MCB 110
First Exam
A TOTAL OF SIX PAGES

NAME:

Student ID Number:

Question	Maximum Points	Your Points
I	30	
II	30	
III	30	
IV	38	
V	22	
	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 (30 points)

Many enzymes discussed in class function by introducing discontinuity in the DNA phosphodiester backbone. Considering ONLY ENDONUCLEASES, in the spaces provided below, list FIVE examples of an enzyme activity that cleaves only once (makes a nick), THREE examples of an enzyme that cleaves at 2 sites in concerted manner (cleaves two strands), and TWO examples of an enzyme that cleaves at 4 or more sites in concerted manner in the course of one complete reaction (as discussed in class). One word answers are acceptable, either the name of a protein OR the specific type of enzyme, but do not duplicate your answers (do not use the name of the protein as one answer and that enzyme type as a second answer).

A ONE cleavage (a nick):

1. MutH

2. TopoI

3. AP endonuclease

4. UvrC *(makes 2 nicks, on one strand, in a consecutive manner)*

5. RAG1/2 *(each makes one nick on one strand)*

6. FEN1

7. RNaseH

8. The enzyme that cleave hairpins during V(D)J recombination.

B TWO cleavage positions:

1. Topo II

2. restriction enzyme

3. RuvC

4. *UvrC and Rag1/2 were also accepted here.*

C FOUR or more cleavage positions:

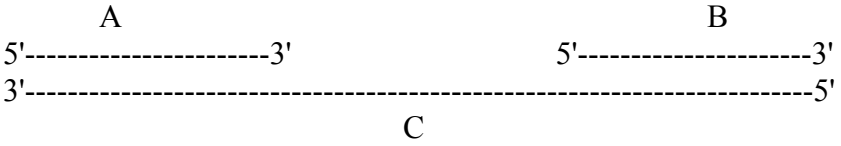
1. site-specific recombinase/ lambda integrase/ Hin invertase

2. transposase

3. *Rag1/2 were also accepted here.*

Question II (30 points)

Consider the three strands of DNA labeled A, B, and C in the illustration below: ---- indicates continuous backbone, there is a central region of C that is single-stranded, all of A and B are base-paired to C. Just to be extra clear, this is a linear DNA with two ends and a central gap.



A. (+16 points total) Based on the specificities described in class, indicate which strand(s) (A, B, and/or C) would be acted on by the following enzymes when they initially encountered the A+B+C annealed DNA substrate.

1. DNA Pol I polymerase domain
A

2. The exonuclease activity specific to DNA Pol I
B

3. The DNA Pol I exonuclease proofreading activity shared by many polymerases
A

4. The exonuclease that processes damaged DNA for loading of Rad51
A and C

B. (+14 points total) Based on the specificities described in class, indicate which junction of double-stranded and single-stranded DNA would be bound by the following protein or protein complex. Use one of two answers: either "A+C" or "B+C" as an indication of the junction at the 3' end of the A strand (this is "A+C") or the junction at the 5' end of the B strand (this is "B+C"). For your answers to this part B, don't concern yourself with the length of the single-stranded region in C (assume that it is long) or whether C has a free 3' end (it does as drawn above); it is the polarity of strands at the junction that matters for giving the correct answer. Each answer is +3 points.
ALSO, indicate the biological function of the protein or protein complex (+2 points) and why binding specificity for one junction polarity matters for this biological function (+2 points). BRIEF answers please; no need for whole sentences.

1. gamma complex
A+C because the 3'OH/5' overhang are where a sliding clamp should be loaded to recruit polymerase

2. BRCA2
B+C because the recessed 5' end/3' overhang (can say free 3' end) should be loaded with Rad51 (OK to say RecA although that is the E. coli protein and BRCA2 is eukaryotic only) {for strand exchange prior to homologous recombination - don't need to include that for full credit}

Question III (30 points)

A. (+18 points total) DnaB loading for genome replication of a circular chromosome requires three events listed below (1-3). For each event, indicate the cooperating protein factor for DnaB (+2 points each). Explain the mechanism by which the cooperating protein factor accomplishes the listed event, including any role of ATP binding and/or ATP hydrolysis (+4 points each).

{don't need to state words in parentheses for full credit}

1. Transient 'opening' of the hexameric DnaB helicase ring

DnaC {hexamer} binds DnaB to change ring conformation {to loadable form }; ATP binding by DnaC promotes DnaB interaction, with ATP hydrolysis required for release of DnaC {full credit for saying both parts about ATP binding AND ATP hydrolysis}

2. Creation of single-stranded DNA at the replication origin

DnaA {cooperative binding} changes DNA structure to promote formation of an adjacent region of single-stranded DNA {at the AT-rich repeats}; ATP binding by DnaA promotes DNA interaction, with ATP hydrolysis required for DNA release

3. DnaB recruitment to/loading on the replication origin single-stranded DNA

DnaA physically interacts with the helicase {DnaBC complex} to recruit/position it for loading on single-stranded DNA; ATP binding by DnaA promotes DNA interaction, with ATP hydrolysis required for DNA release

B. (+12 points total) Helicases accomplish the unfavorable strand separation of a duplex by using the energy of ATP hydrolysis to 'step' or 'track' along one strand of DNA, thereby displacing the other strand. DNA binding proteins other than helicases also can favor duplex strand separation or strand exchange. Among the several examples discussed in class, pick **ONE** example of a protein that is **NOT A HELICASE** and yet accelerates strand separation or strand exchange (+4). Indicate how that protein favors what would otherwise be an unfavorable strand separation or strand exchange, including any role(s) of ATP binding or hydrolysis (+8). A few sentences should be sufficient!

1. RecA. Cooperative protein-protein interaction forces DNA within the RecA filament to become underwound/undertwisted/stretched, which favors sampling of bound single-strand with the complementary polarity strand of the bound duplex.

2. DnaA. Cooperative protein-protein interaction forces a wrapping of DNA around the DnaA multimer {that traps a {positive} supercoil}, favoring melting/unpairing/strand dissociation of the adjacent AT-rich repeats

3. A topoisomerase; by introduction of negative supercoils/negative change in linking number, reduced linking number would favor strand separation. Type II would be the best answer (can move away from Lko), but correct rationale for a type I would also be an acceptable answer for full credit.

Question IV (36 points)

For each pathway of repair listed below, indicate THREE proteins that are SPECIFIC to that repair pathway (+2 each) and the function of that protein in just a few words (+1).

A. Nucleotide excision repair

1. UvrA recognizes/binds site of damage {UvrAB complex}

2. UvrB recognizes/binds site of damage {UvrAB complex}

3. UvrC endonuclease activity

4. any of the mammalian XP proteins are also OK, as long as the right function is given
(UvrD is not acceptible - shared with MR)

B. Mismatch repair

1. MutS recognizes/binds the mispair

2. MutL links MutS to MutH

3. MutH endonuclease {nicks unmethylated A in GATC sequence motif}

4. DAM methylase methylates template strand to differentiate between old and newly synthesized strands.
(UvrD is not acceptible - shared with NER)

C. Base excision repair

1. UDG cleaves glycosidic linkage of a uracil base in DNA

2. AP endonuclease cleaves phosphodiester backbone at an abasic site

3. Phosphodiesterase removes sugar-phosphate unit missing the base

4. Pol I 5'-3' exonuclease activity would be a creative answer - with good rationale, full credit.
(Pol I and ligase are not acceptable - shared with NER and beyond)

D. Homologous recombination

1. RuvA binds Holliday junction

2. RuvB branch migration/creation of heteroduplex/helicase activity

3. RuvC Holliday junction resolution/cleavage; duplex separation

4. RecA {filament} would be an OK answer, as long as the role of RecA* in SOS repair is distinguished (such as by saying RecA filament for this answer or saying "unactivated" RecA)

Question V (22 points)

A. (+12 points) Many mobile elements can be present in a genome. There are dramatic differences in the consequences of excision of a mobile element that moves out of the genome (that excises itself from the donor site) by site-specific recombination (SSR) compared to transposition (TPN). List two differences in the element-flanking DNA ends IMMEDIATELY following excision of a mobile element by SSR versus TPN. Note that this question is NOT ASKING about the fate of the mobile element itself! HINT: the two differences could describe (1) the state of the ends (are there ends?) and (2) same sequence or different sequence of element flanking DNA (repeats?) Few-word answers are anticipated.

1.
SSR: no ends/ligated ends
TPN: blunt ends not ligated

2.
SSR: no direct repeat at junction
TPN: {direct} repeat left behind in element-flanking DNA ends

B. (+10 points) Site-specific recombinase enzymes and transposase enzymes typically function as subunit tetramers. One reason is to have multiple protein-DNA recognition contacts. Provide a second reason for their function as subunit multimers, considering the total number of strand cleavage requirements for a SSR or TPN reaction.

1. site-specific recombinase:
four coordinated strand cleavages by four coordinated active sites

2. transposase:
at least four strand cleavages (at donor and target sites)
sequence-specific recognition requirement is also an OK answer