

- Q4 True
- Q5 Human insulin
- Q6 1991
- Q7 False
- Q8 Restriction endonucleases
- Q9 True
- Q10 Golden rice

#### Section C: Critical Thinking

*Answers are not provided in this section as the students are encouraged to write their own viewpoints.*

## CHAPTER 2 GENES & GENOMICS

### Section A: Descriptive Type

#### Q1. What is the cell theory?

**Answer:** The idea of the cell theory is coined by a Frenchman, H.J. Dutrochet, and the credit for formulating the cell theory is given to a German botanist, M.I. Schleiden and a German zoologist, T. Schwann, who clearly outlines the basic features of the theory in 1839. Moreover, in the year 1858, R. Virchow extended the cell theory and suggested that all living cells arise from pre-existing living cells. To prove Virchow hypothesis, Louis Pasteur performed experiments suggested that living things are composed of cells and all living cells are arise from pre-existing cells. There is an exception that does not fit into cell theory, such as viruses which may be defined as an infectious subcellular and ultramicroscopic organism. The viruses are simple as they lack the internal organization which is the main characteristics of a living cell and due to this unique characteristics, viruses, mycoplasma, viroids, and prions do not easily fit in the definition of a cell theory. In addition, there are other organisms in protozoa and algae, which also don't fit in the definition of cell theory.

#### Q2. Describe the characteristics of a prokaryotic cell.

**Answer:** The prokaryote cell is simpler than a eukaryote cell, lacking a nucleus and most of the other organelles of eukaryotes. There are two kinds of prokaryotes: bacteria and archaea; they share a similar overall structure. A prokaryotic cell has three architectural regions: (a) on the outside, flagella and pili project from the cell's surface. These are

structures (not present in all prokaryotes) made of proteins that facilitate movement and communication between cells; (b) enclosing the cell is the cell envelope generally consisting of a cell wall covering a plasma membrane though some bacteria also have a further covering layer called a capsule. The envelope gives rigidity to the cell and separates the interior of the cell from its environment, serving as a protective filter. Though most prokaryotes have a cell wall, there are exceptions, such as *Mycoplasma* (bacteria) and *Thermoplasma* (archaea)). The cell wall consists of *peptidoglycan* in bacteria, and acts as an additional barrier against exterior forces. It also prevents the cell from expanding and finally bursting (cytolysis) from osmotic pressure against a hypotonic environment. Some eukaryote cells (plant cells and fungi cells) also have a cell wall; (c) inside the cell is the cytoplasmic region that contain the cell genome (DNA) and ribosomes and various sorts of inclusions. A prokaryotic chromosome is usually a circular in a shape with an exception is that of the bacterium *Borrelia burgdorferi*, which causes Lyme disease, DNA is linear in shape. One of the distinct features of prokaryote is the absence of nucleus in the cytoplasm; DNA is usually condensed in a *nucleoid*. Prokaryotes can carry extra-chromosomal DNA elements called *plasmids*, which are usually circular. Plasmids enable additional functions, such as antibiotic resistance. The presence of plasmid DNA in bacteria not only made bacteria a distinct from animal or plant but also put the bacteria in the unique position as an important genetic engineering tool.

### Q3. Explain Mendelian's genetics.

**Answer:** For thousands of years, farmers and herders have been selectively breeding their plants and animals to produce more useful hybrids. It was somewhat of a hit or miss process since the actual mechanisms governing inheritance were unknown. Knowledge of these genetic mechanisms finally came as a result of careful laboratory breeding experiments carried out over the last century and a half. By the 1890's, the invention of better microscopes allowed biologists to discover the basic facts of cell division and sexual reproduction. The focus of genetics research, then shifted to understanding what really happens in the transmission of hereditary traits from parents to children. A number of hypotheses were suggested to explain heredity, but Gregor Mendel, a little known Central European monk, was the only one who got it more or less right. His ideas

published in 1866 but largely went unrecognized until 1900, which was long after his death. While Mendel's research was with plants, the basic underlying principles of heredity that he discovered also apply to people and other animals because the mechanisms of heredity are essentially the same for all complex life forms. Through the selective cross-breeding of common pea plants (*Pisum sativum*) over many generations, Mendel discovered that certain traits show up in offspring without any blending of parental characteristics. For instance, the pea flowers are either purple or white--intermediate colors do not appear in the offspring of cross-pollinated pea plants. Mendel observed seven traits that were easily recognized and apparently only occurred in one of two forms:

- Flower color is purple or white;
- Flower position is terminal
- Stem length is long or short
- Seed shape is round or wrinkled
- Seed color is yellow or green
- Pod shape is inflated or constricted
- Pod color is yellow or green

This observation that these traits do not show up in offspring plants with intermediate forms were critically important because the leading theory in biology at the time was that inherited traits blend from generation to generation. Most of the leading scientists in the 19th century accepted this "blending theory." Charles Darwin proposed another equally wrong theory known as "pangenesis". This held that hereditary "particles" in our bodies are affected by the things we do during our lifetime. These modified particles were thought to migrate via blood to the reproductive cells and subsequently could be inherited by the next generation. This was essentially a variation of Lamarck's incorrect idea of the "inheritance of acquired characteristics." Mendel picked common garden pea plants for the focus of his research because they can be grown easily in large numbers and their reproduction can be manipulated. Pea plants have both male and female reproductive organs. As a result, they can either self-pollinate themselves or cross-pollinate with another plant. In his experiments, Mendel was able to select cross-pollinate purebred plants with particular traits and observe the outcome over many generations. This was the

basis for his conclusions about the nature of genetic inheritance. He came to three important conclusions from these experimental results (i)- That the inheritance of each trait is determined by "units" or "factors" that are passed on to descendants unchanged (these units are now called genes), (ii)- That an individual inherits one such unit from each parent for each trait, and (iii) That a trait may not show up in an individual but can still be passed on to the next generation. It is important to realize that, in this experiment, the starting parent plants were homozygous for pea seed color. That is to say, they each had two identical forms (or alleles) of the gene for this trait--2 yellows or 2 greens. The plants in the F1 generation were all heterozygous. In other words, they each had inherited two different alleles--one from each parent plant. It becomes clearer when we look at the actual genetic makeup, or genotype, of the pea plants instead of only the phenotype, or observable physical characteristics. Note that each of the F1 generation plants inherited a Y allele from one parent and a G allele from the other. When the F1 plants breed, each has an equal chance of passing on either Y or G alleles to each offspring. With all of the seven pea plant traits that Mendel examined, one form appeared dominant over the other. Which is to say, it masked the presence of the other allele? For example, when the genotype for pea seed color is YG (heterozygous), the phenotype is yellow. However, the dominant yellow allele does not alter the recessive green one in any way. Both alleles can be passed on to the next generation unchanged.

[Q4. Explain supercoiling in a DNA molecule.](#)

**Answer:** One of the interesting characteristics of DNA is its ability to form a coil like structure and this process of making coiling is called as DNA supercoiling. With DNA in its "relaxed" state, a strand usually circles the axis of the double helix once every 10.4 base pairs, but if the DNA is twisted the strands become more tightly. If the DNA is twisted in the direction of the helix, this is positive supercoiling, and the bases are held more tightly together. If they are twisted in the opposite direction, this is negative supercoiling, and the bases come apart more easily. In nature, most DNA has slight negative supercoiling that is introduced by enzymes called topoisomerases. These enzymes are also needed to relieve the twisting stresses introduced into DNA strands during processes such as transcription and DNA replication.

[Q5. Describe the role of DNA polymerase in replication.](#)

**Answer:** DNA polymerases are a family of enzymes that carry out all forms of DNA replication. A DNA polymerase can only extend an existing DNA strand paired with a template strand; it cannot begin the synthesis of a new strand. To begin synthesis of a new strand, a short fragment of DNA or RNA, called a primer must be created, and paired with the DNA template. Once a primer pairs with DNA to be replicated, DNA polymerase synthesizes a new strand of DNA by extending the 3' end of an existing nucleotide chain, adding new nucleotides matched to the template strand one at a time via the creation of phosphodiester bonds. The energy for this process of DNA polymerization comes from two of the three total phosphates attached to each unincorporated base. Free bases with their attached phosphate groups are called nucleoside triphosphates. When a nucleotide is being added to a growing DNA strand, two of the phosphates are removed and the energy produced creates a phosphodiester bond that attaches the remaining phosphate to the growing chain. The energetic of this process also help explain the directionality of synthesis if DNA were synthesized in the 3' to 5' direction, the energy for the process would come from the 5' end of the growing strand rather than from free nucleotides. DNA polymerases are generally extremely accurate, making less than one error for every  $10^7$  nucleotides added. Even so, some DNA polymerases also have proofreading ability; they can remove nucleotides from the end of a strand in order to correct mismatched bases. If the 5' nucleotide needs to be removed during proofreading, the triphosphate end is lost. Hence, the energy source that usually provides energy to add a new nucleotide is also lost.

#### Q 6 What are topoisomerases and helicases?

**Answer:** Topoisomerases are enzymes with both nuclease and ligase activity. These proteins change the amount of supercoiling in DNA and some of these enzyme work by cutting the DNA helix and allowing one section to rotate, thereby reducing its level of supercoiling; the enzyme then seals the DNA break. Other types of these enzymes are capable of cutting one DNA helix and then passing a second strand of DNA through this break, before rejoining the helix. Topoisomerases are required for many processes involving DNA, such as DNA replication and transcription. Helicases are proteins that are a type of molecular motor. They use the chemical energy in nucleoside triphosphates, predominantly ATP, to break hydrogen bonds between bases and unwind the DNA

double helix into single strands. These enzymes are essential for most processes where enzymes need to access the DNA bases.

#### Q7. How does DNA methylation occur?

**Answer:** DNA methylation has been proven by research to be manifested in a number of biological processes such as regulation of imprinted genes, X chromosome inactivation, and tumor suppressor gene silencing in cancerous cells. It also acts as a protection mechanism adopted by the pathogen DNA mainly bacterial against the endonuclease activity that destroys any foreign DNA. The expression of genes is influenced by how the DNA is packaged in chromosomes, in a structure called chromatin. Base modifications can be involved in packaging, with regions that have low or no gene expression usually containing high levels of methylation of cytosine bases. For example, cytosine methylation produces 5-methylcytosine, which is important for X-chromosome inactivation. The average level of methylation varies between organisms - the worm *Caenorhabditis elegans* lacks cytosine methylation, while vertebrates have higher levels, with up to 1% of their DNA containing 5-methylcytosine. Despite the importance of 5-methylcytosine, it can deaminate to leave a thymine base, methylated cytosine are therefore particularly prone to mutations. Other base modifications include adenine methylation in bacteria and the glycosylation of uracil to produce the "J-base" in kinetoplastids.

#### Q 8 What is a PCR?

**Answer:** Polymerase chain reaction (PCR) is a technique to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating thousands of millions of copies of a particular DNA sequence. PCR is developed in 1983 by Kary Mullis, and now it is most common and indispensable technique used in medical and biological research labs for a variety of applications. These include DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes; the diagnosis of hereditary diseases; the identification of genetic fingerprints, and the detection and diagnosis of infectious diseases. The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers which are basically short DNA fragments containing sequences complementary to the target region along with a DNA polymerase are key

components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified. PCR can be extensively modified to perform a wide array of genetic manipulations. Almost all PCR applications employ a heat-stable DNA polymerase, such as Taq polymerase, an enzyme originally isolated from the bacterium *Thermus aquaticus*. This DNA polymerase enzymatically assembles a new DNA strand from DNA building blocks, the nucleotides, by using single-stranded DNA as a template and DNA oligonucleotides which is also called as DNA primers, which are required for initiation of DNA synthesis. The vast majority of PCR methods use thermal cycling, i.e., alternately heating and cooling the PCR sample to a defined series of temperature steps. These thermal cycling steps are necessary first to physically separate the two strands in a DNA double helix at a high temperature in a process called DNA melting. At a lower temperature, each strand is then used as the template in DNA synthesis by the DNA polymerase to selectively amplify the target DNA. The selectivity of PCR results from the use of primers that are complementary to the DNA region targeted for amplification under specific thermal cycling conditions. Most PCR methods typically amplify DNA fragments of up to ~10 kilo base pairs (kb), although some techniques allow for amplification of fragments up to 40 kb in size. The PCR is commonly carried out in a reaction volume of 10–200 µl in small reaction tubes (0.2–0.5 ml volumes) in a thermal cycler. The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction (see below). Many modern thermal cyclers make use of the Peltier effect which permits both heating and cooling of the block holding the PCR tubes simply by reversing the electric current. Thin-walled reaction tubes permit favorable thermal conductivity to allow for rapid thermal equilibration. Most thermal cyclers have heated lids to prevent condensation at the top of the reaction tube. Older thermocyclers lacking a heated lid require a layer of oil on top of the reaction mixture or a ball of wax inside the tube.

**Q9 How does forensic DNA profiling done with a PCR tool?**

**Answer:** Forensic scientists can use DNA in blood, semen, skin, saliva or hair found at a crime scene to identify a matching DNA of an individual, such as a perpetrator. This process is called genetic fingerprinting, or more accurately, DNA profiling. In DNA

profiling, the lengths of variable sections of repetitive DNA, such as short tandem repeats and minisatellites, are compared between people. This method is usually an extremely reliable technique for identifying a matching DNA. However, identification can be complicated if the scene is contaminated with DNA from several people. DNA profiling was developed in 1984 by British geneticist Sir Alec Jeffreys, and first used in forensic science to convict Colin Pitchfork in the 1988 Enderby murders case. People convicted of certain types of crimes may be required to provide a sample of DNA for a database. This has helped investigators solve old cases where only a DNA sample was obtained from the scene. DNA profiling can also be used to identify victims of mass casualty incidents. On the other hand, many convicted people have been released from prison on the basis of DNA techniques, which were not available when a crime had originally been committed.

#### Section B: Multiple Choice

- Q1 100 trillion
- Q2 Leeuwenhoek
- Q3 No
- Q4 Plasmids
- Q5 Mitochondria
- Q6 True
- Q7 Both contain their own genome
- Q8 Fungi
- Q9 True
- Q10 True
- Q11 Genes
- Q12 Sugar
- Q13 Positive
- Q14 Protein synthesis
- Q15 Ribosome
- Q16 False
- Q17 Polymerase
- Q18 DNA mutation
- Q19 Kary Mullis

## Q20 DNA

### Section C: Critical Thinking

*Answers are not provided in this section as the students are encouraged to write their own viewpoints.*

## CHAPTER 3 PROTEINS & PROTEOMICS

### Section A: Descriptive Type

#### Q1: Explain the function of proteins as enzymes.

**Answer:** The best-known role of proteins in the cell is as enzymes, which catalyze chemical reactions. Enzymes are usually highly specific and accelerate only one or a few chemical reactions. Enzymes carry out most of the reactions involved in metabolism, as well as manipulating DNA in processes such as DNA replication, DNA repair, and transcription. Some enzymes act on other proteins to add or remove chemical groups in a process known as post-translational modification. About 4,000 reactions are known to be catalyzed by enzymes. The rate acceleration conferred by enzymatic catalysis is often enormous as much as  $10^{17}$ -fold increase in rate over the uncatalyzed reaction in the case of orotate decarboxylase. The molecules bound and acted upon by enzymes are called substrates. Although enzymes can consist of hundreds of amino acids, it is usually only a small fraction of the residues that come in contact with the substrate, and an even smaller fraction 3 to 4 residues on average that are directly involved in catalysis. The region of the enzyme that binds the substrate and contains the catalytic residues is known as the active site.

#### Q2. Explain the role of proteins in cell signaling.

**Answer:** Many proteins are involved in the process of cell signaling and signal transduction. Some proteins, such as insulin, are extracellular proteins that transmit a signal from the cell in which they were synthesized to other cells in distant tissues. Others are membrane proteins that act as receptors whose main function is to bind a signaling molecule and induce a biochemical response in the cell. Many receptors have a binding site exposed on the cell surface and an effectors domain within the cell, which may have enzymatic activity or may undergo a conformational change detected by other proteins within the cell. Antibodies are protein components of adaptive immune system whose main function is to bind antigens or foreign substances in the body, and target them for