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:		
RNase H	RNA:DNA duplex	N
FEN1 (5' flap endonuclease)	5' single-stranded overhang from a duplex	N
AP endonuclease	abasic site OR missing base	N
UvrC (or XP-F/XP-G)	distorted B-form DNA bound by UvrAB (XP-A/D)	N
MutH	dsDNA GATC, non-methylated strand	N
Topoisomerase I (type I topo)	dsDNA with Lk unequal to Lk_o	Y
Endonuclease activity that disrupts 2 strands:		
Restriction endonuclease	specific sequence of dsDNA	N
Topoisomerase II (type II topo)	supercoil OR two dsDNA	Y
RuvC	Holliday junction OR 4-way duplex junction	N
Acceptable alternatives:		
RAG1/2	two signal sequence ends (don't need to say 12/23 spacer combo for full credit)	N
Endonuclease activity that disrupts 4 strands:		
Site-specific recombinase	two SSR binding sites	Y
RAG1/2	two signal sequence ends (don't need to say 12/23 spacer combo for full credit)	N
Transposase (could also disrupt more than 4)	transposon IR end(s) AND target dsDNA end(s)	N
Acceptable alternatives:		
Transposon-encoded Resolvase	binding sites within a duplicated transposon	Y

Note: Depending on how this question was read, RAG1/2 was given as an answer for 2 or 4 strands; no credit for putting it in both!

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	DNA	DNA	None
2. Pol III	DNA	DNA or RNA	beta OR sliding clamp
3. Pol alpha	DNA	RNA (OK to say DNA too; must say RNA)	primase
4. Pol V	damaged DNA	DNA	beta OR sliding clamp (can say Pol III also)
5. Telomerase	RNA	DNA (don't need to say chromosome end repeat)	None

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).

DnaA wraps DNA to favor melting of adjacent region. (note that DnaA is required and as an ATPase is an enzyme, but don't need to decribed the role of ATP here)

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

5'-3' exonuclease activity from the broken dsDNA end generates a single-stranded 3' overhang.

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

Pol I 5'-3' exonuclease activity removes the RNA primer.

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

Answers such as:

Must have a continuous region of strand exchange long enough for the heteroduplex to be stable instead of spontaneously (rapidly) reverting back to the parental duplex. *OR*

Without protein-protein interactions, a sufficient length of DNA bound by RecA would not be held in a conformation that allows strand sampling for homology.

2. DnaA

Answers such as:

DnaA protein-protein interactions change the structure of bound dsDNA, wrapping it in a manner that favors melting of the AT-rich adjacent sequence. *OR*

Need to ensure that only oriC is used as the origin of replication, so need to stabilize bound DnaA by protein-protein as well as protein-DNA interactions.

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

Answers such as:

Need to load SSB onto long but not necessarily short regions of ssDNA template that will need to be replicated by Pol III. *OR*

Need to saturate ssDNA with SSB to prevent (energetically favorable) secondary structure formation *OR* to allow it to be efficiently used as template by Pol III.

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	5' phosphate, 3' OH at a nick
2. Human DNA ligase IV	dsDNA break with Ku bound to each end
3. Transposase	transposon 3' ends joined to target 5' ends

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. Base excision repair (BER)
B. AP endonuclease, cleaves (phosphodiester) backbone
Phosphodiesterase, removes sugar-phosphate backbone unit (without base)
NOTE: not OK to say UDG/UNG as this repair reaction starts downstream of that enyzme
C. 1-30

2. A double-stranded DNA break in a high copy number plasmid in *E. coli*.
A. Homologous recombination
B. RecA, (initial) strand exchange [not the best answer due to RecA* role in SOS response, but acceptable for full credit because RecA* is a different form of the protein]/ RAD51 same answer
RuvA, HJ binding
RuvB, HJ helicase
RuvC, HJ resolvase
Also OK to say "5'-3' exonuclease that acts at the dsDNA end to generate 3' overhang"
C. more than 30

3. 3. A nucleotide misincorporation by Pol delta (not corrected by proofreading).
A. Mismatch repair
B. MutS (or a human paralog name), mispair recognition
MutL (or a human paralog name), can say activates MutS, endonuclease, anything reasonable
NOTE: not OK to say MutH - there isn't a the relevant mechanism of strand discrimination.
C. more than 30

4. The product of Pol V synthesis over a pyrimidine dimer in *E. coli*.
A. Nucleotide excision repair (NER)
B. UvrA, (complex with UvrB) recognizes damage/distorted B-form DNA
UvrB, (complex with UvrA) recognizes damage/distorted B-form DNA
(OK to say UvrAB as one answer too)
UvrC, endonuclease/ nicks damaged strand
NOTE: not OK to say UvrD because it is not unique to NER
C. 1-30

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: ssDNA (at least 50 nt, don't have to say)

approximate minimal length of heteroduplex generated: ~50 bp

requirement for heteroduplex strand complementarity: perfect/absolute

2. RuvAB

starting structure requirement: Holliday junction (HJ)

approximate length of heteroduplex generated: ~1000 bp/one or a few kbp

requirement for heteroduplex strand complementarity: limited mismatch tolerance

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

RecA: ATP hydrolysis allows disassembly of RecA filament/polarity of strand exchange/similar

RuvB: ATP hydrolysis drives strand separation/HJ translocation/other similar answer

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).

Gene conversion/mismatch repair/formation of mispairs/ similar answer

D. Transposition and site-specific recombination differ in the consequences of element excision. Indicate which reaction is perfectly reversible (2 points).
SSR

For the other reaction, what strand cleavage specificities account for the lack of perfect reversibility? (4 points)
Transposase cleaves the target duplex with a 5' staggered cut (and pastes each donor IR 3' end to a target 5' end, leaving a gap on either side of the inserted transposon that is then replicated; it is sufficient to say the first part without the rest of it).