MCB 110
Spring 2017 Exam 1
SIX PAGES

NAME:

SID Number:

Question	Maximum Points	Your Points
I	28	
II	32	
III	32	
IV	30	
V	28	
	150	

PLEASE WRITE your NAME or SID number on each page.
This exam must be written in PEN if you want the option of a regrade.
DO NOT USE WHITE-OUT: ask for a clean page of exam to start over if you need it.

Question I (28 points)

A. (+16 points) Considering the many examples discussed in class, list 8 enzymes with a the stated nuclease specificities (give 2, 2, or 4 examples respectively, as requested below). Note that there are more correct answers than necessary to complete the list, but list ONLY the requested number of enzymes. Alternate names for the same nuclease activity are not separate answers.

1. Exonuclease activity that disrupts a phosphodiester bond on only one strand of a duplex
Give two answers:
(i)

(ii)

2. Endonuclease activity that acts on undamaged duplex DNA in a sequence-specific manner (other than restriction endonucleases – don't use those in your answers)
Give two answers:
(i)

(ii)

3. Endonuclease activity that is structure-specific but not sequence-specific (OK to list a nuclease that needs to be recruited by another protein to its DNA cleavage site)
Give four answers:
(i)

(ii)

(iii)

(iv)

B. (+6 points) Would each of these proteins or protein complexes bind a 5' AND/OR 3' single-stranded overhang junction with duplex DNA? Use only these answers: 5', 3', both, neither.

1. Clamp loader

2. BRCA2

3. DnaB

C. (+ 6) List three proteins or protein complexes that can slide on DNA without complete dissociation. Include at least one single-stranded and one double-stranded DNA binding factor. OK to include proteins that slide with or without ATP involvement.

1. Double-stranded DNA

2. Single-stranded DNA

3. Your choice double-stranded or single-stranded

Question II (32 points)

A. (+16 points) In class, some DNA polymerases were described that did NOT act in canonical DNA replication (i.e. they do not synthesize primer or replicate an entire leading or lagging strand). Name FOUR, and for EACH indicate general cellular FUNCTION. You can count as separate answers an *E.coli* polymerase and a eukaryotic polymerase with same general cellular function.

1.

2.

3.

4.

B. (+16 points) Diverse enzymes join DNA ends to (re)create an intact phosphodiester backbone. For each example below, answer the questions about the backbone ligation/repair reaction.

1. *E. coli* DNA ligase

(i) What are the 5' and 3' chemical groups required for DNA ends to be joined by ligase activity?

(ii) Describe the use of ATP.

2. Ligase IV

(i) What is one of the accessory proteins or protein complexes that recruits this ligase to DNA?

(ii) What is this ligase's specificity for DNA that is distinct from *E. coli* DNA ligase?

3. Topoisomerase type I

(i) By what number(s) and in what direction(s) will linking number be changed by this enzyme?

(ii) Why is ATP unnecessary for the reaction?

4. *E. coli* DNA gyrase (a type II topoisomerase that works ahead of the replication fork)

(i) By what number(s) and in what direction(s) will linking number be changed by this enzyme?

(ii) Why does replication fork progression require DNA gyrase activity?

Question III (32 points)

A. (+20 points) Proteins bind DNA with sequence and structure specificity and impose changes in DNA conformation upon binding. For EACH factor listed below, answer THREE questions:

1. What is structure and/or sequence **of DNA** that protein will recognize for initial binding? Answer for the DNA BEFORE it is bound to the protein.
2. How is **DNA structure** changed by enzyme **binding**? Answer for protein-DNA complex, not anything subsequent to enzyme release of bound DNA.
3. What provides the favorability for change in DNA structure induced by protein binding?

(a) DNA adenosine methyltransferase (Dam1)

1.

2.

3.

(b) A DNA helicase

1.

2.

3.

(c) DnaA

1.

2.

3.

(d) RecA

1.

2.

3.

B. (+12 points) DnaB loading at *oriC* requires two additional proteins. Describe all of the roles of those two proteins at *oriC* and the roles of ATP binding and hydrolysis for each.

1.

2.

Question IV (30 points)

For each of 1-5 below, give answers for A and B:

A. (+2 points) Give ONE example of a form of DNA damage that will be fixed by the listed type of DNA repair. Be as specific as necessary in description of the DNA substrate to make the substrate *most suited to this pathway compared to any other repair pathway covered in class*.

B. (+4 points) State one protein or its specialized enzyme activity (i.e. "nuclease" is not a sufficient answer) SPECIFIC for ONLY this repair pathway, AND in one sentence describe the function/activity of that protein.

1. Mismatch repair

A.

B.

2. Nucleotide excision repair

A.

B.

3. Base excision repair

A.

B.

4. Homologous recombination

A.

B.

5. Classical NHEJ

A.

B.

Question V (28 points)

A. (+ 14 points) Strand exchange reactions in *E. coli* homologous recombination are mediated by many proteins including RecA and RuvB. For each protein, answer EACH of the THREE questions below.

1. What is the exchanged strand(s) length? As answers use only 0, 5, 50, or 1000 base-pairs.
2. What DNA structure does the protein bind? Include any important element of DNA structure AND any other DNA-interacting proteins required for recruitment.
3. Describe the roles of protein ATP binding and ATP hydrolysis.

(a) RecA

1.

2.

3.

(b) RuvB

1.

2.

3.

B. Site-specific recombination

1. (+3 points) Each reaction has 100% probability of recombinant ends. How does the catalysis of SSR ensure this specificity? One sentence could be a sufficient answer.

2. (+3 points) Why is there no requirement for DNA ligase? Only need one sentence to answer.

C. Transposition

1. (+4 points) Transposase makes double-strand cuts at the edges of the transposon donor DNA. Are these cuts blunt (even), 3' overhang, or 5' overhang? What implication does this have for the transposon-excised donor DNA?

2. (+4 points) Transposase makes a double-strand cut in the target DNA. Is the cut blunt, 3' overhang, or 5' overhang? What implication does this have for the transposon-flanking target DNA?