

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

### Study questions - Pre-lab

#### 1. Genetics of PTC sensitivity

PTC, or **1. phenylthiocarbamide**, 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. bitter** taste in the air as the powder diffused around him. His colleague Noller, on the other hand, tasted **3. nothing**. Intrigued by the vast differences in their tasting Fox and Noller went on to explore the phenomenon further and to find an **4. explanation** 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. differences** 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. generation** to **7. generation** in a Mendelian fashion. At the DNA level, the two most common **8. alleles** for PTC differ in three **9 nucleotides**. Each of these nucleotide differences is known as a SNP, for **10. single nucleotide polymorphisms**.

#### 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.

- a. What genotypes would you expect in the progeny? What phenotypes?

*Genotypes: T/T, T/t, and t/t (1:2:1)*

*Phenotypes: Taster and Non-Taster (3:1)*

- b. What type of cross is this? What allelic frequencies would you expect?

*Monohybrid cross*

*T/T: 25%, T/t: 50%, and t/t: 25%*

Punnett Square		Parent 1	
		Gametes	T
Parent 2	T	T/T	T/t
	t	t/T	t/t

### 3. Neurophysiology of taste

Taste receptors, or gustatory cells, are found in the **1. mouth** clustered in a group called a **2. gustatory papillae**. Gustatory papillae convey to the brain whether a food is salty or sweet, among other qualities. Humans have about **3. 60** different receptors for bitter taste. These receptors are transmembrane proteins of the **4. G-protein-coupled receptor** family.

### 4. Predict your taster phenotype and genotype

- Predict whether or not you will exhibit the PTC taster phenotype.
- If you are a taster of PTC, what are your possible genotypes at the *TAS2R38* locus?  
*PAV/AVI or PAV/PAV (T/t or T/T)*
- In which ways can single nucleotide polymorphisms (SNPs) affect the function of a gene?  
*Non-sense mutations (truncated protein) and mis-sense mutations (amino acid substitution) are the most common ways in which gene function can be affected. Other ways include changes to gene regulation or expression.*
- What do we mean when we say a SNP is *associated* with a certain phenotypic trait?  
*We mean that it's not necessarily causal to the phenotypic trait. The SNP segregates with the trait, but it may or may not be its underlying cause (for example, the SNP DNA may be in very close proximity to the DNA underlying the trait).*
- Why do you think the three SNPs in the *TAS2R38* gene are inherited together (haplotype)?  
*The three SNPs are genetically linked as these nucleotides lie in relative close proximity to one another in the TAS2R38 DNA sequence. The AVI and PAV haplotypes are by far the most common TAS2R38 haplotypes. They may have been conserved in evolution because the combination of particular amino acids at multiple sites may be crucial for TAS2R38 receptor function. Despite the predominance of PAV and AVI, "breaking" the genetic linkage between*

*these nucleotide variants can happen during chromosomal recombination in meiosis, leading to up to at least 6 known TAS2R38 haplotypes (although PAV and AVI are by far the most common haplotypes).*

## 5. G-Protein Coupled Receptors (GPCRs)

- a. What is the subcellular localization of GPCRs?

*Plasmatic membrane (integral membrane proteins)*

- b. List 3 known functions of GPCRs.

*Sensory perception (e.g., olfaction, taste, vision)*

*Neurotransmitter signaling in the brain (e.g., some types of glutamate receptors)*

*Chemokine signaling in the immune system*

- c. List 3 types of cells that might be rich in GPCRs.

*Taste receptor neurons*

*Olfactory receptor neurons*

*Yeast cells*

## Study Questions - During the Lab

### 1. DNA Extraction

- a. During DNA extraction we heated the cheek cells to 95°C. Why?  
*To help lyse the cells.*
- b. Explain the role of the following components of a DNA extraction buffer:
  - **Detergent:** *disrupt the organization of lipids in the plasma membrane*
  - **Chelating agent:** *sequester (chelate) divalent metal ions that are necessary cofactors of nucleases (enzymes that can degrade DNA.)*
  - **Basic (alkaline) ions:** *an alkaline environment contributes to DNA denaturation.*

### 2. Polymerase Chain Reaction (PCR)

- c. What is happening to DNA molecules at each step of the PCR cycle?
  - **Denaturation:** *DNA strands of template separate through breakage of H bonds between nitrogenous bases on opposing strands.*
  - **Annealing:** *primers bind to complementary sequence on template DNA through H-bond formation between primer and template complementary bases.*
  - **Extension:** *elongation of primers, from their 3' ends, through the addition of complementary nucleotides catalyzed by Taq DNA polymerase and formation of phosphodiester bonds*
- d. What is the purpose of the following PCR mix components?
  - **dNTPs:** *provides a source of deoxynucleotides for primer elongation, and consequently, new strand synthesis*
  - **Mg<sup>+2</sup>:** *a co-factor necessary for Taq DNA polymerase function*

- **Taq DNA polymerase:** *catalyzes the primer elongation*

e. What temperature do you think is optimal for most enzymes?

*Many enzymes prefer temperatures in the 36-39°C, the body temperature of most mammals*

- What feature of Taq DNA polymerase makes this enzyme uniquely suited for PCR?  
*Taq DNA polymerase was discovered from a thermophilic bacterium, *Thermus aquaticus*, that lives in thermal vents like the geysers in Yellowstone National Park. Hence, it has the ability to withstand the high denaturation temperatures in a 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?

*During every PCR cycle, the strands of each DNA molecule separate, primers anneal and elongate to yield two DNA molecules. So, each PCR cycle doubles the number of DNA molecules.*

*The amount of additional DNA after a certain number of PCR cycles can be calculated using the mathematical expression  $2^N$ , where N is the number of cycles. So, for 30 cycles,  $2^{30} = 1,073,741,824$ , or just over a billion (109) times more molecules than the initial amount.*

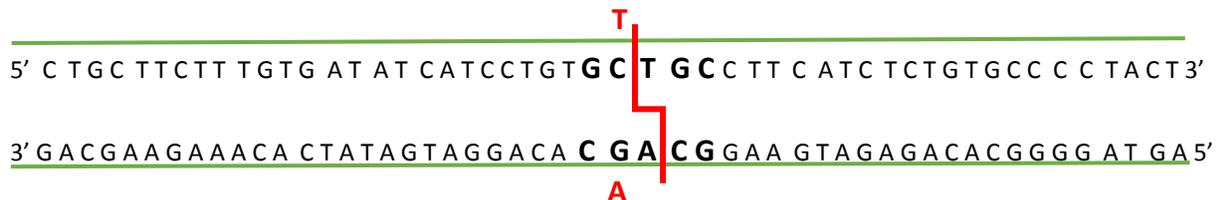
g. Which of the following is NOT a characteristic of PCR primers?

*(C.) PCR primers are single-stranded oligonucleotides*

### 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?



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

*Phosphodiester bond*

- c. Why is this enzyme called a *restriction endonuclease*? How is it different from an *exonuclease*?  
*Nuclease enzymes catalyze the hydrolysis of single nucleotides present in a chain of DNA. Endonucleases can cleave the bonds (“cut”) within the middle of a DNA molecule. Exonucleases cleave the nucleotides at the ends of the DNA molecule. In chemical terms, exonuclease causes the hydrolysis of a nucleotide at the ends of the DNA molecule, where a free 3’ or 5’ hydroxyl group is present in the polynucleotide chain, while the exonuclease does not require a free 3’ or 5’ hydroxyl group to cause hydrolysis of the polynucleotide chain.*
- d. Why do we keep enzymes on ice when not in use? What does ice do to enzymatic activity?  
*To preserve their enzymatic activity by slowing down catalysis.*
- e. After the restriction digest, what is the expected size of the DNA fragments for the “Taster” allele?  
*~150 base pairs and ~100 base pairs (bp)*

## 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)  
*25% T/T, 50% T/t, 25% t/t*
- e. Speculate what adaptive advantage might exist for animals able to taste PTC  
*This phenotype is of evolutionary interest because the ability to taste PTC is correlated with the ability to taste other bitter substances, many of which are toxic. Animals with bitter taste sensitivity (“tasters”) might be better equipped to avoid toxic substances in their diet.*
- f. Speculate what adaptive implications might exist for humans able or unable to taste PTC  
*Variation in PTC taste sensitivity has been correlated with dietary preferences that may have significant health effects. For example, PTC is similar in structure to isothiocyanates and goitrin,*

*both of which are bitter substances found in cruciferous vegetables like cabbage and broccoli. Hence, variable aversions to these compounds may have important implications for food acceptance and food intake. For example, a study in an Italian population showed that AVI/AVI nontaster homozygotes consumed more cruciferous vegetables than individuals carrying a single copy of the PAV taster haplotype.*

- g. Do you think the allelic frequencies of Taster and non-Taster vary across populations? Why?

*Ethnic groups have been shown to differ in their frequencies of TAS2R38 haplotypes. For example, Africans have higher levels of genetic and phenotypic diversity at the TAS2R38 locus relative to non-Africans. One study found the AAI haplotype at frequencies as high as 8% and 19% in some African populations. The dominant PAV taster haplotype is hypothesized to have played a role in detecting potentially toxic substances during hominid evolution. However, the selective force maintaining common AAV, AAI, and AVI haplotypes remains unclear. Common PAV, AAI, and AVI haplotype variation may be maintained at high frequencies in response to selective pressures unrelated to diet.*

- h. Why might someone with a heterozygous genotype find that they are unable to taste PTC?

*Studies have shown that polymorphisms in TAS2R38 can account for ~85% of the variance in PTC tasting ability. The expression of the phenotype is strongly influenced by other factors, such as gender (women are more sensitive to PTC and are more likely to be super-tasters than men). Other factors besides genetics can influence the sense of taste, for example temperature, aging, colds, flu, and allergies. Even hunger can influence someone's sense of taste, although bitter taste does not seem to be influenced by that!*