA : 5'-
Amino Acid :

Variant 2

Coding Strand : 5'-
Complement : 3'-TTC TCC GTC GGT GAC TCG-5'
mRNA : 5'-
Amino Acid :

nonpolar polar basic acidic (stop codon)

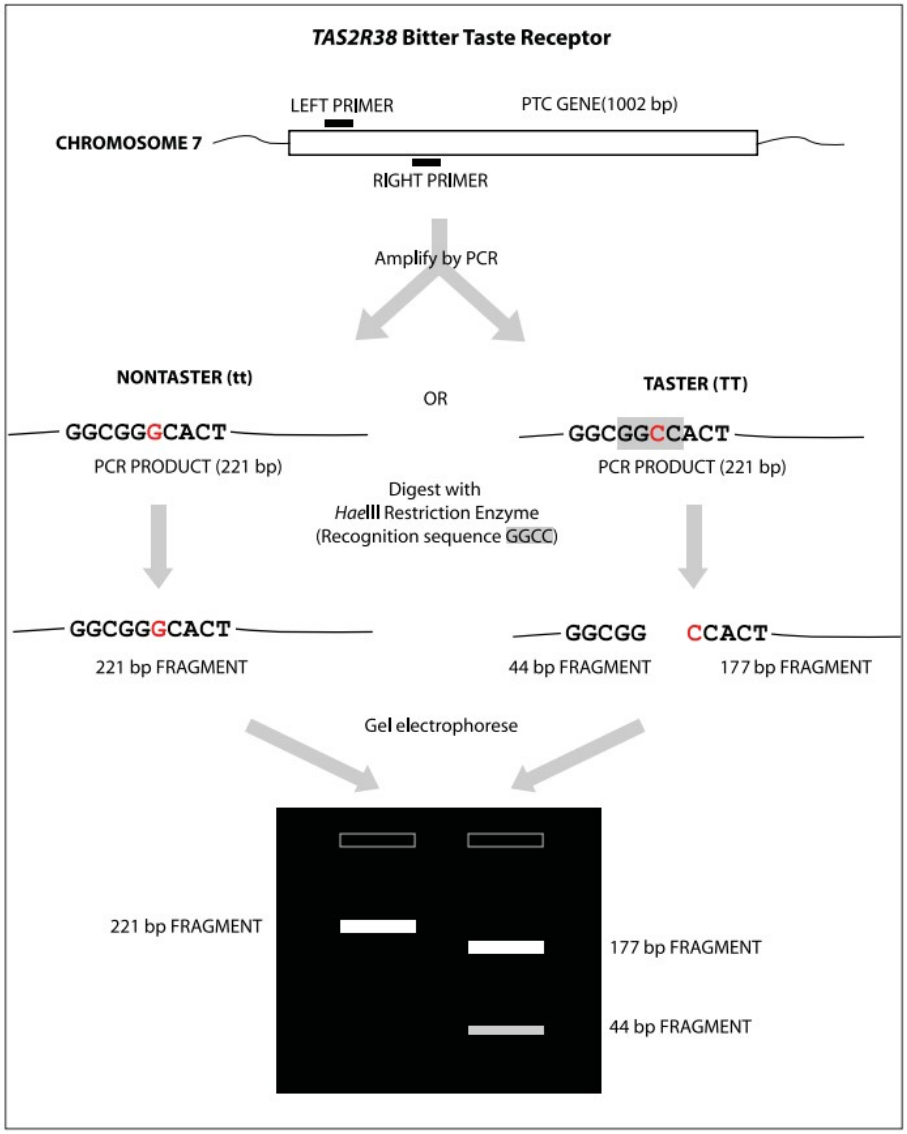
Standard genetic code

1st base	2nd base								3rd base
	T		C		A		G		
T	TTT	(Phe/F) Phenylalanine	TCT	(Ser/S) Serine	TAT	(Tyr/Y) Tyrosine	TGT	(Cys/C) Cysteine	T
	TTC		TCC		TAC		TGC		C
	TTA	(Leu/L) Leucine	TCA		TAA	Stop (*Ochre*)	TGA	Stop (*Opal*)	A
	TTG		TCG		TAG	Stop (*Amber*)	TGG	(Trp/W) Tryptophan	G
C	CTT		CCT	(Pro/P) Proline	CAT	(His/H) Histidine	CGT	(Arg/R) Arginine	T
	CTC		CCC		CAC		CGC		C
	CTA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CTG		CCG		CAG		CGG		G
A	ATT	(Ile/I) Isoleucine	ACT	(Thr/T) Threonine	AAT	(Asn/N) Asparagine	AGT	(Ser/S) Serine	T
	ATC		ACC		AAC		AGC		C
	ATA		ACA		AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine	A
	ATG[A]	(Met/M) Methionine	ACG		AAG		AGG		G
G	GTT	(Val/V) Valine	GCT	(Ala/A) Alanine	GAT	(Asp/D) Aspartic acid	GGT	(Gly/G) Glycine	T
	GTC		GCC		GAC		GGC		C
	GTA		GCA		GAA	(Glu/E) Glutamic acid	GGA		A
	GTG		GCG		GAG		GGG		G

Activity: PCR Genotyping the *TAS2R38* PTC receptor

5′-CCTTCGTTTTCTTGGTGAATTTTTGGGATGTAGTGAAGAGGCGG-3′ (Forward Primer)

5'-AGGTTGGCTTGGTTTGCAATCATC-3' (Reverse Primer)



1. PCR the individual samples
2. Pour 2% agarose into casting apparatus in refrigerator
    - 2 gels per class need to be made → 100ml of TBE with 2g agarose
    - add 5µl SYBR safe solution into the molten agarose before casting
    - place 2 sets of combs into the gel → at one end and in the middle
3. remove 10µl into a fresh PCR tube
4. add 1µl of *HaeIII* enzyme into new tube
5. incubate in PCR machine for 10 minutes at 37°C
6. load gel with DNA ladder, Digested and Undigested
7. Run gel at 120V for 20 minutes
8. Visualize on UV transilluminator