Introduction –

Bitter Taste Perception

Mammals are believed to be able to distinguish five basic tastes: sweet, sour, bitter, salt and umami. Taste perception is a two-step process. Taste receptors interact with stimuli (usually water soluble chemicals). Then the taste cell initiates a signal which is transmitted to the brain, resulting in taste perception.

Bitter taste perception may be used as a defence mechanism against the ingestion of potential poisons. Many harmful compounds such as synthetic chemicals, inorganic ions and rancid fats have a bitter taste.

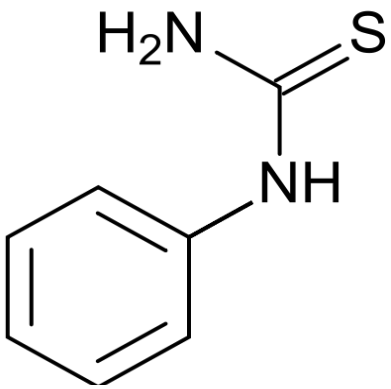

Figure 5.4: PTC molecule

An interesting observation, made at the chemical company DuPont in the 1930's, first showed that there is a genetic basis to taste perception. A scientist, Arthur Fox, had synthesised some phenylthiocarbamide (PTC, Figure 5.1), and some of the PTC dust escaped into the air. His colleague, complained that the dust had a bitter taste, but Fox tasted nothing. Further studies showed that the inability to taste PTC is a recessive trait in humans. PTC does not occur in food, but related compounds do. This is thought to affect food choice.

The gene for the PTC taste receptor, named *TAS2R38*, was identified in 2003. DNA sequencing studies have identified three nucleotide positions that vary in the human population called a Single nucleotide polymorphism (SNP). These tiny changes in the DNA sequence determine an individual's ability (or inability) to taste PTC.

In this practical you will determine the genotype of your own *TAS2R38* gene. You will obtain a sample of your cheek cells using a mouth swab, and extract DNA from the cells. Polymerase Chain Reaction (PCR) is used to amplify a short region of the *TAS2R38* gene. The PCR product is digested with the *HaeIII* restriction enzyme (similar to Lab 3 – Restriction digest). This enzyme's recognition site is at one of the SNPs. The dominant allele will be cut by the enzyme, while the recessive allele will not. We can distinguish between taster (dominant) and non-taster (recessive) traits by looking at the result of the restriction digestion on an agarose gel.

Polymerase Chain Reaction:

In nature, most organisms copy their DNA using the same method. This process begins by 'unzipping' the two strands in the DNA double helix. Then, enzymes called DNA polymerase makes a copy, using each strand as a template. PCR mimics this process, in a tube.

A PCR involves three steps, each carried out at different temperatures:

Denaturing (95°C):

This step separates the two strands of the DNA double helix. This is done by heating the tube to 95°C which breaks the Hydrogen bonds between the bases.

Annealing (50-70°C):

Because the primers cannot bind to the single stranded DNA at such high temperatures, the tube is cooled to 50-70°C. The primers then bind to the DNA templates. The temperature that this step is carried out at depends on the nucleotide sequence of the primers. This primers used in this Lab bind at 67°C.

Extension (72°C):

The final step of the reaction is to synthesise a copy of the DNA template. The enzyme *Taq polymerase* is used to do this. *Taq* is a DNA polymerase derived from the thermophilic bacterium *Thermus aquaticus.* This bacterium lives in hot springs and therefore its enzymes are able to withstand the high temperatures required for PCR in particular the denaturing step at 95°C. Human DNA polymerase enzymes would be denatured at such high temperatures. The optimum working temperature for *Taq* is of 72°C. When the temperature in the tube rises to 72°C, the *Taq* DNA polymerase begins adding nucleotides to the primer, making a complimentary copy of template.

This completes the three steps of the PCR cycle with two double helix DNA strands where there was only one before. In this experiment, the PCR will run through this cycle 35 times, leading to exponential amplification of a single DNA strand.

PCR beads:

The PCR bead provided contains a dry mixture of Taq polymerase, as well as dNTPs and a reaction buffer. To this, you need to add your primers and your DNA template.

dNTPs are a mixture of nucleotide triphosphates: deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxycytidine triphosphate (dCTP) and deoxythymidine triphosphate (dTTP). These provide the building blocks for DNA replication.

The reaction buffer ensures the proper conditions for the PCR reaction such as maintaining the pH and increasing Taq activity.

The primers are short strands of DNA (20-40 base pairs) that marks the start and end point to be copied. Taq then adds the nucleotides from this point, because DNA polymerases can only add new nucleotides to an existing strand of DNA.