Name: _____ Date: _____

AP Biology Exam Review: DNA, Protein Synthesis & Biotechnology

Helpful Videos and Animations:

1. Bozeman Biology: DNA Replication
2. Bozeman Biology: DNA and RNA - Part 1
3. Bozeman Biology: DNA and RNA - Part 2
4. Cold Spring Harbor Lab Animation: Griffith / Avery, McCarty, and Macleod Experiments
5. McGraw-Hill Animation: Hershey Chase Experiment
6. Bozeman Biology: Transcription and Translation
7. McGraw-Hill Animation: Transcription
8. McGraw-Hill Animation: Translation
9. McGraw-Hill Animation: Intron Removal by Spliceosomes containing snRNP's (small nuclear riboproteins)
10. McGraw-Hill Animation: Lytic vs. Lysogenic Cycle of Viral Infection
11. Sumanas Animation: Life Cycle of HIV, a Retrovirus
12. McGraw-Hill Animation: Bacterial Transduction Using a Temperate Phage
13. Bozeman Biology: Mechanisms of Genetic Variation in Prokaryotic vs. Eukaryotic Cells
14. Sumanas Animation: Trp Operon (Repressible Operon)
15. Sumanas Animation: Lac Operon (Inducible Operon)
16. Bozeman Biology: Operon
17. Bozeman Biology: Gene Regulation in Prokaryotic vs. Eukaryotic Cells
18. Sumanas Animation: Gel Electrophoresis
19. McGraw-Hill Animation: Restriction Enzymes (AKA Restriction Endonucleases)
20. McGraw-Hill Animation: Restriction Fragment Length Polymorphisms
21. Sumanas Animation: Polymerase Chain Reaction (PCR)
22. Cold Spring Harbor Lab Animation: Bacterial Transformation
23. Bozeman Biology: Response to External Environments

Relevant Objectives:

27. Describe the structure of DNA
28. Describe the experiments leading to the discovery of DNA as the genetic material
29. Describe the process of DNA replication, including: leading and lagging strand, enzymes involved in replication, the semi-conservative model, primers, and telomeres
30. Describe how mutations in the DNA can arise and the process of DNA repair enzymes
31. Explain how DNA is converted into a protein.
32. Describe the process of transcription and how this produces a modified mRNA product based off of DNA.
33. Describe the process of translation and how this leads to the production of a polypeptide from an mRNA sequence
34. Describe how proteins are modified and sent to the correct location
35. Explain the different types of mutations and how they can affect protein formation
36. Describe the makeup of a virus
37. Explain how viruses use host cells to replicate
38. Be able to differentiate between the lytic and lysogenic phases of bacteriophage replication
39. Explain how retroviruses differ from other viruses, and describe their mode of replication
40. Describe how vaccines prevent viral infection
41. Explain how viroids and prions can be infectious agents
42. Explain how bacteria can transfer DNA (3 ways) and how this influences evolution
43. Explain how bacteria can evolve quickly
55. Explain how recombinant DNA is made and inserted into an organism
56. Describe the potential uses for recombinant DNA technology
57. Explain how to screen for and select bacteria that has undergone transformation for intended gene
58. Explain what cDNA is and describe the uses of cDNA and DNA libraries
59. Describe the process of gel electrophoresis and its uses
60. Describe the process of PCR and its uses
61. Explain why genetic engineering is a highly debated issue, and describe both sides' views on the topic
82. Describe the organization of an operon
83. Explain how prokaryotes regulate gene expression using operons
84. Describe the difference between a repressible operon and an inducible operon, and give an example of each
85. Explain how genes are regulated in eukaryotes by the following methods: DNA packaging, transcriptional regulation, post-transcriptional regulation, translational regulation, post-translational regulation

Topic Outline:

1. DNA History
    - Be able to describe the experiments leading to the discovery of DNA as the cell's genetic material. Key scientists and experiments include
        - Franklin, Watson, Crick, Wilkins – structure of DNA
        - Griffith – bacterial transformation, genetic material
        - Hershey / Chase – sulfur and phosphorus tagged viruses, showed DNA passed not proteins
        - Avery, MacLeod, McCarty – tried transformation after knocking out macromolecules (RNA, DNA, proteins, lipids, carbohydrates) transformation NOT successful if DNA knocked out

2. Structure of DNA
    - Deoxyribose nucleic acid
    - Double helix (two twisted stsrands) made of nucleotides (monomers)
    - Nucleotide = phosphate + 5C deoxyribose sugar + nitrogen base
    - Antiparallel strands- one runs 3' to 5' the other runs 5' to 3', sides of phosphates and sugars
    - (backbone), rungs of paired bases with hydrogen bonds in between
    - Purines (adenine, guanine; double rings) pair with Pyrimidines (cytosine, uracil, thymine; single ring)
    - A & T – double H bond
    - C & G – triple H bond
    -

3. Location of DNA
    - In eukaryotes DNA is found in nucleus on multiple linear chromosomes (a chromosome IS a strand of DNA with proteins etc. associated).
    - In prokaryotes DNA is not in a nucleus and is usually a single circular chromosome
    - Prokaryotes, viruses, and eukaryotes (yeast) can contain plasmids (small extra-chromosomal DNA that is double stranded DNA)

4. DNA replication
    - Process of making exact copies of DNA (i.e. for mitosis or meiosis)
    - Process is semi conservative (original strand is copied)
    - Steps
        A. Enzyme (helicase) unzip strands by breaking hydrogen bonds
        B. "Spare" nucleotides are added bidirectionally to bond complementarily with use of DNA polymerases (DNA pol)
        C. DNA pol only can add to the 3' end of DNA and new DNA is made in the 5' to 3'direction
        D. Replication bubbles open up and a replication fork is created because bubble is in half and it has one side 3/5 and one 5/3
        E. RNA primers must be laid down to start process (RNA primase makes primers)
        F. Leading strand makes DNA continuously (Read 3→5, laid down 5→3)
        G. Lagging strand makes DNA discontinuously (Read 5→3, must flip strand to orient correctly), Okazaki fragments
        H. Lagging strand requires enzyme (ligase) to fuse fragments

5. RNA
    - Ribonucleic acid
    - Single stranded, different sugar called ribose, different base called uracil INSTEAD of thymine
    - Base pair rules in RNA, A-U and C-G
    - messenger RNA or mRNA carries information from DNA to the ribosome
    - transfer RNA or tRNA bind amino acids and are used in translation at ribosome
    - ribosomal RNA or rRNA acts as an enzyme in the ribosome aiding in forming peptide bonds – likely one of the first enzymes (ribozyme)

6. Transcription
    - making mRNA in nucleus
    - enzyme RNA pol reads the DNA in 3' to 5' direction and synthesizes complementary mRNA in 5' → 3' direction
        - Ex. 3' to 5' DNA is ATG CAT then the 5' to 3' mRNA made will be UAC GUA
    - Steps
        A. Initiation – Promoter is where RNA pol binds and begins
        B. Elongation – adding of RNA nucleotides, does not stay attached to DNA
        C. Termination – ends when RNA pol reaches a termination sequence

7. mRNA editing
    - introns spliced out (cut out) using spliceosomes (snRNP's)
        - alternative splicing leads to many proteins from one mRNA
    - add polyA tail to 3'
    - add GTP cap to 5'
    - each 3 nucleotides are called a codon
    - go to ribosome (free or in rough ER)

8. Translation
    - mRNA code is read and matched with tRNA (brings amino acids) to construct a polypeptide using the ribosome
        - Ex. mRNA codon is AAA then tRNA anticodon will be UUU and will have a corresponding amino acid for that codon of mRNA
    - 3 steps: Initiation, Elongation, Termination (see notes)
    - If in ER then: polypeptide is released into ER, then to Golgi complex, vesicle to cell membrane, then exocytosis (may be given signals for exit/destination)
    - Free ribosomes typically make products for the cell and are not exported – go to other organelles, used in cytoplasmic reactions

9. Mutations and Increasing Genetic Diversity
    - Changes to the DNA sequence are not all harmful, some can increase genetic variability → more possible forms of traits so that not all organisms can be killed off by any one factor (ex: a disease that kills all tall people)
    - They can be spontaneous errors in replication or they can be caused by mutagens (environmental factors like radiation, chemicals, cigarette smoke, etc.)
    - If a mutagen causes changes in genes that regulate the cell cycle/cell division it is considered a carcinogen (a cancer-causing factor)
    - Some mutations are neutral (happen in introns that do not code for proteins)
    - Some mutations are harmful (change protein function in a negative way)
    - Types of Mutations:
        A. Point mutation – change in one base pair of a gene (substitution: replace one base with another)
        B. Silent – changes one base, but codes for the same amino acid (due to redundancy)
        C. Missense – codes for another amino acid (changes protein sequence and usually function)
        Example: sickle cell disease, one T substituted for A in the gene coding for hemoglobin protein
        D. Nonsense – code changes to a stop codon (makes a nonfunctional protein that is terminated early)
        E. Frameshift mutation – the mutation effects all nucleotides/codon groupings farther along the DNA/RNA code, typically caused by insertion or deletion
            - Insertion – adding extra nucleotides (causes a frameshift if you are not adding exactly three extra bases)
            - Deletion – removing nucleotides (causes a frameshift if you are not removing exactly three bases)
            Example: O blood type allele involves a deletion in the A blood type code

10. Viruses – protein coating with nucleic acids (ssDNA, ssRNA, dsDNA or dsRNA) inside. Needs host to replicate.
    - Viral Replication
        - Viruses inject DNA or RNA into host cell
        - Viruses have highly efficient replicative capabilities that allow for rapid evolution
        - Viruses replicate via the lytic cycle, allowing one virus to produce many progeny simultaneously
        - Virus replication allows for mutations to occur through usual host pathways.
        - RNA viruses lack replication error-checking mechanisms, and thus have higher rates of mutation
        - Related viruses can combine/recombine information if they infect the same host cell
        - Some viruses are able to integrate into the host DNA and establish a latent (lysogenic) infection
        - HIV is a well-studied system where the rapid evolution of a virus within the host contributes to the pathogenicity of viral infection.
        - Genetic information in retroviruses is a special case and has an alternate flow of information: from RNA to DNA, made possible by reverse transcriptase, an enzyme that copies the viral RNA genome into DNA. This DNA integrates into the host genome and becomes transcribed and translated for the assembly of new viral progeny.

11. Bacterial Reproduction and Genetic Recombination
    - Transformation – bacteria uptakes DNA from another bacteria
    - Transduction – virus transfers DNA between two bacteria
    - Conjugation – bacterial "sex"
    - Transposition – "jumping genes"

12. Prokaryotic Gene Regulation
    - Bacteria are prokaryotic with a single circular chromosome
    - Bacteria express all the genes needed for a product (more than one gene at a time)
    - Organization includes the promoter region of DNA, operator, and structural genes
    - Trp operon = repressible; anabolic pathway; used to make enzymes that help make tryptophan if none is present
        - Repressor is naturally INACTIVE so it will make tryptophan
        - Repressor only becomes ACTIVE when trp (called corepressor) is in excess and binds to repressor changing its shape
    - Lac operon = inducible; catabolic pathway; used to make enzyme to break down lactose when it is available
        - Repressor is naturally ACTIVE so it will block gene transcription unless lactose (called inducer) binds and makes repressor INACTIVE

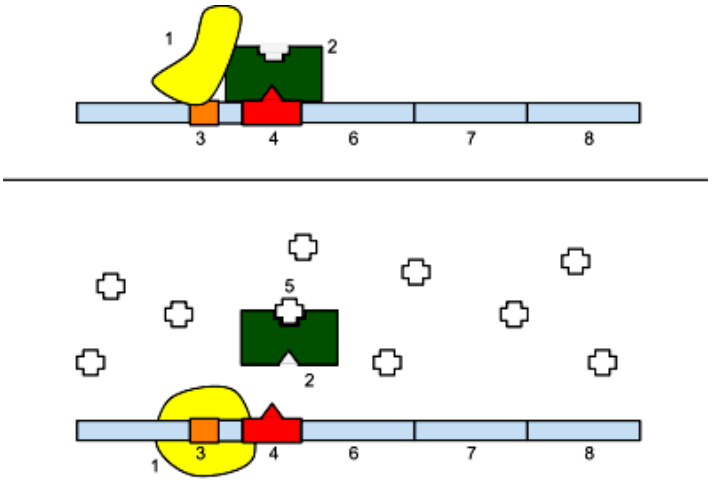
13. Eukaryotic Gene Regulation
    - Enhancers- Areas on genome that are non-coding that are located at a distance from a promoter Transcription factors / activators can bind to these areas and cause transcription of certain genes (turns on)
    - mRNA Degradation by RNAi - mRNA has a life span in the cytoplasm (can last a few hours to a week) (turns off)
    - RNA processing (intron splicing, 3' poly a tail, 5' cap) (turn on and alter expression)
    - Histone Acetylation (turn on)
    - DNA methylation (turn off)
    - Translation Repressors (turn off)
    - Posttranslational modifications- folding, cleaving, etc. (alter expression)

14. Creation of Recombinant DNA and Bacterial Transformation
    - Toolkit includes plasmid (piece of round DNA from bacteria/yeast) or other vector such as viruses; restriction enzymes; host cell (usually bacteria like E. coli)
    - Restriction enzymes cut genes at restriction sites to make blunt or sticky ends
    - Cut gene of interest with same enzyme to get same ends
    - Use ligase to seal gene of interest into the plasmid
    - Insert vector into host
    - Used to clone and make copies or to produce a foreign protein such as HGH or insulin

15. Polymerase Chain Reaction (PCR)
    - Used to make large amounts of clones of DNA without using a host; heat which opens; use a primer to mark the place in the sequence where Taq polymerase begins replication; cool; repeat

16. Gel Electrophoresis
    Used to look at unique pattern created by fragments of DNA; cut DNA using enzyme; load into a gel; turn on electricity; DNA runs from negative to positive; larger chunks move less; unique for each person if testing variable areas of DNA (ex: RFLP's); can be used for protein or mRNA too

Practice Multiple Choice Questions:



Questions 1 and 2. With regard to the operon pictured to the right, the image on top shows the operon in its normal state, and the image on the bottom shows the operon in the presence of molecule #5 (looks like a + sign). The identities of some of the molecules shown in the picture are given below.

1. RNA polymerase
3. Promoter
4. Operator
6, 7, and 8. Genes of the operon

****Note: In the picture on top, RNA polymerase is UNABLE to bind correctly to the promoter region and initiate transcription of the genes of the operon****

1. What type of operon is shown in the image, and how do you know?
a. An inducible operon; it is usually off but can be turned on.
b. An inducible operon; it is usually on but can be turned off.
c. A repressible operon; it is usually off but can be turned on.
d. A represible operon; it is usually on but can be turned off.

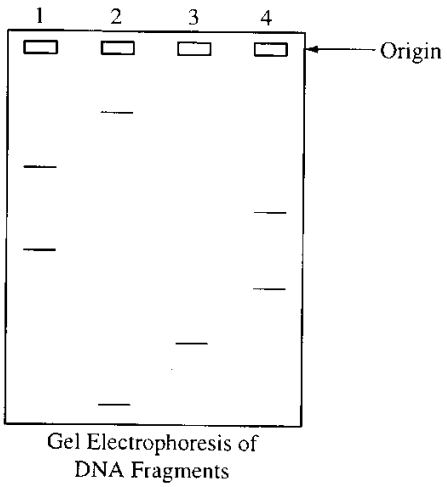
2. What is the role of molecule #5 in regulating the operon?
a. It is an inducer, which is used to inactivate the repressor protein (#2) and prevent it from binding to the operator.
b. It is an inducer, which is used to activate the repressor protein (#2) and allow it to bind to the operator.
c. It is a repressor, which is used to inactivate the repressor protein (#2) and prevent it from binding to the operator.
d. It is a repressor, which is used to activate the repressor protein (#2) and allow it to bind to the operator.

3. Why is an anabolic operon usually repressible?
a. It is used to break down a molecule in the environment (ex: maltose sugar) so it should usually be on.
b. It is used to break down a molecule in the environment (ex: maltose sugar) so it should usually be off.
c. It is used to build an essential molecule in the cell so it should usually be on.
d. It is used to build an essential moelcule in the cell so it should usually be off.

4. Adding acetyl groups to the histone proteins interacting with the DNA of the insulin gene causes the DNA to coil less tightly. What will be the effect on gene expression?
a. This will prevent expression of the insulin gene and result in decreased amounts of insulin protein produced.
b. This will prevent expression of the insulin gene and result in increased amounts of insulin protein produced.
c. This will facilitate expression of the insulin gene and result in decreased amounts of insulin protein produced.
d. This will facilitate expression of the insulin gene and result in increased amounts of insulin protein produced.

5. How can multiple types of antibodies be synthesized from the same "antibody gene"?
a. Changing the tightness of coiling of the DNA can result in the creation of different antibody proteins.
b. Changing the speed of transport of mRNA out of the nucleus can result in the creation of different antibody proteins.
c. Changing which introns are spliced out of the pre-mRNA can result in the creation of different antibody proteins.
d. Changing the regulatory proteins that bind to the 5' end of the mRNA and prevent ribosome attachment can result in the creation of different antibody proteins

6. The electrophoretic separation of the pieces of DNA in each of the four samples shown to the right was achieved because of differential migration of the DNA fragments in an electric field. This differential migration was caused by the
a. relative amounts of radioactivity in the DNA
b. number of cleavage points per fragment
c. size of each fragment
d. overall positive charge of each fragment



Gel Electrophoresis of DNA Fragments

7. The DNA in this sample was labeled with ^{32}P in order to
a. stimulate DNA replication
b. inhibit the uptake of unlabeled ATP
c. show which fragments included the 5' end and which fragments included the 3' end
d. visualize the fragments

A scientist is using an ampicillin-sensitive strain of bacteria that cannot use lactose because it has a nonfunctional gene in the *lac* operon. She has two plasmids. One contains a functional copy of the affected gene of the *lac* operon, and the other contains the gene for ampicillin resistance. Using restriction enzymes and DNA ligase, she forms a recombinant plasmid containing both genes. She then adds a high concentration of the plasmid to a tube of the bacteria in a medium for bacterial growth that contains glucose as the only energy source. This tube (+) and a control tube (-) with similar bacteria but no plasmid are both incubated under the appropriate conditions for growth and plasmid uptake. The scientist then spreads a sample of each bacterial culture (+ and -) on each of the three types of plates indicated below.

8. If no new mutations occur, it would be most reasonable to expect bacterial growth on which of the following plates?
a. 1 and 2 only
b. 3 and 4 only
c. 5 and 6 only
d. 4, 5, and 6 only
e. 1, 2, 3, and 4 only

	Glucose Medium	Glucose Medium with Ampicillin	Glucose Medium with Ampicillin and Lactose
Bacterial strain with added plasmid (+)	#1	#2	#3
Bacterial strain with no plasmid (–)	#4	#5	#6

9. The scientist used restriction enzymes for what purpose in the experiment?
a. To make the plasmid small enough to transform cells
b. To make cuts in the plasmid DNA
c. To make the plasmid enter the cells
d. To enable the fragments of DNA to form covalent bonds
e. To enable the plasmid to recognize the bacterial cells

	Lactose Medium	Lactose Medium with Ampicillin
Bacterial strain with added plasmid (+)	#7	#8
Bacterial strain with no plasmid (–)	#9	#10

10. If the scientist had forgotten to use DNA ligase during the preparation of the recombinant plasmid, bacterial growth would most likely have occurred on which of the following?
a. 1 and 2 only
b. 1 and 4 only
c. 4 and 5 only
d. 1, 2, and 3 only
e. 4, 5, and 6 only

11. If the scientist used the cultures to perform another experiment as shown above, using medium that contained lactose as the only energy source, growth would most likely occur on which of the following plates?
a. 10 only b. 7 and 8 only c. 7 and 9 only
d. 8 and 10 only e. 9 and 10 only

12. Actinomycin D is an antibiotic drug that inhibits protein synthesis by blocking transcription. In some cells, the application of the drug does not affect the synthesis of certain proteins. Which of the following best explains such an occurrence?
a. Not all proteins need tRNA molecules for their synthesis.
b. The proteins that are made are using mRNA synthesized before application of the drug.
c. Nuclear proteins do not require the cytoplasmic machinery of ribosomes.
d. Protein synthesis is blocked in the cytoplasm at the ribosome level.

. . . glycine-serine-glycine . . .

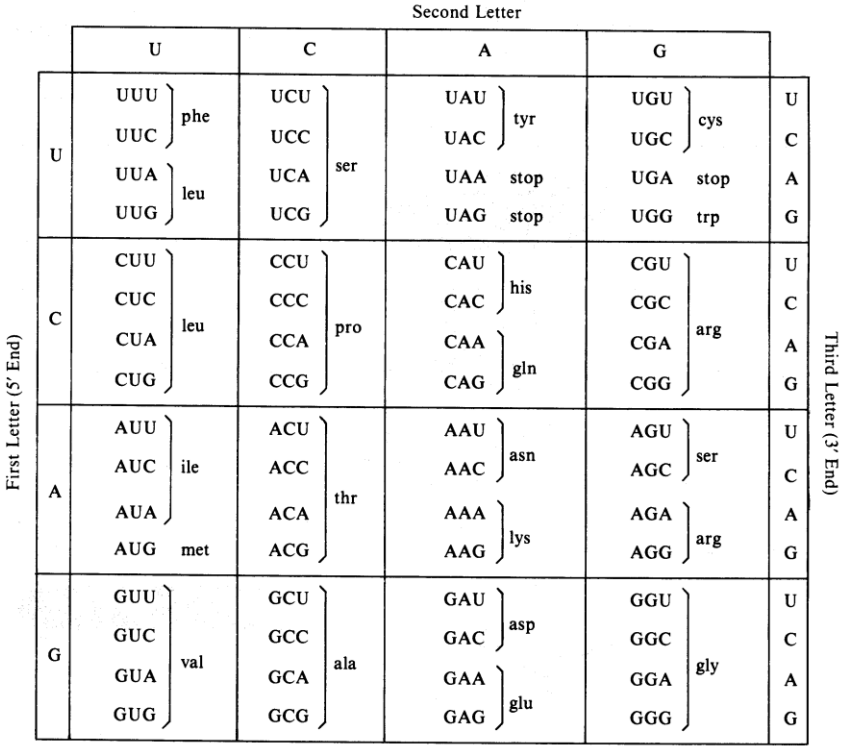
13. Which of the following DNA strands will code for the amino acid sequence shown above?
a. . . . ACTCCTTCT . . .
b. . . . TCTCCGTCG . . .
c. . . . CCGTCGACT . . .
d. . . . CCTTCGCCT . . .

14. A single substitution in the third position would have the greatest probability of mutational effect on the codon
a. GUU
b. AUU
c. CGU
d. AUG
e. CCC

15. What would be the sequence of bases of an mRNA molecule that was transcribed from the sequence of DNA bases shown below?

GTAGTAGGT

a. GTAGTAGGT
b. CAUCAUCCA
c. UCGUCGUUC
d. AUGAUGAAU
e. CATCATCCA



<u>Questions 16-20.</u> Refer to the following list to answer the following questions. The answers may be used once, more than once, or not at all.
a. transcription
b. translation
c. transformation
d. replication
e. reverse transcription

16. Process in which a protein is assembled at a ribosome.

17. Process in which naked DNA is taken up by a bacterial or yeast cell.

18. Process in which RNA is produced by using a DNA template.

19. Process that results in the production of cDNA from an RNA molecule.

20. Process in which DNA is produced by using a DNA template.

21. DNA replication can be described as
a. semiconservative
b. conservative
c. degenerative
d. dispersive
e. radical

22. In DNA replication, DNA polymerase catalyzes the reaction in which
a. The double helix unwinds
b. The sugar-phosphate bonds of each strand are broken
c. A phosphate group is added to the 3'-carbon or 5'-carbon of ribose
d. A nucleotide with a base complementary to the base on the template strand is added to the new DNA strand
e. Two nucleotide strands come together and intertwine to form a double helix

23. The replacement of glutamine by valine at a specific position in the beta chains of hemoglobin leads to sickle cell anemia. This change represents which of the following mutational events?
a. DNA base-pair substitution
b. DNA base-pair deletion
c. DNA base-pair addition
d. Chromosomal deletion
e. Frame-shift mutation

Questions 24-27. Refer to these scientists famous for their work with DNA.
a. Hershey and Chase
b. Griffith
c. Rosalind Franklin
d. Avery, McCarty, MacLeod

24. Discovered transformation in bacteria.

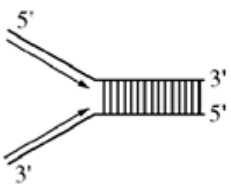
25. Showed that DNA was the genetic material by doing transformation experiments while knocking out different macromolecules.

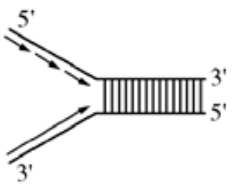
26. Proved that the nuclear material in a bacteriophage, not the protein coat, infects a bacterium.

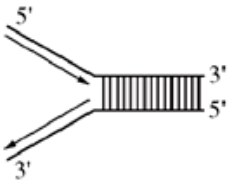
27. The first to analyze DNA by x-ray crystallography, proposed DNA was helical.

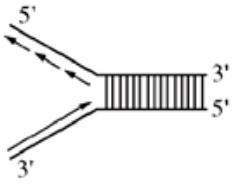
28. Once transcribed, eukaryotic RNA normally undergoes substantial alteration that results primarily from
a. removal of exons
b. removal of introns
c. addition of introns
d. combining of RNA strands by ligase

29. When DNA replicates, each strand of the original DNA molecule is used as a template for the synthesis of a second, complementary strand. Which of the following figures most accurately illustrates enzyme-mediated synthesis of new DNA at a replication fork?

a.


b.


c.


d.


30. If guanine makes up 28% of the nucleotides in a sample of DNA from an organism, then thymine would make up _____ % of the nucleotides.
a. 28
b. 56
c. 22
d. 44

31. Prions are
a. bacteriophages that cause disease
b. infectious proteins
c. a bacterium that infects viruses
d. the cause of sickle cell anemia

Practice Long Response Questions: Make an outline of the information you would include in each of these essays.

1. Describe how recombinant DNA technology can be used to accomplish the following:
a. The creation of human insulin protein to treat diabetes.
b. The creation of golden rice, which is a transgenic plant (meaning it contains DNA from two different organisms) that has been given the gene for beta carotene (vitamin A) production using a bacterial vector.

2. Meiosis reduces chromosome number and rearranges genetic information.
a. Explain how the reduction and rearrangement are accomplished in meiosis.
b. Several human disorders occur as a result of defects in the meiotic process. Identify ONE such chromosomal abnormality; what effects does it have on the phenotype of people with that disorder? Describe how this abnormality could result from a defect in meiosis.
c. Production of offspring by parthenogenesis or cloning bypasses the typical meiotic process. Describe either parthenogenesis or cloning and compare the genomes of the offspring with those of the parents.

3. 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
- gene regulation
- cell division

4. All humans are almost genetically identical. However, every person has a unique DNA fingerprint. Explain this contradiction.

Thinking Practice Questions:

Compare the two DNA sequences shown below. Transcribe them into mRNA and translate them into an amino acid sequence.

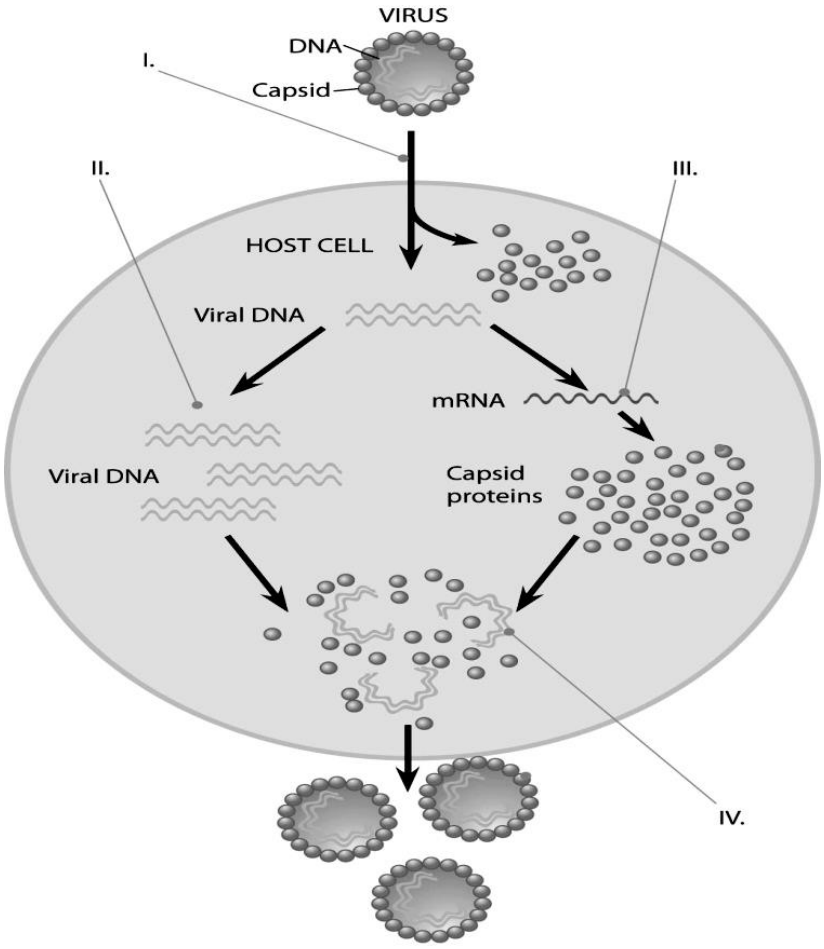
GTG CAC CTC ACA CCA GAG GAG (Normal Hemoglobin)

GTG CAC CAC ACA CCA GTG GAG (Sickle Cell Hemoglobin)

a. Circle any differences there are in the DNA, RNA and amino acid sequences that might exist between these two sequences.
b. Identify the type of mutation that is represented AND EXPLAIN, IN DETAIL, what effect this would have on the protein/pigment.

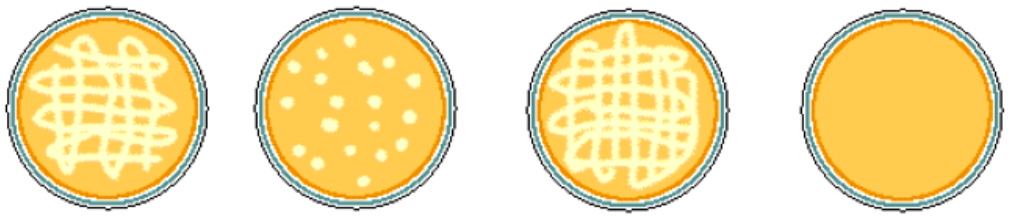
2. In prokaryotic cells, translation begins before transcription is finished. Give two reasons why this would not be possible in eukaryotic cells.

3. Describe the processes occurring at each of the numbered positions (I, II, III, and IV) in the diagram below.



Viral reproductive cycle

4. In a molecular biology laboratory, a student obtained competent *E. coli* cells and used a common transformation procedure to induce the uptake of plasmid DNA with a gene for resistance to the antibiotic kanamycin. The results below were obtained.



a. What is the purpose of Plate IV?
b. Explain the growth you see and the type of bacteria (transformed vs. non-transformed or both) that would be on Plate 1.
c. Explain the growth you see and the type of bacteria (transformed vs. non-transformed or both) that would be on Plate II.
d. If the student repeated the experiment, but the heat shock was unsuccessful and the plasmid was unable to be transformed, for which plates would growth be expected? Explain your answer.