Objectives for Test Eight: Chapter 13 – 14, 15.2, 17.1
DNA, Protein Synthesis, Gene Regulation & Biotechnology

You should be able to:

1. Identify the scientists who contributed "pieces of the genetic puzzle," and the basic information they discovered
    a. Griffith
    b. Avery
    c. Hershey & Chase
    d. Chargaff
    e. Franklin
    f. Crick & Watson
    g. Meselson & Stahl
2. Explain the experiments conducted by Griffith and then Avery, and the information they discovered.
3. Identify and label the parts of a DNA nucleotide.
4. Describe the structure of DNA in detail.
5. Explain the experiment of Meselson & Stahl that concluded DNA replication was semiconservative.
6. List the steps involved in the process of DNA Replication. In addition, describe
    a. Where and why it takes place.
    b. The enzymes involved and their specific roles.
    c. The difference between replicating the leading and the lagging strands.
    d. Proof reading of DNA
    e. Why DNA replication is termed "semiconservative"
    f. Appropriate terms for this objective includes: origin of replication, replication fork, helicase, single-stranded binding proteins, topoisomerase, primase, primer, DNA polymerse III, DNA polymerase I, leading strand, lagging strand, Okazaki fragments, ligase, 5′ → 3′ direction
7. Recognize that DNA can proofread and fix errors. Identify items can increase errors (like radioactivity, X-rays, chemicals, cigarette smoke chemicals, etc.)
8. Explain how telomeres protect DNA as cells replication for many generations. Explain the role of telomerase in normal cells and why it's level might be high in cancer cells.
9. Identify differences in DNA replication between prokaryotes and eukaryotes.
10. Explain the relationship between a molecule of DNA and a chromosome. How can such large molecules of DNA fit into small cells? (Use histones, nucleosomes, fibers, and looping)
11. Identify structural differences between DNA and RNA.
12. LAB: DNA extraction from strawberries: Explain the role of salt, dish soap, ethanol, and the glass rod.
13. Describe the genetic code. Given a sequence of DNA (on the template strand), list the sequence of bases for the mRNA made from it, and the sequence of amino acids that results when the mRNA is translated in the cytoplasm.
14. Describe how protein synthesis is related to gene expression. Distinguish between translation and transcription.
15. Distinguish between mRNA, tRNA (with its anticodon) and rRNA and describe their roles in protein synthesis.
16. Describe the structure of a eukaryotic ribosome.
17. Explain the redundancy but not ambiquity in the RNA code.
18. Describe the steps in the process of Transcription. Include the role of the
    a. Template strand (is it read 5′→3′ or 3′→5′?)
    b. Promotor
    c. Terminator
    d. codon
    e. RNA polymerase
    f. Transcription factors
    g. mRNA
    h. introns/exons
19. Tell how translation differs in eukaryotes and prokaryotes.
20. Explain the process that a pre-mRNA molecules go through before leaving the nucleus (the splicing of introns and exons with spliceosomes & snRNPs; addition of G-cap and poly A tail)
21. One gene can be spliced in several difference ways (alternative RNA splicing). Why is this important?
22. Describe the steps in the process of Translation. You may find it convenient to divide the steps into those of initiation, elongation and termination.
23. What is the advantage of a polyribosome?

24. Proteins will either stay with the cell in the cytoplasm or they will become part of the endomembrne system or be exported (secreted) by the cell. How does a protein "know" where to go?
25. Describe several types of mutations and explain how they affect the protein made from the mutated DNA. Identify at least one real human disorder cause by each type of mutation. Include:
    a. base pair substitution (point mutations; missense, nonsense, silent)
    b. reading frameshift mutations (insertions/deletions)
    c. Triple repeats
26. Describe the role of each of the following in prokaryotic gene expression: regulatory gene, repressor molecule (active or inactive), promotor, operator, operon, structural gene, RNA Polymerase.
27. Distinguish between negative gene control (both repressible and inducible systems) and positive gene control.
28. Distinguish between repressor proteins and activator proteins using the Lac operon and Trp operon as examples.
29. Describe several ways that gene expression in eukaryotes is regulated such as
    a. Chromatin structure and the packaging of DNA
    b. Modifying histones with methyl and acetyl groups
    c. Epigenetic inheritance (good review!)
    d. Transcription factors (proteins), enhancers & activators, silencers & repressors
    e. Alterative gene splicing
    f. Rate of mRNA degradation
30. Describe the technique of electrophoresis. Why/how does it separate pieces of DNA?
31. Explain how restriction enzymes and electrophoresis can be used to for genetic screening, forensics, paternity testing and the like. (DNA Fingerprinting). Given an electrophoresis gel, analyze the results.
32. What is recombinant DNA? How are plasmids, restriction enzymes, ligase, and vectors involved in creating and then using recombinant DNA?
33. Describe some practical applications of Biotechnology. (See our presentation for examples)
34. What is the purpose of Polymerase Chain Reaction (PCR) and how does it work? (We will discuss this on Monday in class).
35. Explain what a GMO (Genetically modified organism) is and how they are produced.
36. Regarding viruses:
    a. Describe the structure of typical bacteriophage.
    b. Explain how a typical virus (like a bacteriophage) reproduces in a host cell.
    c. How can a virus be host (species, cell type) specific?
    d. Restriction endonucleases were discovered in viruses. For what to the viruses use them?
    e. How does a lysogenic virus reproduce differently than a lytic virus? How can this difference, as well as viruses in general, be useful for biotechnology?
    f. Discuss the life cycle of the HIV virus. HIV makes and uses the enzyme reverse transcriptase. What does HIV do with it and what can we now use for in biotechnology applications?
37. Explain PCR (Polymerase Chain Reaction). How can it be useful to biotechnology procedures?
38. Regarding AP Lab #8: Biotechnology: Bacterial Transformation
    a. What is bacterial transformation?
    b. What is the purpose of each of the steps in the process?
        i. Exposure to the pGLO plasmid
        ii. Incubation in transformation solution (contains salts, $CaCl_2$)
        iii. Heat Shock at 42 oC
    c. What is the purpose of each of the genes on the pGLO plasmid? What would happen if any of these genes were removed from the plasmid prior to transformation?
        i. Origin of replication
        ii. GFP gene
        iii. Amp resistance gene
        iv. Arabinose gene and operator (operon)
    d. Why was the GFP gene inserted within the arabinose operator and not somewhere else on the plasmid? Why did exposure to arabinose sugar cause the transformed bacteria to make GFP?
    e. Explain the results expected and/or obtained on each of the plates (LB, LB/amp, LB,/amp/ara) with the –pGLO and +pGLO bacteria. Why did some plates have a lawn of growth, some colonies and some no growth at all?
    f. How could a researcher use these techniques to make enzymes useful in manufacturing the enzymes to make dyes for denim jeans, for making Human Growth Hormone, or clotting factor VIII to treat hemophilia?
    g. Design a well controlled experiment to evaluate how the length of the heat shock or the type of salt, the concentration of plasmid or some other variation in the procedure could affect the rate of transformation.

h. Identify several causes of mutations. Select one and design an experiment to test whether this can cause mutation to any of the genes on the pGLO plasmid.
i. When we looked at our LB/amp and LB/amp/ara plates with the +pGLO bacteria, we noticed many "satellite" colonies (very small colonies growing in the vicinity of the larger colonies). Why might these satellite colonies be growing (Hint: Ampicillin has a limited life span; it degrades rather quickly at warmer temps). How could you tell whether or not these satellite colonies are transformed?
j. Transformation is an example of horizontal gene transfer rather than vertical gene transfer. What might this mean?
k. How does this experiment illustrate the concept that "the environment can affect gene expression."
l. **Calculate transformation efficiency** given values for the procedure (See your practice problems).
m. (Not for this test, but our next unit is on natural selection. How does transformation provide a variation that can be acted on by natural selection?)

39. Regarding AP Lab #9: Biotechnology: Restriction Enzyme Analysis of DNA
    a. Identify questions that can be answered using restriction enzyme analysis of DNA (DNA profiling). (See AP Lab manual p. 112 if you are unsure).
    b. What is a restriction enzyme? Where do they cut DNA? Given a piece of DNA and a specific restriction enzyme wit it's restriction site, predict where it will cut the DNA and the size of the ices that will result. Predict where these pieces will end up when used in electrophoresis.
    c. How can Restriction Enzyme Analysis be used to diagnosis sickle cell trait and sickle cell anemia?
    d. Given an electrophoresis gel which contains a DNA ruler (DNA fragments of known sizes), use a semi-log graph to construct a standard curve and then use it to **estimate the fragment sizes** of other fragments.
    e. Does DNA move toward the positive or negative end of an electrophoresis gel? Why?
    f. How would RFLP patterns (Restriction Fragment Length Polymorphism patterns) compare between identical twins? Fraternal twins?
    g. This type of analysis has important social and ethical implications. What are some of these concerns and questions? (See page 123 in your AP Lab manual). This is an important objective.
    h. Where in the genome are most of the genetic differences between individuals located? Are they in the genes themselves or the noncoding regions between the genes? Why would this be the case?
40. Each chapter has some multiple choice questions and a few other additional questions at its end. Give these a try. You might see them again!

Some Examples of Short Free Response Questions: (2-3 sentences): These might be the actual questions.

1. Any of the above questions OR parts of the essays below could be turned into a short answer questions. Calculation type questions are highlighted in bold and underlined.
2. From Essay #207 (b) **Explain** how bacteria can be altered to make genetically engineered products.
3. From Essay #170 (b) The relationship of structure to function is one of the major themes in biology. For the structure/function pair of mRNA structure/protein synthesis **describe** the structure and then **explain** how the function is related to the structure.
4. From Essay 166 C The unit of genetic organization in all living things is the chromosome. **How** does the function and structure of the chromosome differ in prokaryotes compared to eukaryotes?
5. Protein-large complex molecules-are major building blocks of all living organisms. **Discuss** the following in relation to proteins: The roles of DNA and RNA in protein synthesis.
6. From Essay #134 (b). The survival of organisms depends on regulatory mechanisms at various levels. Explain how the expression of a gene is regulated. (This was one of three parts on this essay question).

Possible ESSAY Questions

219. Information flow in cells can be regulated by various mechanisms.
    a. **Describe** the role of THREE of the following in the regulation of protein synthesis:
        - RNA splicing
        - repressor proteins
        - methylation
        - siRNA
    b. Information flow can be altered by mutation. **Describe THREE** different types of mutations and their effect on protein synthesis.
    c. I**dentify** TWO environmental factors that increase the mutation rate in an organism, and discuss their effect on the genome of the organism.
    d. Epigenetics is the study of heritable changes in the phenotype caused by mechanisms other than changes in the DNA sequence. **Describe** ONE example of epigenetic inheritance.

206. Certain human genetic conditions, such as sickle cell anemia, result from single base-pair mutations in DNA.

(a) **Explain** how a single base-pair mutant in DNA can alter the structure and, in some cases, the function of a protein.

(b) **Explain**, using a specific example, the potential consequences of the production of a mutant protein to the structure and function of the cells of an organism.

~~(c) **Describe** how the frequency of an allele coding for a mutant protein may increase in a population over time.~~

197. **Describe** how a plasmid can be genetically modified to include a piece of foreign DNA that alters the phenotype of bacterial cells transformed with the modified plasmid. **Describe** a procedure to determine which bacterial cells have been successfully transformed.

196. The flow of genetic information from DNA to protein in eukaryotic cells is called the central dogma of biology.

(a) **Explain** the role of each of the following in protein synthesis in eukaryotic cells.

1. RNA polymerase
2. Spliceosomes (snRNPs)
3. Codons
4. Ribosomes
5. tRNA

(b) Cells regulate both protein synthesis and protein activity. **Discuss** TWO specific mechanisms of protein regulation in eukaryotic cells.

(c) The central dogma does not apply to some viruses. **Select** a specific virus or type of virus and **explain** how it deviates from the central dogma.

183. A molecule of messenger RNA (mRNA) has just been synthesized in the nucleus of a human cell.

(a) **What** types of modifications may occur to this RNA before it leaves the nucleus?

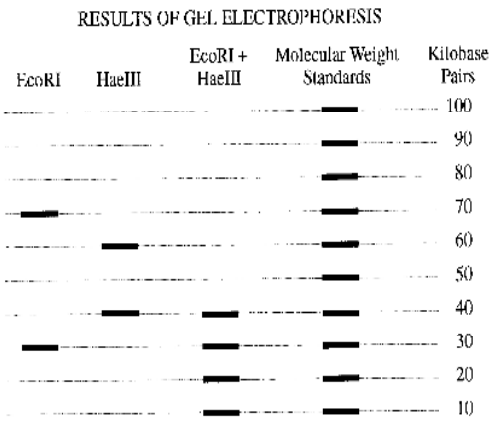
(b) Once in the cytoplasm, **how** is the mRNA translated to a protein?

(c) If the cell is a secretory cell, **how** is the protein from part (b) eventually targeted, packaged, and secreted to the exterior of the cell?

76. Experiments by the following scientists provided critical information concerning DNA. Describe each classical experiment and indicate how it provided evidence for the chemical nature of the gene.

a. Hershey and Chase-bacteriophage replication
b. Griffith and Avery, MacLeod, and McCarty-bacterial transformation
c. Meselson and Stahl-DNA replication in bacteria

180. A bacterial plasmid is 100 kb in length. The plasmid DNA was digested to completion with two restriction enzymes in three separate treatments: EcoRI, HaeIII, and EcoRI + HaeIII (double digest). The fragments were then separated with electrophoresis, as shown.

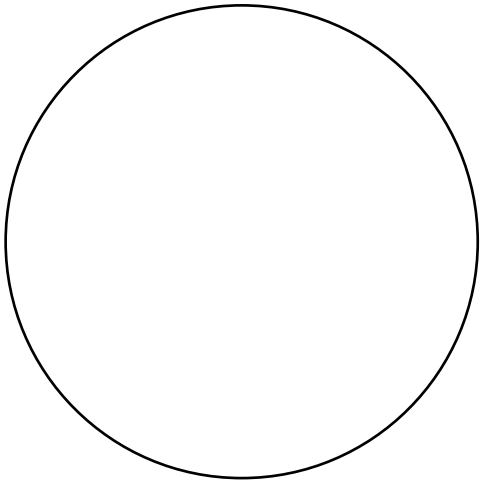


(a) Using the circle provided, **construct** a labeled diagram of the restriction map of the plasmid. **Explain** how you developed your map.

(b) **Describe** how:

- recombinant DNA technology could be used to insert a gene of interest into a bacterium
- recombinant bacteria could be identified
- expression of the gene of interest could be ensured

(c) **Discuss** how a specific genetically modified organism might provide a benefit for humans and at the same time pose a threat to a population or ecosystem.



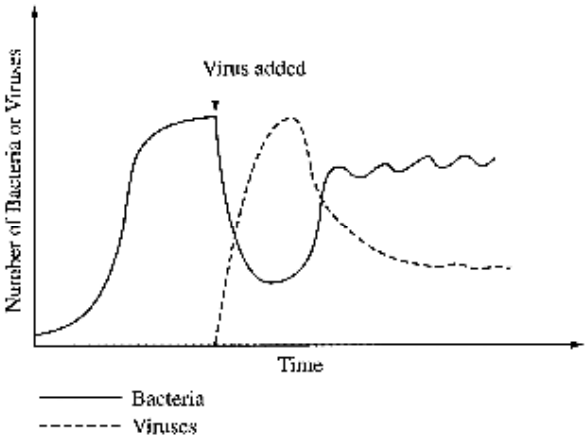
160A. A difference between prokaryotes and eukaryotes is seen in the organization of their genetic material.

(a) **Discuss** the organization of the genetic material in prokaryotes and eukaryotes.

(b) **Contrast** the following activities in prokaryotes and eukaryotes.

- Replication of DNA
- Transcription or translation
- Gene regulation
- Cell division

156A. Bacteria were cultured in a system that allowed for the continual addition of fresh nutrients and the removal of waste products. Bacteriophage (virus) were added at the time shown and the following population changes were observed (illustrated in the graph to the right).



(a) **Describe** and explain the observed results.

(b) **Discuss** the infection cycle of a DNA virus from attachment to lysis.

(c) **Describe** how the genome of a retrovirus like HIV (Human Immunodeficiency Virus) becomes incorporated into the genome of the host cell.

153. The human genome illustrates both continuity and change.

(a) **Describe** the essential features of two of the procedures/techniques below. For each of the procedures /techniques you describe, **explain** how its application contributes to understanding genetics.

- The use of bacterial plasmid to clone and sequence a human gene
- Polymerase chain reaction (PCR)
- Restriction fragment length polymorphism (RFLP) analysis

(b) All human are nearly identical genetically in coding sequences and have many proteins that are identical in structure and function. Nevertheless, each human has a unique DNA fingerprint. **Explain** this apparent contradiction.

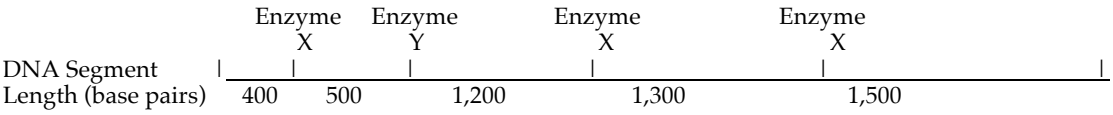
147. Information transfer is fundamental to all living organisms. For **<u>two</u>** of the following examples, explain in detail how the transfer of information is accomplished.

- ~~The genetic material in one eukaryotic cell is copied and distributed to two identical daughter cells~~
- A gene in a eukaryotic cell is transcribed and translated to produce a protein
- The genetic material from one bacterial cell enters another via transformation, transduction, **or** conjugation

138. By using the techniques of genetic engineering, scientists are able to modify genetic material so that a particular gene of interest from one cell can be incorporated into a different cell.

1. Describe a procedure by which this can be done.
2. Explain the purpose of each step of your procedure.
3. Describe how you could determine whether the gene was successfully incorporated.
4. Describe an example of how gene transfer and incorporation have been used in a biomedical or commercial application.

128. The diagram below shows a segment of DNA with a total length of 4,900 base pairs. The arrows indicate reaction sites for two restriction enzymes (enzyme X and enzyme Y).



(a) Explain how the principles of gel electrophoresis allow for the separation of DNA fragments.

(b) Describe the results you would expect from the electrophoretic separation of fragments from the following treatments of the DNA segment above. Assume that the digestions occurred under appropriate conditions and went to completion.

I. DNA digested with only enzyme X
II. DNA digested with only enzyme Y
III. DNA digested with enzyme X and enzyme Y combined
IV. Undigested DNA

(c) Explain both of the following.

(1) The mechanism of action of restriction enzymes

(2) The different results you would expect if a mutation occurred at the recognition site for enzyme Y.

94. Describe the production and processing of a protein that will be exported from a eukaryotic cell. Begin with the separation of the messenger RNA from the DNA template and end with the release of the protein at the plasma membrane.

88. Describe the biochemical composition, structure, and replication of DNA. Discuss how recombinant DNA techniques may be used to correct a point mutation.

82. Describe the operon hypothesis and discuss how it explains the control of messenger-RNA production and the regulation of protein synthesis in bacterial cells.