BCH 4054 **HOUR TEST 4** **NAME**_____
April 11, 2001

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(9) 1. A circular DNA plasmid of length 1040 bp is supercoiled with a twist (T) value of 100 and a linking number (L) of 94.

(a) What is the value of the writhing number (W)?

(b) Is the plasmid negatively or positively supercoiled?

(c) What effect would topoisomerase I have on L, T, and W?

(d) What effect would DNA gyrase and ATP have on L, T, and W?

(e) Ethidium bromide is an intercalating agent that inserts between the stacked base pairs, separating the stacks and causing local unwinding that decreases the value of T. What effect would ethidium bromide have on the migration rate of the plasmid during electrophoresis?

(f) If part of the plasmid were to undergo a transition from B-DNA to Z-DNA, what would be the effect on L, T, and W?

(7) 2. Complete the following table by identifying the DNA structures described:

Helix Direction:	Left	Right	Right
Base Pairs/Turn	12	10.4	11
Base Tilt	9^o	1.2^o	19^o
Diameter (Å)	1.84	2.37	2.55
Name of Structure	_____	_____	_____

Which helical structure does double stranded RNA form?

(10) 3. The following proteins have been identified as being involved in homologous recombination in bacteria: RecA, RecB, RecC, RecD, RuvA. RuvB, RuvC. Their synthesis is stimulated when there is extensive DNA damage, and they function to carry out recombination as a repair mechanism. The repair mechanism involves the intermediate formation of a Holliday junction.

(a) Diagram a Holliday junction, labeling the ends of the DNA strands to show the directionality of each strand.

(b) Select from the above lists the protein or proteins that carry out the following processes during recombination.

_____ Components of a complex that recognizes breaks in the DNA, unwinds the DNA to form "rabbit ears", then cleaves near a chi site to generate a single strand stretch of DNA.

_____ Forms a polymeric helical structure that binds both a single strand and double strand of DNA.

_____ Catalyzes "strand displacement" of one strand of double stranded DNA by a stretch of homologous single strand DNA.

_____ Recognizes and binds to the Holliday structure.

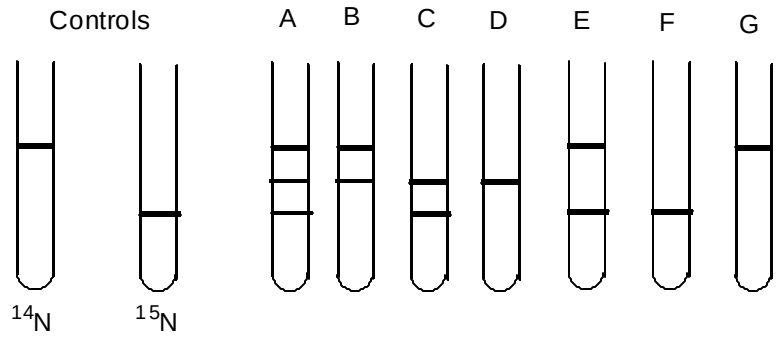
_____ A second protein that binds to the first at the Holliday structure and catalyzes branch migration.

_____ Displaces the two proteins from the Holliday structure, and "resolves" the structure into the recombinant DNA by nuclease action.

(6) 4. Methylation of DNA plays a variety of roles in regulation, repair recognition, and gene activation. However, regions of DNA that have a high content of 5-methyl cytosine have higher mutation rates than other regions of DNA. Why do you think this is so?

(6) 5. Why do cells exposed to visible light following irradiation with ultraviolet light have a greater survival rate than cells kept in the dark after such irradiation?

(10) 6. In the Meselson-Stahl experiment, bacteria were grown in culture media labeled with ^{15}N, then transferred to ^{14}N media, and cells were harvested at various times after transfer. The DNA was isolated and analyzed in a density-gradient ultracentrifugation experiment where heavy (^{15}N labeled), light (^{14}N labeled), and hybrid (mixed labeling) DNA can be separated. The diagram shows control centrifugation tubes indicating the banding positions of light and heavy DNA. The other tubes represent several possible banding patterns that might have been observed in the experiment. (DNA quantities in each band are not represented).



Identify the banding pattern observed under the following conditions. (Put the letter of the appropriate tube in the blank next to the condition).

_____ DNA taken from cells before transfer to the ^{14}N media.

_____ DNA taken from cells one generation after transfer.

_____ DNA taken from cells two generations after transfer.

The banding patterns observed supported the **semiconservative model** for DNA replication. Suppose that instead DNA was replicated by the **conservative** model. Then predict what the banding pattern would be for:

_____ DNA taken from cells one generation after transfer.

_____ DNA taken from cells two generations after transfer.

(12) 7. DNA Polymerase I from bacteria has three enzymatic activities.

(a) Explain what each does during DNA replication:

5'-3' polymerase

3'-5' exonuclease

5'-3' exonuclease

(b) Explain how Polymerase I participates in the normal DNA replication process in bacteria.

(c) Explain how Polymerase I participates in DNA repair in bacteria.

(6) 8. DNA polymerase III from bacteria has a much greater "processivity" than polymerase I. What is meant by the term "processivity", and describe the structural feature of polymerase III that accounts for this property.

(8) 9. What is the function of NAD^+ in the DNA ligase reaction in bacteria? Explain chemically what happens to it.

(10) 10. (a) Termination of replication of circular DNA in bacteria occurs when the replication forks meet. There may be a need to separate the molecules if they are concatenated. (interlocked). How is this separation accomplished?

(b) Termination of replication of linear DNA in eukaryotes faces an entirely different problem. Describe the problem and how it is handled.

(6) 11. The drug AZT inhibits the enzyme reverse transcriptase. What is the function of this enzyme, and why has this drug become very important in recent years?

(10) 12. When DNA polymerase inserts a new nucleotide into the growing DNA chain, a mistake in base pairing can be made if the base happens to be in the wrong tautomeric form. There are two mechanisms to correct this mistake. Describe them.