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Exploring the Human Genome: DNA Analysis through Use of PCR and Bioinformatics

Introduction:

Humanity is full of intricate variances. The differences that separate one individual from another are both vast and minute, ranging from an obvious difference in phenotype to a subtle mutation of an allele. In order to examine a tiny piece of the human genome, this experiment prepared the TAS2R38 gene for analysis through use of PCR (Polymerase Chain Reaction) and bioinformatics. The TAS2R38 gene regulates the ability of receptor proteins on an individual's tongue to recognize bitter molecules found on the surface of taste cells. A human's ability to taste phenylthiocarbamide (PTC), a bitter substance, is reliant upon this gene: if one is recessive they are unaffected by the taste while if one is homozygous dominant or heterozygous dominant they are affected by the taste to differing extents (ie: strong, moderate, etc). The change in genotype is caused by three single nucleotide polymorphisms (SNPs) in an individual's genome. In a typical population, 70% are tasters while 30% are not ("Using a Single Nucleotide Polymorphism to Predict Bitter-Tasting Ability" 1).

PCR has become a popular biological tool by creating a relatively simple way to amplify DNA strands based upon regulatory DNA processes. It utilizes temperature cycles and primers, synthetic oligonucleotides, to amplify a target sequence of DNA ("Polymerase Chain Reaction" 1). The first temperature cycle, at approximately 94-96 degrees Celsius, denatures the DNA strand so that the hydrogen bonds between the nucleotides break and the DNA becomes single-stranded. The second temperature cycle, at approximately 50-65 degrees Celsius anneals the synthetic primers to the target DNA sequence. The third temperature cycle, at approximately 72 degrees Celsius, induces the *Taq* polymerase to attach to the primers and synthesize both sequences of single-stranded DNA. This process is repeated until essentially only the target sequence of DNA is being synthesized by the *Taq* polymerase (through many cycles) ("Polymerase Chain Reaction (PCR) Biology Animation Library").

Restriction enzymes were also necessary for this experiment. After the desired target sequences of DNA are created through use of PCR, the restriction enzymes, which can be likened to endonucleases, cleave the DNA at specific nucleotides. The term endonuclease implies that the enzyme possesses the capability to cut within the DNA strand, leaving either blunt or sticky ends ("Restriction Enzymes"). These endonucleases are added to the PCR sample after it goes through the required number of cycles to create the target DNA fragment for gel electrophoresis. The restriction enzymes cut out the selected portion of the DNA sequence so this portion can be analyzed on a DNA gel. They 'digest' the replicated fragment, cutting where the recognition sequence is found on the strand. The fragment determines where the bands show up on the agarose DNA gel ("Restriction Enzyme Digestion and Gel Electrophoresis of your PTC Gene PCR products" 2). Gel electrophoresis is used to determine the size of an individual DNA fragment. During this process, an electrical field of negative and positive charges is applied to the gel matrix that separates DNA samples based upon the length of the strand and charge. Since all of our DNA samples are negatively charged, size is the significant determining factor.