MCB 110
First Exam
A TOTAL OF SIX PAGES

NAME:

Student ID Number:

Question	Maximum Points	Your Points
I	36	
II	35	
III	27	
IV	28	
V	24	
	150	

Please write your name or student ID number on every page.

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

Question I (36 points)

Nucleases disrupt the phosphodiester backbone. Considering the many examples of nuclease function discussed in class, list the indicated number of enzymes with endonuclease activity that disrupt 1, 2, or 4 nucleic acid strands during a biological reaction (9 answers, 1 point each). Note that there are more correct answers than necessary to complete the list, but please ONLY list one enzyme per answer space A-I. No duplicate answers (alternate names for the same type of enzyme). FOR EACH ANSWER, state what features of the substrate are required to recruit the nuclease activity (nucleic acid strands and polarity, any bound protein required AT THE TIME OF NUCLEASE ACTION; 2 points each of 9) AND state whether the nuclease action involves a covalent protein-DNA intermediate (1 point each of 9).

Enzyme:	Features of substrate:	Covalent protein-DNA?

Endonuclease activity that disrupts 1 strand:

A.

B.

C.

D.

Endonuclease activity that disrupts 2 strands:

E.

F.

G.

Endonuclease activity that disrupts 4 strands:

H.

I.

Question II (35 points)

A. Among the factors that specialize a polymerase for its cellular roles, three features include the available template, primer, and pre-acting or pre-loaded protein. For the polymerases listed below, indicate the typical features of template(s), primer(s), and (if present) the directly interacting protein that carried or recruited the polymerase to its typical template-primer substrate (5 answers, 4 points each). For template and primer, simply indicate DNA and/or RNA. For accessory protein, indicate NONE or the name of the interacting protein that would typically (as discussed in class) bring or load the polymerase to the site of its action.

	Template:	Primer:	Interacting protein
1. Pol I			
2. Pol III			
3. Pol alpha			
4. Pol V			
5. Telomerase			

B. DNA polymerases and strand exchange reactions typically require single-stranded DNA to initiate. Different mechanisms can be used to generate the single-stranded region of DNA. For the contexts listed below, describe how single-stranded DNA template is generated (3 answers, 5 points each). Indicate any required enzyme and how this enzyme creates single-stranded DNA.

1. *E. coli oriC* (the origin of DNA replication).

2. A double-stranded DNA break that will be repaired by homologous recombination.

3. A nick at the site of an RNA primer resulting from Okazaki fragment synthesis in *E. coli.*

Question III (27 points)

A. Some DNA binding proteins use cooperative binding to achieve an important requirement of their cellular function. For the examples below, describe what cooperative binding accomplishes that non-cooperative binding could not (3 questions, 4 points each).

1. RecA

2. DnaA

3. *E. coli* single-stranded DNA binding protein (SSB).

B. Diverse enzyme activities can join DNA ends to create an intact phosphodiester backbone. For the examples listed below, describe the features of the backbone ligation/repair reaction including all important specificities of the reaction. Be sure to indicate the specific DNA structure(s) or sequences that will be joined OR the bound protein that **determines the specificity of the reaction** (3 questions, 5 points each).

DNA structure/bound proteins:

1. *E. coli* DNA ligase

2. Human DNA ligase IV

3. Transposase

Question IV (28 points)

For each of 1-4 below, answer A, B, and C:

A. (2 pts) What type of DNA repair will fix this type of error, damage, or break?
B. (3 pts) State one protein **SPECIFIC to THIS repair reaction.** Briefly state its function.
C. (2 pts) How much DNA will be synthesized as part of the specific repair process that you listed in (A)? To make it simple, choose between these options: 0, 1-30, or more than 30 nucleotides.

1. The product of hydrolysis across a glycosidic linkage.
A.

B.

C.

2. A double-stranded DNA break in a high copy number plasmid in *E. coli*.
A.

B.

C.

3. A nucleotide misincorporation by Pol delta (not corrected by proofreading).
A.

B.

C.

4. The product of Pol V synthesis over a pyrimidine dimer in *E. coli*.
A.

B.

C.

Question V (24 points)

A. Homologous recombination involves two distinct types of strand exchange reaction mediated in *E. coli* by RecA or RuvAB. Compare the starting DNA structure requirements and heteroduplex outcomes of these two distinct types of strand exchange reaction to indicate any difference in heteroduplex length or extent of strand complementarity. Whole sentences are not necessary; just fill in a few words after the colons (2 points each first answer, 1 point each other answer = 8 points)

1. RecA
starting structure requirement:

approximate minimal length of heteroduplex generated:

requirement for heteroduplex strand complementarity:

2. RuvAB
starting structure requirement:

approximate length of heteroduplex generated:

requirement for heteroduplex strand complementarity:

B. Explain the (different) use of ATP hydrolysis by RecA versus RuvB. (3 points each = 6 points)

RecA:

RuvB:

C. If homologous recombination (HR) occurred between regions including sites normally bound by a site-specific recombinase (SSR), even if recombinant ends were produced as a consequence of homologous recombination, there is a possible difference in outcome from the HR reaction compared to the SSR reaction. What is it? You can answer in 2 words or a short description (4 points).

D. Transposition and site-specific recombination differ in the consequences of element excision. Indicate which reaction is perfectly reversible (2 points). For the other reaction, what strand cleavage specificities account for the lack of perfect reversibility? (4 points)