A TOTAL OF SIX PAGES

NAME:

STUDENT ID:

Question	Maximum Points	Your Points
I	36	
II	32	
III	26	
IV	28	
V	28	
	150	

Please write your **name** and **student ID** number at the top of every page.

Note that this exam must be written in **INK** if you want the option of a regrade. DO NOT USE WHITE-OUT. Do not write on the back of a page. You can ask for a fresh copy of the exam if you run out of space due to answer revisions.

NAME: **Student ID#:**

Question I (36 points)

Numerous enzymes act on a phosphodiester bond in the DNA backbone to change DNA structure.

A. Some enzymes that act on DNA form a covalent protein-DNA intermediate.
(a) (3 pts) Considering just the phosphodiester bond that is cleaved, what does formation of the COVALENT intermediate preserve that simple hydrolysis of the phosphodiester backbone does not?

Bond energy/ DNA topology or linking number

(b) (18 pts) Indicate as (1) and (2) below **two types of enzyme** that form covalent linkage to the DNA backbone (a specific name is OK or a type of enzyme is OK to list, but it is not OK to give two different names for the same enzyme). Note that there could be more than two correct answers. **For each of (1) and (2) answer the following questions (i) and (ii):**
(i) Considering the enzyme function described in class, how many subunits of an active enzyme form a covalent protein-DNA intermediate? In other words, how many individual DNA strand cleavages occur through a covalent protein-DNA intermediate?
(ii) How is covalent linkage essential for the specificity of DNA rearrangement? Answer with reference to the *FINAL PRODUCTS* of the enzyme reaction, not the intermediate. Very short answers are sufficient, if they account for the specificity of enzyme function.

1. Site-specific recombinase/ lambda integrase ("integrase" alone is not sufficiently descriptive)
(i) four
(ii) 100% chance of recombinant ends

2. Type I topoisomerase
(i) one
(i) change linking number by 1 (OK to say oblige specific change in linking number without "1")

3. Type II topoisomerase/ gyrase/ TopoIV
(i) two
(i) change linking number by 2 (OK to say oblige specific change in linking number without "2")

B. (15 pts) Several endonucleases were described that cleave the backbone of B-form double-stranded DNA *without* a covalent protein-DNA intermediate. Indicate as (1) (2) and (3) below **three different enzymes** that act as an endonuclease on what is initially B-form duplex DNA (no DNA damage or junctions - but anything that is regular B-form is included). There are more than three correct answers. **For each of (1)-(3),** considering enzyme function described in class, how many individual DNA strand cleavages does the enzyme perform to generate *FINAL PRODUCTS*?

1. Restriction enzyme 2
2. MutH 1
3. Transposase 6
4. RAG1/2 (or just RAG or something similar) 4

NAME: **Student ID#:**

Question II (32 points)

Numerous proteins affect DNA without any disruption of a phosphodiester bond.

A. (14 pts) Helicases use the energy of ATP hydrolysis to step forward on their bound DNA. This ATP-dependent "translocation" on DNA can generate different products and have many different biological functions. For the two helicases listed below, indicate (i) how the helicase changes the structure of product DNA, (ii) a biological function of this helicase, and (iii) an interacting protein necessary to give the helicase your indicated biological function.

1. DnaB
(i) duplex to single-stranded
(ii) replication fork movement/ genome replication
(iii) DNA polymerase III/ DnaB/ DnaC/ clamp loader/ primase (need to answer only one) DnaA

2. RuvB
(i) create heteroduplex/ exchange parental dsDNAs to heteroduplex dsDNAs/ duplex to duplex
(ii) branch migration/ homologous recombination
(iii) RuvA (not OK to say RuvC)

B. (18 pts) Cooperative protein-DNA interaction or DNA binding by a multimeric complex can alter DNA structure in a manner dependent on the protein-protein interactions. **For each of the three DNA binding proteins below, answer questions (i) (ii) and (iii).**
(i) Is there is a role for ATP binding and/or hydrolysis? (+1)
(ii) What is the change in DNA structure induced directly by protein binding? (+3)
(iii) Why is this change in structure important for the protein's biological function (there could be many answers to this; indicating just one function of the protein is sufficient)? (+2)

1. RecA filament
(i) Yes/ ATP binding increases affinity for DNA, ATP hydrolysis decreases affinity
(ii) Unwinding/ stretching/ reduced stacking/ similar answers
(iii) Necessary to promote homology sampling/ to allow strand exchange

2. DnaA filament
(i) Yes/ ATP binding increases affinity for DNA, ATP hydrolysis decreases affinity
(ii) Positive supercoil/DNA wrapping (only partial credit for AT-rich repeat melting, but full credit for answering only positive supercoiling or giving both answers)
(iii) Favor melting of adjacent AT-rich DNA/ allow loading of helicase

3. Histone complex (eight tightly interacting histone subunits)
(i) No
(ii) Negative supercoil (OK to just say supercoil)
(iii) DNA compaction/ dissociation can promote DNA melting/ other answers possible

NAME: **Student ID#:**

Question III (26 points)

A. (16 pts) DNA polymerases have properties that specialize them for distinct biological functions.
(i) What is one biological function of this polymerase?
(ii) What is the polymerase processivity? Pick one of these options: 2-5, 5-200, or >200 nucleotides.
(iii) List one cellular factor **bound to the protein or protein complex DURING ELONGATION** that is required for its recruitment and/or activity and/or processivity.

1. DNA polymerase III
(i) genome replication/ mismatch repair/ double-strand break repair
(ii) >200
(iii) sliding clamp/beta, (the questions asks for a protein bound DURING ELONGATION)

2. DNA polymerase V
(i) SOS repair/ error-prone repair/ rescue stalled Pol III/ bypass lesion in front of stalled Pol III
(ii) 2-5
(iii) sliding clamp/beta

3. DNA polymerase alpha
(i) Extend RNA primer [to give replicative polymerases [Pol delta/epsilon] a B-form DNA substrate]
(ii) 5-200
(iii) primase

4. Telomerase reverse transcriptase protein
(i) telomere synthesis/ end replication
(ii) 5-200
(iii) telomerase RNA

B. (10 pts) DNA ligases also have different properties. For the two DNA ligases listed below, indicate what requirements the protein has to act on its DNA substrate. Answer specifically for DNA structure AND whether additional protein factors are required (Yes or No is sufficient answer for the latter).

1. DNA ligase (bacteria) or DNA ligase I (eukaryotes)
Nick in DNA. [NO additional protein factors required]

2. DNA ligase IV (eukaryotes)
Break in DNA/ duplex ends. Requires recruitment factors [Ku, but don't need to say this explicitly]

NAME: **Student ID#:**

Question IV (28 points)

For each of 1-4 below, give answers for A-C:
A. What pathway of repair removes this DNA damage?
B. 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.
C. Indicate EVERY nuclease activity required for repair of this DNA lesion - you can answer either the name of the protein(s) or enough detail about biochemical activity to specify unique function. If answering by function, make sure to include exo versus endo nuclease activity. Important: consider nuclease activity to be defined as breaking a phosphodiester bond.

1. Uracil in DNA
A. Base excision repair/BER
B. 1
C. AP endonuclease AND phosphodiesterase
Pol I is not a great answer if "1" nucleotide was indicated above, but at least partial credit given because it has 5'-3' exonuclease activity that could replace phosphodiesterase
WRONG answer: UDG, uracil DNA glycosylase/glycosidase; this hydrolyzes a glycosidic linkage, not the phosphodiester backbone

2. Pyrimidine intrastrand dimer
A. Nucleotide excision repair/NER
B. 2-40
C. UvrC (nicks twice, but don't need to say this)/ XP-F and XP-G

3. A guanosine paired with thymidine
A. Mismatch repair/MR
B. more than 40
C. MutH (bacteria) or MutL (eukaryotes) - OK to say either without explanation
AND single-stranded DNA exonuclease (can say 5'-3' OR 3'-5' OR don't specify OR say both) or say explicitly ExoVII, ExoX, ExoI AND/OR RecJ (these are labeled on the lecture slides but were not discussed in detail)

4. A double-stranded DNA break in cells that do not have NHEJ
A. homologous recombination/HR
B. more than 40
C. 5'-3' exonuclease that acts on double-stranded DNA
AND RuvC for Holliday junction resolution (nicks twice, but don't need to say this)

NAME: **Student ID#:**

Question V (28 points)

A. (20 pts) V(D)J recombination DNA rearrangements are fundamental for our adaptive immunity. The recombinase RAG1/2 complex evolved from a transposase. **For each of (1) and (2) below, answer (i) (ii) and (iii).** Assume transposition by the cut-and-paste mechanism covered in class.
(i) What DNA sequence features does the active enzyme complex recognize directly in the course of one reaction cycle? Here and below, **answer for only one V-D-J junction join**, not the entire process of antibody locus rearrangements, and answer for the **entire process of cut-and-paste transposition**.
(ii) Neither process is fully reversible in the cell. Briefly explain why.
(iii) Name another enzyme that must also act to complete the process

1. RAG1/2 complex
(i) **Inverted repeats** [heptamer/nonamer sequences] with **different spacer lengths**/ recombination signal sequences [RSSs] with 12 and 23 bp spacer
(ii) Sequence between the RSSs is deleted/ RSSs deleted in the reaction/ mutagenic or sloppy joining
(iii) NHEJ machinery (Ku, DNA ligase IV - NOT sufficient to say just "ligase")/ polymerase activity/ hairpin opening enzyme/
Full credit but not ideal answer since not *essential* for the junction joining: TdT or terminal [deoxynucleotidyl] transferase

2. Transposase
(i) **Inverted repeats [flanking the transposon]** AND **non-specific duplex** [at the target site]
(ii) Direct repeat is left behind at donor DNA site/ direct repeat is introduced at the target site
(iii) DNA polymerase, ligase (can say just "ligase" or DNA ligase I), NHEJ machinery (Ku, DNA ligase IV) OR homologous recombination machinery [to repair the donor site]

B. (8 pts) A DNA intermediate that is a Holliday junction forms during both homologous recombination (HR) and site-specific recombination (SSR).

1. What is a Holliday junction?
four-way dsDNA junction (OK to just draw this) [involving two homologous duplex DNAs]

2. What protein is bound to the HR Holliday junction?
RuvA

3. What protein is bound to the SSR Holliday junction?
The recombinase/ site-specific recombinase/ lambda integrase