Section 8.2: DNA Sequencing

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1. (a) DNA primers anneal to the complementary single DNA strands and initiate the synthesis of a new DNA strand. They are responsible for the specificity of the target region of DNA that is to be amplified.
(b) Taq polymerase withstands the high temperature variations created in the PCR without getting denatured.
(c) Cycling through three different temperatures is required for the three different steps to occur in a sequence inside the PCR and result in the amplification of the target DNA. Different processes occur at each temperature. For example, at higher temperatures the hydrogen bonds break whereas at lower temperatures the primers can anneal to the complementary sequences.

2. PCR technology has revolutionized genetic testing by making it faster and cheaper to amplify DNA. As well, PCR technology does not require the use of organisms to clone the DNA. Society has benefitted by the fact that DNA screening for forensic and health applications is now much cheaper and faster than ever before. Disadvantages suggested may vary: Sample disadvantage: Because PCR technology allows rapid cloning of DNA without the use of organisms, it is a very powerful technique. In the wrong hands, it could be misused, for example, possibly used for the widespread release of pathogens, or other harmful acts.

3. At higher temperatures the hydrogen bonds break allowing denaturation to occur.

4. One primer must be complementary to the nucleic acid sequence near the 3′ end of the target sequence on one strand, whereas the other must be complementary to the nucleic acid sequence at the 3′ end of the target sequence on the other strand.

5. The negatively charged DNA fragments move away from a negative electrode and move towards the oppositely charged positive electrode in an electric field through the pores of the supporting material of the agarose gel. The movement of the DNA fragments is determined by their size. The DNA stain inserts itself in the DNA fragments and so helps in locating them on the gel. The distances travelled by separated DNA fragments are compared with the distances travelled by known DNA fragments to determine their size.

6. The process of gel electrophoresis is used by research scientists to study gene sequences, in forensic labs for identifying criminals from the blood samples obtained from the crime site, or for settling cases of parental disputes.

7. (a) The 200 bp fragment will travel the fastest because it is the shortest.
(b) The 1800 bp fragment will travel the slowest because it is the longest.
8. The Sanger chain termination method relies on addition of any of the four labelled dideoxynucleotides (ddATP, ddGTP, ddCTP, ddTTP) which if incorporated into the growing DNA strand will terminate the elongation of the strand. The DNA fragments are then separated on gel electrophoresis with a resolution of one nucleotide. The sequence of DNA fragment can then be determined by the order of specific labelling of ddNTPs that correspond to the specific nucleotides of the DNA fragment. This process is now computerized. The whole-genome shotgun method fragments the DNA strands and then uses computer technology to reconstruct the genome.

9. In both structural genomics and functional genomics the genome is sequenced, but these fields analyze the data differently. Structural genomics is more concerned with the nucleic acid sequencing, while functional genomics is more interested in the genes involved.

10. Microarrays add the ability to analyze more than a million samples at a time. In particular, they are very effective at finding mutations by comparison with normal DNA.

11. Answers may vary. Sample answer: *Caenorhabditis* (a nematode) is a simple multi-cellular animal with a set number of cells. *Drosophila* (fruit flies) have been used for years in the study of genetics. *Saccharomyces* (a yeast) represents common single-celled organisms. The Human Genome Project wanted to use organisms that have been widely and previously studied, not rarities or exceptions, because these would have the widest applications in the study of comparative genomics.