KEY **Name:**

Gene Action 411 Winter, 2009 Exam 2

1. (12 points) You repeat Experiment 3 ("Isolation of Mutator Strains") exactly as we did it in lab, plating your culture samples on MinGlu + PG + Xgal, as before. This time, however, you also plate several samples of the culture onto MinLac + PG + Xgal as well.

Would you expect the total number of colonies growing on your MinLac plates to be the same as, greater than, or fewer than the number growing on the MinGlu plates? Briefly explain your answer. (≤ 20 words)

Fewer. Only those lacZ- → lacZ+ revertants arising from the initial mutagenesis will be able to grow on MinLac.

Would you expect the percentage of solid blue colonies appearing on your MinLac plates to be the same as, greater than, or fewer than those growing on the MinGlu plates? Briefly explain your answer. (≤ 20 words)

Greater. Because only lacZ+ cells can grow on MinLac, all colonies will be solid blue.

Would you expect the percentage of identifiable mutator colonies appearing on your MinLac plates to be the same as, greater than, or fewer than those growing on the MinGlu plates? Briefly explain your answer. (≤ 20 words)

Fewer. Because cells are already lacZ+, there's no mutation that will confer a growth advantage, meaning no papillae will form.

2. (12 pts) Some cytosine residues in DNA are methylated to 5-methyl cytosine, which base pairs with guanine. Such sites are hotspots for mutation due to spontaneous deamination, which converts 5-methyl cytosine into thymine. The immediate product of deamination at such a site is a G:T base pair, a mismatch. Why is the mismatch repair system not helpful in preventing mutations due to 5-methyl cytosine deamination? (≤25 words)

The deamination will occur on both old and new strands, usually long after dam methylation. Mismatch repair thus cannot distinguish which base is "wrong".

KEY

3. (18 pts) Diagram the <u>most common</u> series of events leading to increased mutation for the mutator strains listed below. The events leading to increased G:C → T:A mutations in mutM⁻ mutants are shown. Show only the DNA which becomes mutated, use the fewest steps possible, and label the steps ("ox" = oxidation; "rep" = replication; "cdm", cytosine deamination; "dpr", depurination; "MutY" = MutY action; "MutM" = MutM action; "MutT" = MutT action). (Note: "O" = 8-oxoguanine.) (Remember that no repair system is 100% efficient.)

mutM⁻

—AC— / —TG— —ox→ —AC— / —TO— —rep→ —AA— / —TO— —rep→ —AA— / —TT—

3 pts / correct answer

2 pts if pathway is correct but steps are mislabeled

0-1 pts for incorrect, rare, or non-mutagenic events

a. mutT⁻

—AC— / —TG— —rep→ -AC- / -OG- —mutY→ -CC- / -OG- —mutM (or rep)→ -C C- / -G G-

b. mutY⁻

—AC— / —TG— —ox→ -AC- / -TO- —rep→ -AA- / -TO- —rep→ -AA- / -TT-

c. mutT⁻ mutY⁻

—AC— / —TG— —rep→ -AC- / -OG- —rep→ CG / OG —mutM→ -CC- / -GG-

d. ung⁻ (uracil N-glycosidase-minus)

—AC— / —TG— —cdm→ -AU- / -TG- —rep→ -AU- / -TA- —rep→ -AT- / -TA-

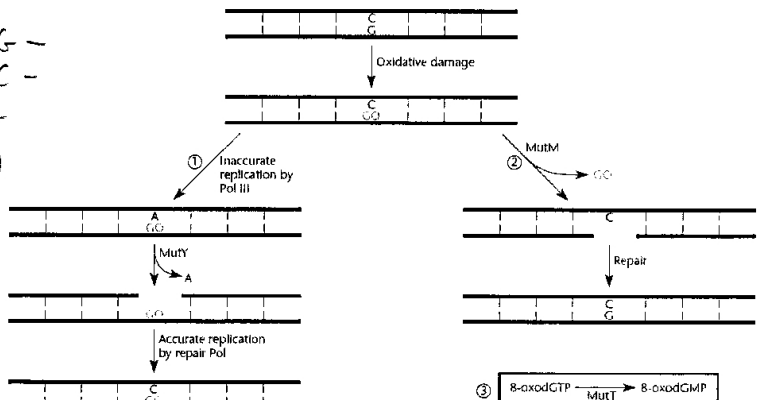
e. lexA⁻ (sulA⁻)

—A— / —T— —dpr→ - / -T- —rep→ -T- / -T- —rep→ -T- / -A-

f. mutS⁻ mutH⁻

—A— / —T— —rep→ -G- / -T- —rep→ -G- / -C-

(pairing of G to evol. T at first replication step)



4. (21 pts) For each of the cases below (A-G), circle the number corresponding to the appropriate answer (1, 2 or 3). For example, for A., if $recA^+$ cells show higher survival after UV exposure than $recA^-$ cells, circle "1". Unless stated otherwise, assume the cells in question are grown in the presence of oxygen in the dark, that all genes and functions not explicitly referred to are wild-type, and that mutations eliminate all functions of the genes affected, e.g., that a $mutT^-$ mutation eliminates all MutT function. (Hints: The mutation frequency after UV exposure is determined by measuring the proportion of cells surviving exposure that express a particular mutant phenotype. SulA is the cell division inhibitor induced as part of the SOS response.)

Mutant phenotype:	Cells that are:		
A. Higher cell survival after UV exposure	1. $recA^+$	2. $recA^-$	3. Neither
B. Higher spontaneous mutation rate	1. $lexA^+$ $sulA^+$	2. $lexA^-$ $sulA^-$	3. Neither
C. Higher spontaneous mutation rate	1. $mutS^-$ $mutH^+$	2. $mutS^-$ $mutH^-$	3. Neither
D. Higher spontaneous mutation rate	1. $mutT^-$ $mutY^+$	2. $mutT^-$ $mutY^-$	3. Neither
E. Higher spontaneous mutation rate	1. error-prone $repair^+$	2. error-prone $repair^-$	3. Neither
F. Higher mutation rate after UV exposure	1. error-prone $repair^+$	2. error-prone $repair^-$	3. Neither
G. Higher cell survival after UV	1. error-prone $repair^+$	2. error-prone $repair^-$	3. Neither

5. (10 pts) There are four repair functions which help cells survive DNA damage caused by ultraviolet irradiation:

1. Photoreactivation

2. Excision repair

3. Recombinational repair

4. Error-prone repair

There exist bacterial viruses with single-stranded DNA genomes. When the single-stranded DNA enters a cell, it is copied into double-stranded DNA as a first step in infection. The double-stranded DNA is eventually used to generate single-stranded DNA for packaging into virus particles, which are released from cells. Imagine that such a single-stranded DNA virus is exposed to UV light prior to infection. Which two of the four repair functions listed above would you expect to be most important for allowing virus survival after irradiation? Circle the appropriate numbers above. (Assume that all repair functions are constitutively active in cells.)

6. (12 pts) Imagine a mutagenic compound which causes a low rate of cleavage of thymine residues from DNA (analogous to spontaneous loss of purine residues ("depurination"), except that it's induced by the compound). Which of the following base pair changes would be increased by treatment with the compound of *E. coli* cells already induced for the SOS response? (Circle your choices.) (Hint: Assume that induction of the SOS response without treatment solely increases A:T–>T:A and G:C–>T:A mutations.)

a. G:C —> T:A

b. A:T —> G:C

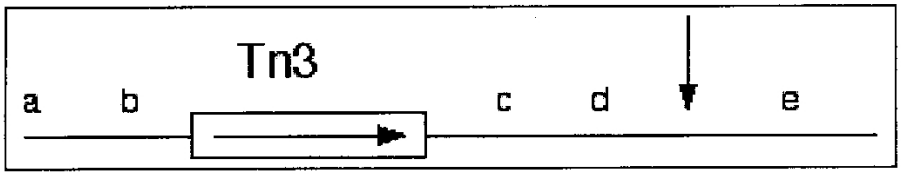
c. G:C —> C:G

d. A:T —> C:G

e. A:T —> T:A

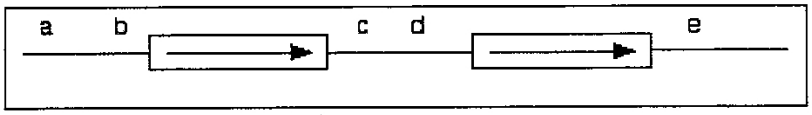
f. None of the above

7. (15 pts). Assume the Tn3 shown in the diagram below transposes to the site indicated by the arrow between d and e. Assume TnpA and TnpR are 100% efficient when active and that resolvase (if present) acts once per transposition event. Assume the diagram represents part of a circular DNA.



a. Draw the structures corresponding to each of the two insertion alternatives for wild-type Tn3. (The first alternative is provided.)

1:



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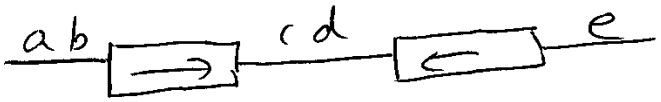
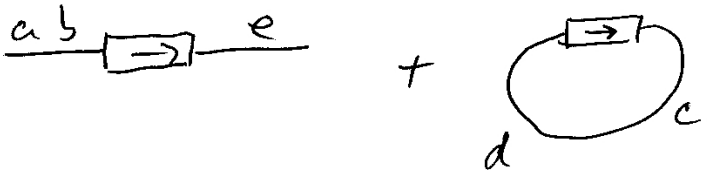


b. Draw the structures corresponding to each of the two insertion alternatives for resolvase–minus (=tnpR$^-$) Tn3.

1:



2: