

MCB 110 SP 2005
Second Midterm
THERE ARE FOUR QUESTIONS (FIVE PAGES)

NAME:

ID Number:

Question	Maximum Points	Your Points
I	40	
II	40	
III	36	
IV	34	
	<hr/>	
	150	

FINAL SCORE:

Your discussion section TA's name:

NOTE: If you think that you might like us to consider a regrade, this exam must be written in PEN.

Question I (40 points)

Nucleases play critical roles in DNA replication, repair and rearrangement. For each type of nuclease specificity described by A-D below, provide TWO examples of an enzyme discussed in class with the appropriate specificity (note: there may be many possible examples but CHOOSE ONLY TWO). FOR EACH EXAMPLE, indicate any important features of cleavage reaction specificity and intermediates.

A. DNA sequence-specific nuclease

(1)

(2)

B. DNA structure-specific nuclease without DNA sequence specificity

(1)

(2)

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

(1)

(2)

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

(1)

(2)

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. (6 pts) Aside from cellular proteins and primer-template, what other two compounds do you add to the assay? Be as specific as necessary in your answer.

B. You discover a highly processive polymerase activity in the crude extract and purify it. You test different primer-template combinations. To your surprise, a single-stranded circular DNA template annealed with a short DNA primer does not allow highly processive elongation. As a control, you initiate the reaction on the linear template then rapidly circularize it – and elongation is highly processive.

(1) (6 pts) Provide a mechanism for the observed differences in processivity based on DNA replication factors described in class.

(2) (13 pts) You use your assay conditions in (A) with single-stranded circular DNA template annealed with a short DNA primer, using purified polymerase AND adding back a crude cell depleted for the polymerase. You discover that crude extract contains an accessory factor that allows purified polymerase to use the circular template while retaining activity on the linear template. What is this new factor? Provide an experimental test to confirm the activity of your proposed new factor.

C. (15 pts) You add *E. coli* SSB to this reaction, thinking that it might stimulate the rate of DNA synthesis. It does not. You add T4 bacteriophage SSB to this reaction, and it does stimulate DNA synthesis. RecA also stimulates the rate of DNA synthesis in the presence of ATP but not in the presence of a non-hydrolyzable ATP analog. What might RecA be doing to promote DNA synthesis, why does it need ATP, and why does the non-hydrolyzable ATP analog inhibit?

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)
- (2)
- (3)

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

- (1)
- (2)
- (3)

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

- (1)
- (2)
- (3)

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 DNA sequencing of the regions would discriminate between the two mechanisms (10 pts).

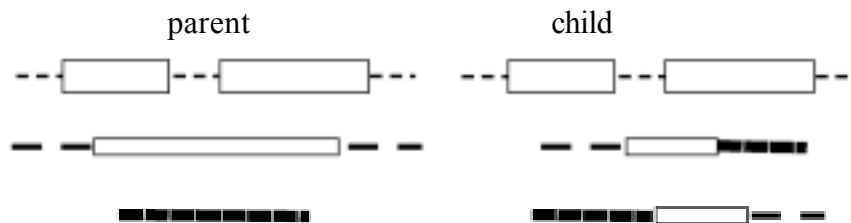
A.



(1) Two mechanisms:

(2) Sequence differences:

B.



(1) Two mechanisms:

(2) Sequence differences: