Biology 100 *Name* _____
Instructor: K. Marr *Lab Section* _____ *Group No.* _____ *Quarter* _____

ALE #10. From Gene to Protein and Biotechnology Practice Problems

Answer the following questions neatly and fully in the spaces provided. ***References***: Chapter 11 (DNA Biology and Technology) and Section 13.3 (Gene Therapy) in *Essentials of Biology by Mader 2nd ed*

1. Compare the differences in RNA and DNA by completing the table below.

	DNA	**RNA**
Number of Strands		
Name of sugar in nucleotides		
Bases present		
Where produced in cell		
Where found in cell		mRNA: tRNA: rRNA:
Name of process that makes it		

2. Fill in the following spaces concerning the "central dogma" of biology: The _____ strand of a gene is a long chain _____. The order of _____ in a gene controls the order of _____ in mRNA, which determines the order of _____ in the protein the gene codes for, which controls the _____ of the protein, which in turn determines the function of that protein. An organism's genotype determines the kind of proteins an organism can make. These proteins determine an organism's _____.

3. Outline the flow of genetic information from DNA to the production of proteins: DNA → RNA → Protein. For each step indicate where it takes place in the cell, the name of each process involved, what is needed for each process to occur, the names of the major enzymes involved, etc.

4. Here is a hypothetical gene showing the sequence of DNA nucleotides for the **coding strand** (i.e. the coding strand is the strand that is transcribed). **IMPORTANT!!** This sequence includes the regions that code for **start** and **stop** in translation—that is, locate both the start codon and stop codon in the mRNA before you translate it into protein!!
Coding strand of DNA: 3' T A G G T A C T G G G G C A T T A A 5'

   a. What would be the sequence of nucleotides in the resulting mRNA if this strand of DNA was transcribed? Label the 5' and 3' ends of the mRNA molecule.

mRNA:

   b. What is the amino acid sequence of the protein that this gene codes for? (You will need the table of codons in your textbook to answer this question.)

The amino acid sequence is:

5. Hemoglobin is a protein in your red blood cells that is responsible for carrying oxygen. A mutation in the gene that codes for hemoglobin leads to a disease called sickle cell anemia. Sickle cell hemoglobin is unable to carry oxygen effectively, resulting in weakness in individuals who inherit one copy of this gene, death in results if the faulty gene is inherited by from both parents. There are over 300 known mutations in the hemoglobin genes. One of these mutations causes a condition called Hemoglobin C disease, which is not as serious as sickle cell anemia. You will need the table of codons in your book to answer some of these questions.)

   a. Below is the part of the sequence from the <u>coding</u> strand of DNA for three variants of the hemoglobin gene. Circle the mutation in the sickle cell sequence and the hemoglobin C sequence.

   - sickle cell anemia**:** **3'...T G A G G A T T C C T C...5'**
   - hemoglobin C disease: **3'...T G A G G A C A C C T C...5'**
   - normal hemoglobin: **3'...T G A G G A C T C C T C...5'**

   b. What is the <u>mRNA</u> nucleotide sequence of the <u>normal</u> hemoglobin gene? Indicate 3' and 5' ends.

mRNA of normal hemoglobin:

   c. What is the <u>amino acid</u> sequence of the <u>normal</u> hemoglobin gene?

The amino acid of normal hemoglobin:

   d. <u>Exactly</u> what is the difference between the normal hemoglobin <u>protein</u> and the hemoglobin <u>protein</u> in a person with sickle cell anemia and a person with hemoglobin C disease?

   - normal hemoglobin vs. sickle cell:

   - normal hemoglobin vs. hemoglobin C:

   e. As mentioned above, there are over 300 known mutations in the hemoglobin genes. While many of these mutations lead to diseases, some of these mutations do not change the ability of the hemoglobin protein to do its job. Explain how it is possible that a mutation in the hemoglobin gene ***<u>does not</u>*** affect how the hemoglobin protein works.

6. Suppose you are a physician interested in cloning the CFTR gene from the cheek cells of healthy people to use in gene therapy trials to treat patients with cystic fibrosis. Starting with a small sample of human DNA (i.e. human cheek cells on a cotton swab) explain how PCR (**P**olymerase **C**hain **R**eaction) could be used to make millions of copies of the CFTR gene. Your explanation should include the roles of each of the following:
    - primers,
    - *taq* DNA polymerase (a heat-stable DNA polymerase from a bacterium that lives in hot springs),
    - DNA nucleotides,
    - repeated cycling of temperatures: 94°C to 55°C to 72 °C and back to 94 °C.
    - In addition to an explanation, include a *labeled diagram* that shows three cycles of the PCR process. You'll find a discussion and illustration of PCR on page 177 in your textbook, *Essentials of Biology by Mader 2nd ed*. Use these pages only as a reference and guide—do not copy this information, rather, reformulate it *in your own words*.

7. Good work! You've cloned the CFTR gene! Now you wish to use it in gene therapy trials to help your patients. The following questions pertain to the use of gene therapy in an attempt to cure them of cystic fibrosis. For a discussion of gene therapy, see Section 13.3 in your textbook, *Essentials of Biology by Mader 2nd ed*.
   a. Describe the steps that would have to occur at the cellular level that would be needed to *cure* a patient with cystic fibrosis using gene therapy. Assume the usage of a nasal spray that contains a viral vector containing the normal CFTR gene.

   b. Would this really be a cure? Explain why or why not.

   c. How would you use gene therapy to insure that the descendants of a CF patient don't have to worry about getting this genetic disease?

   d. What are the challenges facing gene therapy that prevent it from working? Explain.

   e. What are the potential dangers of gene therapy to the patient? Explain.

Connections with Cell Structure and Function….

8. Cystic fibrosis is due to the inheritance of two faulty CFTR genes. The normal CFTR gene codes for a membrane protein found in cells involved with secretion, for example: mucous secreting cells in the respiratory tract, sweat glands in the skin, digestive enzyme secreting cells of the pancreas, etc. The CFTR protein is actually a ***glycoprotein***, that is, it is a protein that has been modified to by the addition of several monosaccharides.

   a. Trace the pathway of the normal CFTR protein from the organelle where the CFTR protein is made to its final location in the cell membrane. *Hints:* What cell organelle makes proteins? Where is this organelle located, cytoplasm or rough ER? Where are the sugar molecules added to the CFTR protein? How does the CFTR protein get there? How does the CFTR get to the plasma membrane?

→	→	→	→ Plasma membrane

   b. Make a sketch of a cell and trace the pathway taken by the normal CFTR protein from where it is made to the plasma membrane.

   c. The function CFTR protein is to pump chloride ions (i.e. salt) into the cells lining the lungs and cells lining various ducts in the body. In cystic fibrosis, the CFTR protein is defective never makes it to the plasma membrane of these cells. As a consequence salt concentration increases resulting in a ***hypertonic solution*** in the alveoli (air sacs) of the lungs, the ducts that carry sweat from sweat glands to the skin, oviducts, vas deferens, etc. Use this information to explain why people with CF experience the following symptoms:

   - Salty sweat
   - Mucous collects in the lungs
   - Chronic respiratory infections
   - Problems digesting food
   - Reduced fertility in women
   - Sterility in males