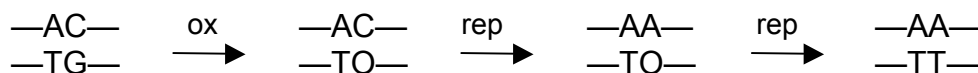


1. (20 pts) Diagram the most common series of events leading to increased mutation for the mutator strains listed below. The events leading to increased G:C → T:A mutations in mutM⁻ mutants are shown. Show only the DNA which becomes mutated, use the fewest steps possible, and label the steps (“ox” = oxidation; “rep” = replication; “rpr” = repair; “MutY” = MutY action; “MutM” = MutM action; “MutT” = MutT action). (Note: “O” = 8-oxoguanine.) (Remember that no repair system is 100% efficient.)

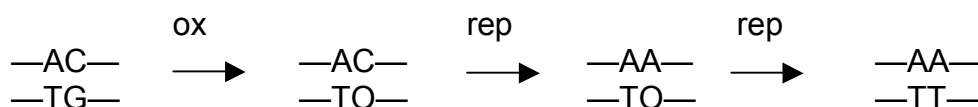
mutM⁻



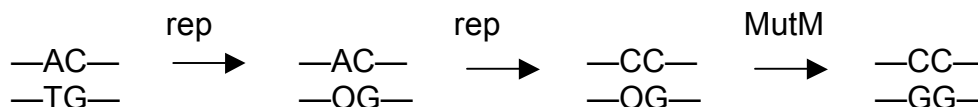
a. mutT⁻



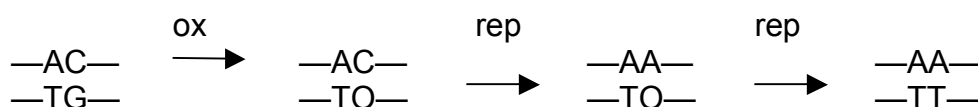
b. mutY⁻



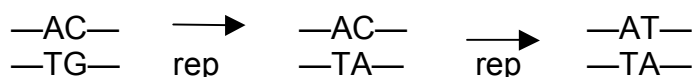
c. mutT⁻ mutY⁻



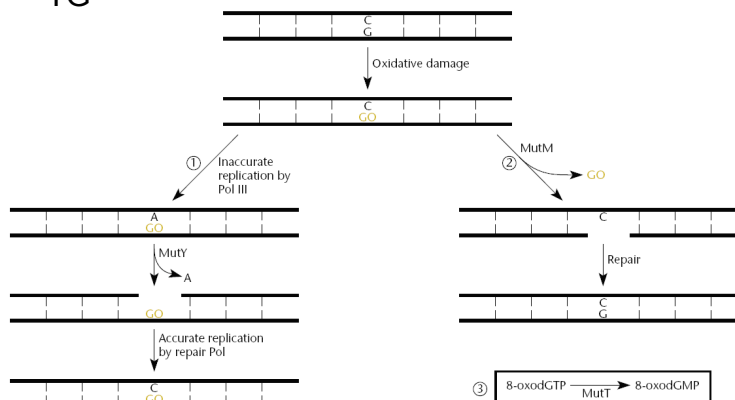
d. mutM⁻ mutY⁻



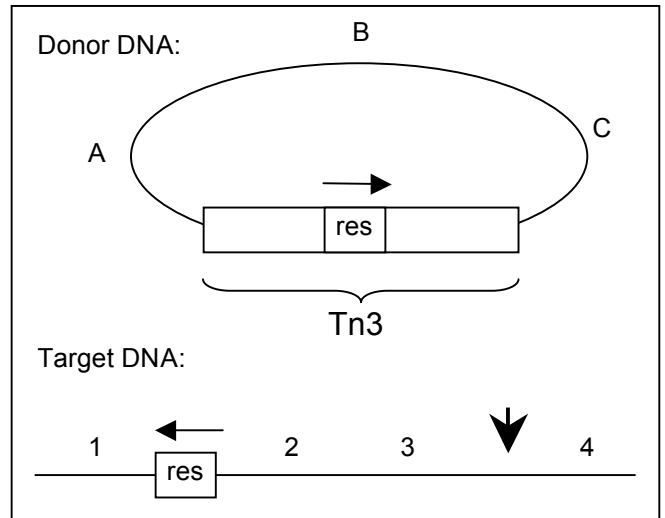
e. mutS⁻ (G:C → A:T change)



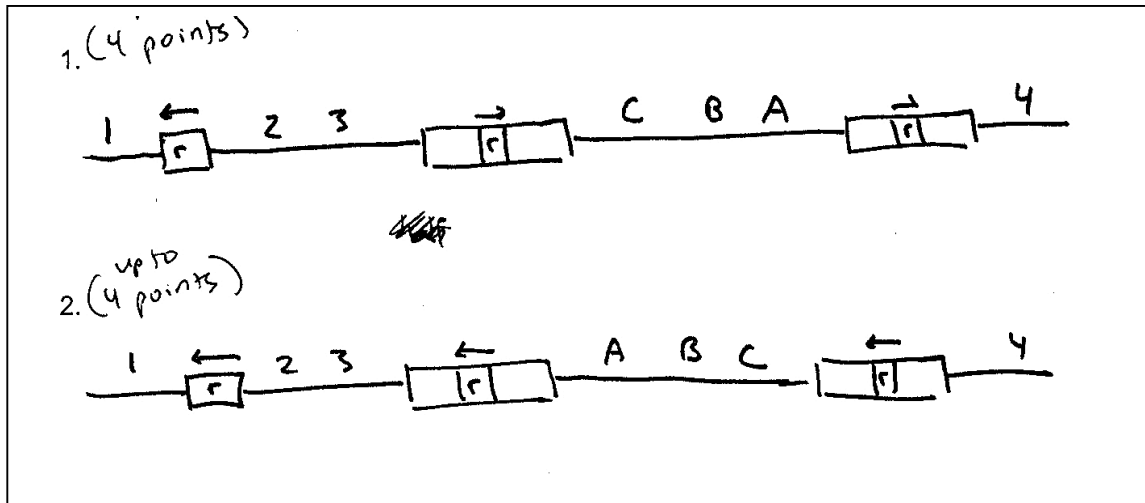
or

$$\begin{array}{c} \text{---AT---} \\ \text{---TG---} \end{array}$$


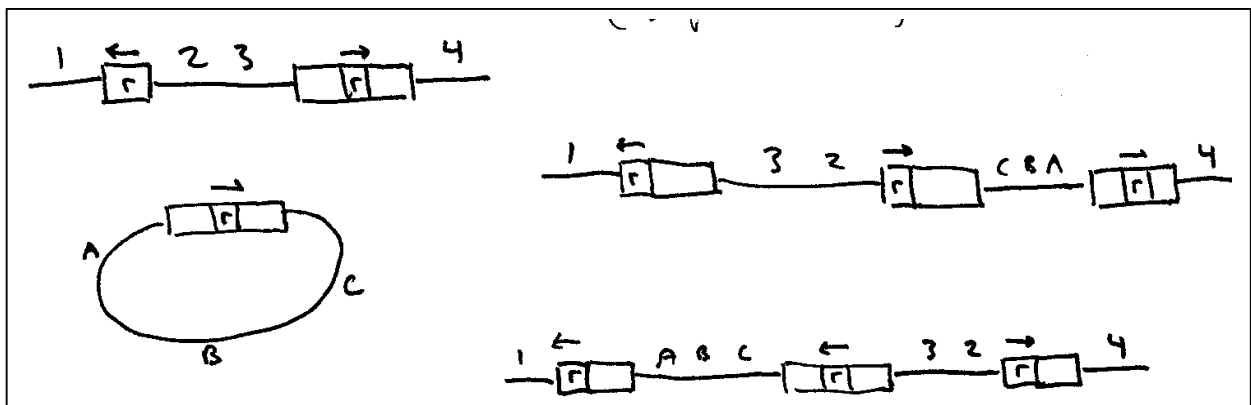
2. (20 pts) Imagine a donor DNA molecule carrying the replicative transposon Tn3 and a linear recipient molecule carrying a Tn3 res site (but no other Tn3 sequences) as shown at the right.



a. A single transposase (TnpA)-mediated event joins the two DNAs at the site indicated by the vertical arrow. The joining can occur in either of two orientations. Draw the two possible joined molecules, showing res site orientations and DNA labels (A-C, 1-4).



b. The TnpA-mediated joining is followed by a single TnpR-mediated event at res sites chosen at random. For the first of the molecules you drew above (1.), draw all possible products which could result from these events, regardless of whether the products can persist in the cell.



Name: _____

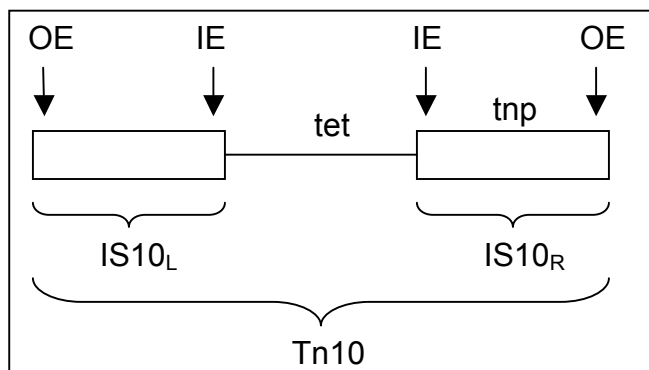
3. (20 pts) For each of the following mutant strains, indicate whether the phenotype indicated would be increased, decreased or unchanged compared to wild-type cells (circle one for each). One answer is given as an example.

Mutant genotype	Phenotype	Expected observation
Photoreactivation ⁻	Frequency of pyrimidine dimers after UV exposure	increased decreased unchanged
<i>dam</i> ⁻	Survival after exposure to the base analog 2-aminopurine	increased decreased unchanged
<i>dam</i> ⁻ <i>mutS</i> ⁻	Survival after exposure to the base analog 2-aminopurine	increased decreased unchanged
<i>dam</i> ⁻ <i>mutH</i> ⁻	Mutation rate after exposure to the base analog 2-aminopurine	increased decreased unchanged
<i>mutT</i> ⁻	T:A → G:C transversion frequency	increased decreased unchanged
<i>mutM</i> ⁻	T:A → G:C transversion frequency	increased decreased unchanged
<i>lexA</i> ⁻	Spontaneous A:T → T:A transversion frequency at depurination* sites	increased decreased unchanged
<i>lexA</i> (noninducible)	Cell division inhibition after UV exposure	increased decreased unchanged
<i>recA</i> ⁻	G:C → T:A transversion frequency at depurination sites after UV exposure	increased decreased unchanged
<i>recA</i> ⁻ <i>lexA</i> ⁻	G:C → T:A transversion frequency at depurination sites after UV exposure	increased decreased unchanged
<i>lexA</i> ⁻	<i>recA</i> gene expression	increased decreased unchanged

*Depurination is the spontaneous loss of purine residues from DNA.

4. (16 pts) Imagine that you isolate a number of Tn10 derivatives and examine their transposition properties. You identify mutants which may be grouped according to the table below. (A score of “+” means that the event occurs at the same or higher rate than wild type.) (Hint: Recall that an IE does not function in transposition with an OE from a different IS element.)

Mutant group	Tn10 transposition	IS10 _L transposition	IS10 _R transposition	Mutation frequency of adjacent gene increased by Tn10
Group 1	+	+	+	+
Group 2	+	+	-	-
Group 3	-	-	-	-
Group 4	-	-	+	+



Consider the sites represented on the Tn10 diagrammed to the left. (“IE” = inside end; “OE” = outside end)

- IS10_L OE transposase binding site
- IS10_L IE transposase binding site
- IS10_R OE transposase binding site
- IS10_R IE transposase binding site
- IS10_L IE Dam methylation site
- IS10_R IE Dam methylation site
- Transposase gene (*tnp*)

Which of the individual sites (a-g) could carry mutations corresponding to each mutant group? Assume mutations can only inactivate the sites (for example, methylation site mutations cannot be methylated). (List all individual possibilities consistent with the phenotype, for example, “Group X: a, b, c”.)

Group 1: e, f

Group 2: d

Group 3: g

Group 4: a

Name: _____

5. (8 pts) Provide two examples from lecture of site-specific recombination (<10 words each).

1. (Replicative) transposon resolution
Flagellar phase variation
2. Phage λ integration
Phage Mu resolution
Cytomic island insertion

6. (16 pts) The phage vector λ Tn/lacZ/in (from our Experiment 4) was used to perform a transposon mutagenesis of a wild-type *E. coli* strain. Following infection with the λ Tn/lacZ/in vector, cells were plated on minimal phenyl-galactoside agar containing chloramphenicol and Xgal (= MinPG + Cm + Xgal). After 48 hours of incubation, a few hundred colonies appeared, all of which were dark blue.

a. Why were no white colonies obtained by this procedure? (≤ 20 words)

Growth on PG requires a functional and active lacZ gene; white colonies are lacZ, so they won't appear.

b. What are two different types of insertion mutations that could give rise to these blue colonies? (Note: Tn vs IS transpositions do NOT constitute two different types of mutations.) (≤ 20 words each)

1. A gene (translational) fusion ~~#~~ with any highly-expressed gene that produces a fusion protein with β -galactosidase activity
2. An insertion that inactivated the lacI gene.