A TOTAL OF SIX PAGES

NAME:

STUDENT ID:

Question	Maximum Points	Your Points
I	28	
II	41	
III	27	
IV	28	
V	26	
	150	

Please write your **name** and **student ID** number (if you have one) at the top of every page.

Note that this exam must be written in **INK** if you want the option of a regrade.

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Question I (28 points)

A. Proteins that bind to DNA directly have different types of DNA recognition specificity. Also, the protein-DNA interaction specificity is often combined with protein-protein interaction to recruit additional factors to the site of DNA recognition. **For each of (a) and (b) below, answer (i) and (ii):**

(i) What is protein that DIRECTLY recognizes the indicated DNA? What features of the DNA are important for recognition specificity (list any requirement for sequence or structure of the bound DNA)?

(ii) State one protein that is recruited to the DNA-bound factor listed in (i) by protein-protein interaction AND indicate the biochemical role of this protein (the type of protein activity is sufficient to answer at the level intended).

(a) Holliday junction

(i)

(ii)

(b) *E. coli* origin of DNA replication

(i)

(ii)

B. Proteins can be loaded onto DNA, for example the *E. coli* beta sliding clamp, that then move from the site of initial recruitment. The beta sliding clamp can recruit several other proteins to DNA with specificity partly dependent on adjacent DNA structure and partly dependent on competition among the interacting proteins. List THREE protein factors/complexes that can bind to the *E. coli* beta sliding clamp as **(a), (b), and (c) below,** and for each **describe how it competes/cooperates** with one of the other factors that you have listed.

(a)

(b)

(c)

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Question II (41 points)

A. Nucleases have recognition specificity for DNA structure and/or sequence.
(a) List TWO **exonuclease activities** that **act on one, single strand of DNA that is PAIRED as a DNA duplex**. Indicate the polarity of the exonuclease activity and its biological function.

(b) List THREE **endonucleases** that **act on one, single strand of DNA that is PAIRED as a DNA duplex**. Indicate all important features of substrate specificity of each endonuclease.

(c) List THREE **endonucleases** that act on a **structure other than a single-stranded or duplex DNA**. Indicate critical features of DNA structure specificity for the enzymes that you list.

B. Many enzymes use a cycle of ATP binding and hydrolysis to function. For the enzymes below, describe how ATP binding AND ATP hydrolysis affect enzyme properties.

(a) DnaA

(b) Gamma complex

(c) RecA

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Question III (27 points)

At a DNA replication fork, multiple enzyme activities are coordinated through physical interaction.

A. List FOUR physically associated ENZYMES at an *E. coli* replication fork.

(a)

(b)

(c)

(d)

B. Other *E. coli* factors involved in replication are NOT physically associated with other proteins at the replication fork. List THREE such factors AND state the biochemical activity that is the contribution of each enzyme to complete genome replication.

(a)

(b)

(c)

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Question IV (28 points)

For each of 1-2 below, give answers for A-C:
A. What is a type of DNA damage that will be fixed by the listed type of DNA repair? Pick only one example of damage, but be as specific as necessary in description of the DNA substrate.
B. State two proteins **SPECIFIC for ONLY this repair pathway** and in one sentence describe the function/activity of each protein.
C. How much DNA will be synthesized during repair of the damage? To make it simple, choose between these options: 0, 1, 2-40, or more than 40 nt.

1. Base excision repair
A

Bi

Bii

C

2. Mismatch repair
A

Bi

Bii

C

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Question V (26 points)

A site-specific recombinase and a transposase each act as tetramers to "move" elements out of sites in a genome. For comparison below, consider the excision of an integrated lambda phage by site-specific recombination (SSR) *versus* the excision of a transposon by non-replicative DNA-only transposition - the mechanism covered in detail in class (TPN).

A. In a genome, the two sites recognized by the recombinase tetramer can have different relative orientations. Indicate the orientation of recombinase binding sites for the SSR AND TPN reactions described above and why the orientation is significant (+4 each). ALSO indicate whether strand cleavages occur within or outside the region of sequence-specific recognition.

SSR orientation:

TPN orientation:

SSR strand cleavage:

TPN strand cleavage:

B. Which strands are rejoined to a new partner by the recombinase enzyme itself? Which are later rejoined by DNA ligase and repair pathways?

SSR:

TPN: