

MCB 110 SP 2005
Second Midterm

ANSWER KEY

Question	Maximum Points	Your Points
I	40	
II	40	
III	36	
IV	34	
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	150	

NOTE: This is my answer key (KC). The graders were given disgression in changing this scoring, if the change was applied uniformly.

Question I (40 points)

For each type of nuclease in A-D below, provide TWO examples of an enzyme with the appropriate specificity (note: there may be many possible examples but CHOOSE ONLY TWO).

+1 name; +3 name and partial answer with nothing wrong; +5 complete answer nothing wrong

A. DNA sequence-specific nuclease

MutH: binds dsDNA at GATC sequence, nicks unmethylated strand of hemimethylated site

Restriction endonuclease: binds dsDNA, cleaves/nicks/cuts both strands

Site-specific recombinase: binds two OR paired recognition sites, makes two staggered cuts OR sequentially nicks both W strands then both C strands

Transposase: binds two terminal inverted repeats, makes two blunt cuts/breaks/nicks (any OK) at outer boundary of terminal inverted repeats

B. DNA structure-specific nuclease without DNA sequence specificity

RuvC: binds Holliday junction, makes 2 symmetric nicks (both W or both C strands)

RnaseH: binds DNA-RNA duplex, nicks (“cuts” OK) RNA strand of DNA:RNA duplex

AP (apurinic, apyrimidinic) endonuclease: binds adjacent to a missing base in dsDNA, nicks adjacent to the missing base ONLY on the damaged strand

DNA pol I 5'-3' exonuclease: binds at gap/3' overhang, nicks the strand with 5'-3' polarity

Proofreading 3'-5' exonuclease: binds preferentially mispaired 3' end of primer-template duplex, nicks the primer strand/strand with 3'-5' polarity

C. DNA endonucleases recruited ONLY by a specific DNA-bound protein

UvrB, UvrC (XP-F, XP-G): binds dsDNA already bound by UvrA (XP-A), nicks damaged DNA strand

D. DNA endonucleases that can cut double-stranded DNA at a site lacking specific sequence or structure

Transposase when it cleaves the target site (MUST say target site because donor-site cut is sequence-specific): binds random dsDNA only when bound to cleaved transposon ends, makes staggered pair of nicks (or break)

Retrotransposon or retroviral integrase: binds random dsDNA only when bound to cleaved transposon/retrovirus ends, makes staggered pair of nicks (or break)

OK to say DNaseI or “nuclease for nick translation”: binds dsDNA, introduces a nick

Type II topoisomerase or gyrase: binds dsDNA, introduces a transient DNA break by forming a covalent protein-DNA intermediate

Question II (40 points)

Intent on making an important contribution to forensic science, you decide to discover a new polymerase for PCR that has better processivity than the DNA pol I-like enzymes that are used currently. You obtain previously uncharacterized bacterial cultures from various sources, produce protein from cell extracts and test for DNA polymerase activity using a short DNA primer annealed to a several kb long single-stranded DNA template.

A. (8 pts)

Magnesium or Mg^{++} AND 5' triphosphate deoxynucleotides (+4 each, no partial credit)

B.

(1) (6 pts)

Polymerase is purified in association with a sliding clamp (+3) which can load on DNA end but not DNA circle (+3)

(2) (11 pts)

Sliding clamp loader (+4). Allow reaction +/- polymerase-depleted extract to proceed part way through replication of the template, recover DNA from the reaction. Clamp should be trapped on circular but not linear template. An acceptable but less ideal answer could say that the new factor should require ATP, so ATP depletion should inhibit replication on circular template WITHOUT inhibition on linear template (+7).

C. (15 pts)

Because both T4 SSB and RecA assist the reaction, their role is likely not protein-specific. Instead, they could remove secondary structure from the linear ssDNA template by binding to ssDNA and then dissociating (+5). *E. coli* SSB might not dissociate without *E. coli* DNA pol III. RecA needs ATP to bind DNA (+5) and ATP hydrolysis to release (+5).

Question III (36 points)

For the DNA repair substrates shown below in each of (A) and (B), describe

- (1) The name of the repair pathway that will fix the damage (3 pts)
- (2) The FIRST protein in the pathway that initially recognizes this damage (4 pts)
- (3) ALL of the activities of this FIRST protein (2) that are useful in directing efficient repair (5 pts)

A. -----G-----
 -----U-----

- (1) **BER or base excision repair**
- (2) **Uracil (DNA) glycosylase**
- (3) **“Removes uracil” OR “cleaves glycosidic linkage” OR “cleaves between uracil and sugar”**

B. -----T[^]T----- A cyclobutane thymine dimer: ANSWER for the repair pathway shared by
 -----AA----- both eukaryotes and prokaryotes

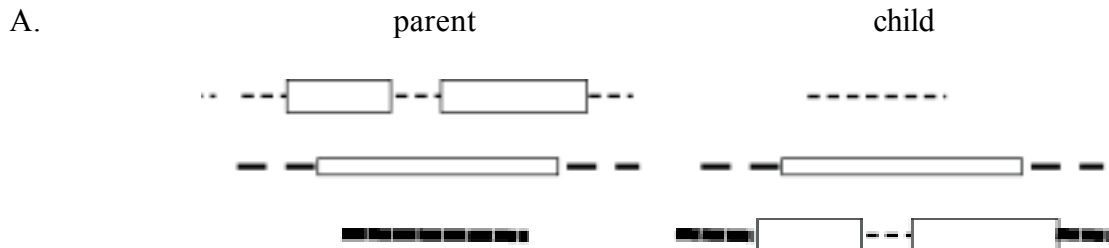
- (1) **NER or nucleotide (oligonucleotide) excision repair**
- (2) **UvrA (full credit for “the binding protein that recognizes bulky adduct distortion of dsDNA”)**
- (3) **Recruits UvrB AND UvrC OR “recruits endonucleases to nick damaged strand on both sides of damage”**

C. -----^{6me}A-----
 ----- C -----

- (1) **MR or mismatch repair (6meA is a methyl mark that DOES NOT distort dsDNA structure)**
- (2) **MutS (“the protein that binds a mispair of normal bases” is also OK)**
- (3) **Recruits MutL to activate MutH (or its eukaryotic equivalent)**

Question IV (34 points)

You are studying a guinea pig family with unusual hair pigmentation. You have tracked the basis for pigment variability to particular regions on three different chromosomes depicted below (chromosome 1, 2, 3 from top to bottom). By sequencing these regions from different animals, you notice two different genome rearrangement events shown in A and B below (from “parent” to “child”). In each of A and B, (1) provide TWO different mechanisms that could have resulted in the genome rearrangement (7 pts) and (2) describe how the DNA sequencing of the regions would discriminate between the two mechanisms (10 pts).

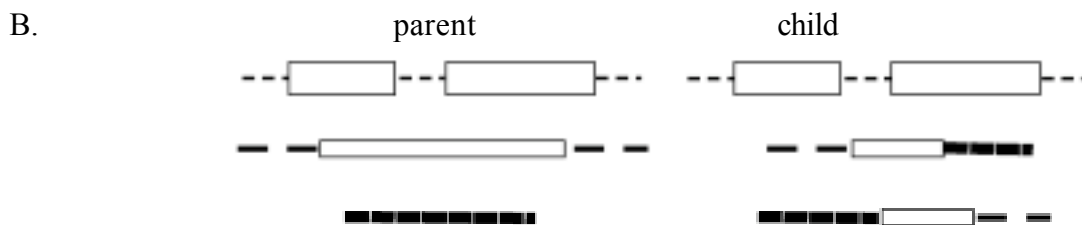


(1) Two mechanisms:

“transposition OR site-specific recombination” (both by excision and reintegration; don’t need to state this though)

(2) Sequence differences:

transposition will introduce a short direct repeat at the target site in chromosome 3; site-specific recombination will not introduce any extra DNA sequence OR transposition will be evidenced by inverted repeats at the edges of the DNA region moved while site-specific recombination will have a direct repeat of sequence at the junctions of the region moved and the same sequence at the site of the new insertion



(1) Two mechanisms:

“site-specific or homologous (general) recombination” (both with recombinant ends; don’t need to state this though)

(2) Sequence differences:

Site-specific recombination will be marked by a short region of similar sequence that allows recombinase binding and very short region of perfect homology for limited strand exchange; homologous recombination will require >50 bp of PERFECT complementarity (Or, although this is an inferior answer, site-specific recombination will occur without gene conversion while homologous recombination could allow nearly gene conversion)