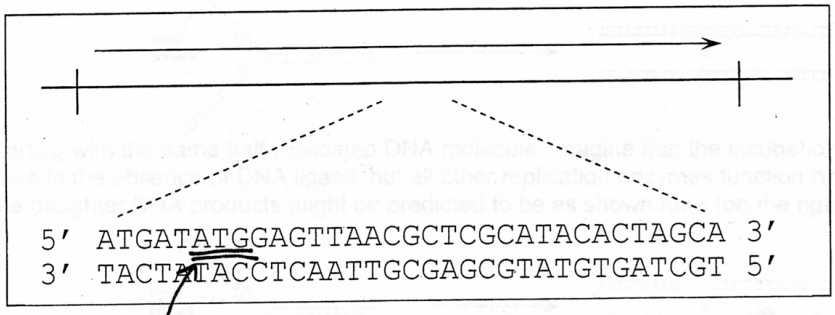
Gene Action Exam 1 Writer Name: Doris Luk

1. (17 pts) Imagine that the following DNA sequence comes from the middle of the wild-type *lacI* open reading frame, as indicated (the arrow shows the direction of transcription and translation of the gene).



a. What amino acid sequence does this DNA sequence encode? (Diagram the codons and the corresponding amino acid sequence below.)

8

-8 correct reading frame (-2, start at ATG)
+3 translation

GAU AUG GAG UUA ACG CUC GCA UAC ACU AGU
Asp Met Glu Leu Thr Leu Ala Tyr Thr Ser

T:A→G:C
C:G→G:C
T:A→G:C
C:G→A:T
C:G→G:C
A:T→C:G

+1 what codons

9

b. On your sequence above, circle the codons which can be converted into codons for arginine by single base pair changes in the DNA, and write the base pair change necessary below each.

If started wrong

AUG AUA UGG AGU
Met Ile Trp Ser

T:A→G:C
T:A→C:G
T:A→G:C → A:T
T:A→A:T → G:C

+5
-1 each wrong/unidentified

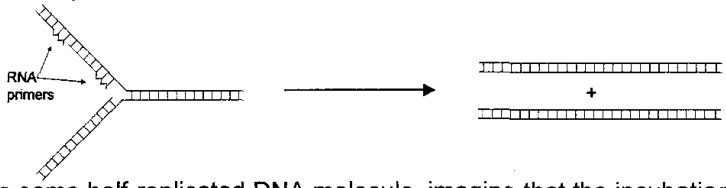
(following same pt distribution as for correct reading frame)

Second Position

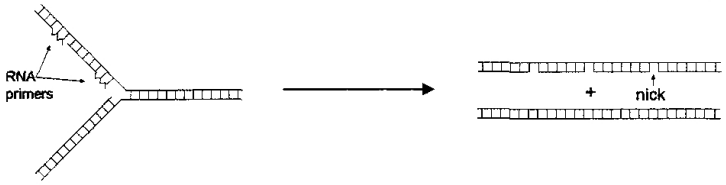
First Position (5′ end)	U		C		A		G		Third Position (3′ end)
U	UUU Phe		UCU Ser		UAU Tyr		UGU Cys		U
	UUC Phe		UCC Ser		UAC Tyr		UGC Cys		C
	UUA Leu		UCA Ser		UAA Stop		UGA Stop		A
	UUG Leu		UCG Ser		UAG Stop		UGG Trp		G
C	CUU Leu		CCU Pro		CAU His		CGU Arg		U
	CUC Leu		CCC Pro		CAC His		CGC Arg		C
	CUA Leu		CCA Pro		CAA Gln		CGA Arg		A
	CUG Leu		CCG Pro		CAG Gln		CGG Arg		G
A	AUU Ile		ACU Thr		AAU Asn		AGU Ser		U
	AUC Ile		ACC Thr		AAC Asn		AGC Ser		C
	AUA Ile		ACA Thr		AAA Lys		AGA Arg		A
	AUG Met		ACG Thr		AAG Lys		AGG Arg		G
G	GUU Val		GCU Ala		GAU Asp		GGG Gly		U
	GUC Val		GCC Ala		GAC Asp		GGC Gly		C
	GUA Val		GCA Ala		GAA Glu		GGA Gly		A
	GUG Val		GCG Ala		GAG Glu		GGG Gly		G

Larry

2. (15 pts) Imagine a bacterial cell with a linear double-stranded DNA molecule that is half-replicated. The cell is incubated just long enough to allow replication to be completed, generating two daughter DNA molecules, as shown here:



Starting with the same half-replicated DNA molecule, imagine that the incubation takes place in the absence of DNA ligase, but all other replication enzymes function normally. The daughter DNA products might be predicted to be as shown here (on the right):

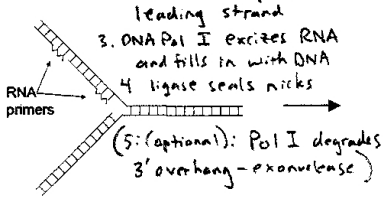


For each of the following functions, predict how the daughter DNA products would look in the absence of the function listed (Draw the products you would expect). In each case, except for the one function listed, assume that all the other replication proteins are present and can act normally.

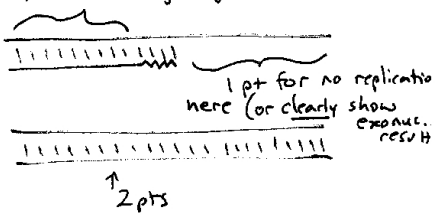
a. DNA primase — 5 pts

1. Helicase unwinds strands
2. DNA Pol III completes leading strand
3. DNA Pol I excises RNA and fills in with DNA
4. ligase seals nicks

(5: (optional): Pol I degrades 3' overhang - exonuclease)



1 pt for filling in (Pol I)
1 pt for sealing (Ligase)



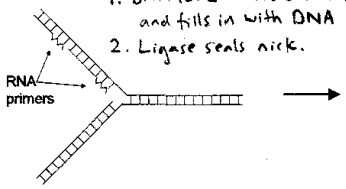
1 pt for no replication here (or clearly show exonuc. result)

↑ 2 pts

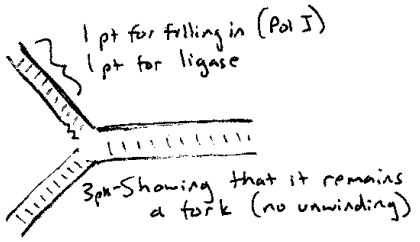
b. DNA helicase — 5 pts

(no unwinding)

1. DNA Pol I excises RNA and fills in with DNA
2. Ligase seals nick.



1 pt for filling in (Pol I)
1 pt for ligase

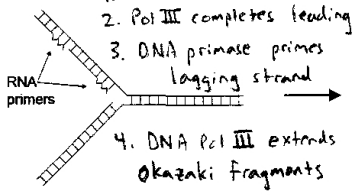


3pts-Showing that it remains a fork (no unwinding)

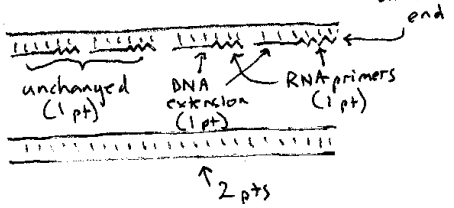
c. DNA polymerase I — 5 pts

1. Helicase unwinds strands
2. Pol III completes leading strand
3. DNA primase primes lagging strand
4. DNA Pol III extends Okazaki fragments

(RNA remains; nicks remain)



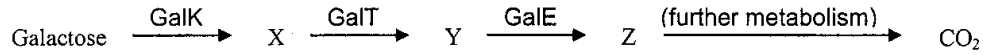
must have an RNA primer at this end



↑ 2 pts

Name: Erin

3. (20 pts) In *E. coli*, metabolism of the sugar galactose is carried out by the enzymes encoded by the *galK*, *galT* and *galE* genes, as shown below. The letters X, Y and Z represent intermediate metabolites in the pathway.



Cells with mutations in *galE* lyse (i.e., cannot grow) in the presence of galactose because the absense of functional GalE enzyme permits accumulation of intermediates X and Y, which are toxic to cells in high concentrations.

Imagine you have isolated a mutant strain with an amber mutation in *galE*. The strain has a complete wild-type *lac* operon (i.e., its lac genotype is *lac*$^+$). Recall that β-galactosidase cleaves lactose to yield galactose and glucose.

You mutagenize the strain and plate it on media that contains lactose and glycerol (an alternative carbon source which does not require *lac* or *gal* genes and which does not affect their expression). Colonies appear.

a. Name <u>two</u> mutations in the *lac* region that could lead to the observed colonies. (<10 words) LacZ$^-$ LacY$^-$ LacO$^-$ LacI^S
(+2) or (+2) or (+2) or (+2) (+4 total)

b. Describe one type of mutation within the *galE* gene that could lead to the observed colonies. (<10 words)
reversion to wild type or single bp change of amber codon (supression) (+4 total)

c. Describe one type of mutation outside of both the *lac* region and the *galE* gene that could lead to the observed colonies. (<10 words)
GalK$^-$ or Amber tRNA supressor (+4 total)
(+4) (+4)

d. If you plate the mutagenized strain on media that contains IPTG, P-Gal (phenyl-β-D-galactoside) and glycerol (no lactose), name the most likely mutation within the *lac* region that will lead to growth. (<10 words) (+4 total)
LacZ$^-$ (+4) LacP$^-$ or LacI^S are less likely and worth (+1)

e. If the *lac* operon used a positive "logic" of regulation, and if the strain was plated as in question (d), name the <u>two</u> most likely types of mutations within the *lac* region that would lead to growth. (<10 words)
LacI$^-$ LacZ$^-$
(+2) (+2)
LacO$^-$ or Δlac (also redundant)
are less likely and worth (1/2 pnt)
(+4 total)

Kendall

4. (13 pts) Imagine a growth condition in which there is no spontaneous deamination of cytosine residues. Under this condition, the most common type of spontaneous DNA change is conversion of adenine in DNA into a non-standard base (call it "X") which base-pairs with cytosine.

+4 a. What would the most common type of spontaneous base pair change leading to mutation be (write the predicted base pair change)?

A:T → G:C +2 for AT → XC

$\left(\frac{A}{T} \rightarrow \frac{X}{T} \rightarrow \frac{X}{C} \rightarrow \frac{G}{C}\right)$

+2 b. Is the base-pair change listed a transition or a transversion?

transition

+7 c. You isolate a mutant in which the frequency of mutations due to the A→X change is greatly increased at all adenine residues. What (hypothetical) function might be defective in the mutant? (<20 words)

The enzyme (similar to ung) that recognizes X as a non-standard base & removes it.

(Because the A → X change is spontaneous, it will occur in double-stranded DNA. It's thus independent of DNA synthesis, meaning that proofreading repair is irrelevant.)

+2 for answers proposing loss of essential processes (like translation) due to excessive AT → GC transitions, as these would be lethal defects, making isolation of the mutant unlikely.

Name: MARK

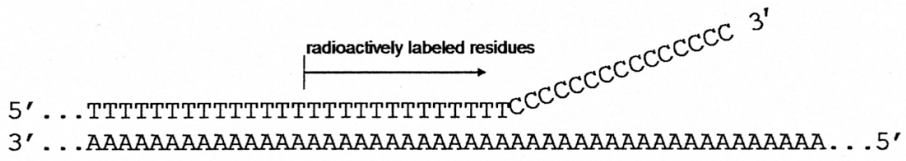
5. (20 pts) The table below lists the diploid genotypes for stable F' strains that carry two copies of the *lac* operon – one on the chromosome and one on the F' factor. For each diploid genotype, indicate in the first column whether active β-galactosidase can be made if lactose is present (write Yes or No); in the second column, indicate whether synthesis of β-galactosidase activity is inducible, constitutive or neither (write I, C or N); in the third column, indicate whether synthesis of lactose permease activity is inducible, constitutive or neither (write I, C or N); and, in the fourth column, indicate whether or not the strain could grow with lactose as the sole carbon source (write Yes or No). Assume that no recombination or additional mutation takes place. Four answers are provided as examples. *I*, *O*, *Z* and *Y* are used for *lacI*, *lacO*, *lacZ*, *lacY*, for simplicity. I^S indicates lac superrepressor, which is non-responsive to lactose.

Diploid genotype	Can active β-galactosidase be made if lactose is present (Yes or No)?	Is synthesis of β-galactosidase activity inducible (I), constitutive (C) or neither (N)?	Is synthesis of lac permease activity inducible (I), constitutive (C) or neither (N)?	Can the cell grow on lactose as the sole carbon source (Yes or No)?	
$I^+ Z^- Y^+ / I^+ Z^+ Y^-$	Yes	I	I	Yes	+2
$I^+ Z^- Y^+ / I^+ O^C Z^- Y^+$	No	N	C	NO	+3
$I^+ Z^+ Y^+ / I^- O^C Z^+ Y^-$	Yes	C	I	Yes	+3
$I^- Z^+ Y^- / I^- Z^+ Y^+$	Yes	C	C	Yes	+3
$I^- Z^+ Y^+ / I^+ O^C Z^- Y^+$	Yes	I	C	Yes	+3
$I^S Z^+ Y^- / I^- Z^- Y^+$	No	N	N	No	+3
$I^+ O^C Z^- Y^+ / I^S Z^+ Y^+$	No	N	C	No	+3

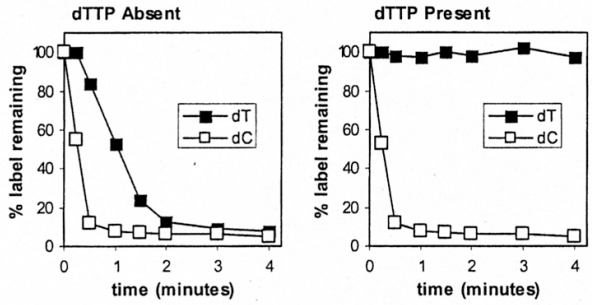
20

KEY INGRID

6. (15 pts) Purified DNA polymerase I from various *E. coli* strains is incubated in the presence of the following DNA substrate in which some of the T and C residues are radioactively labeled, as shown:

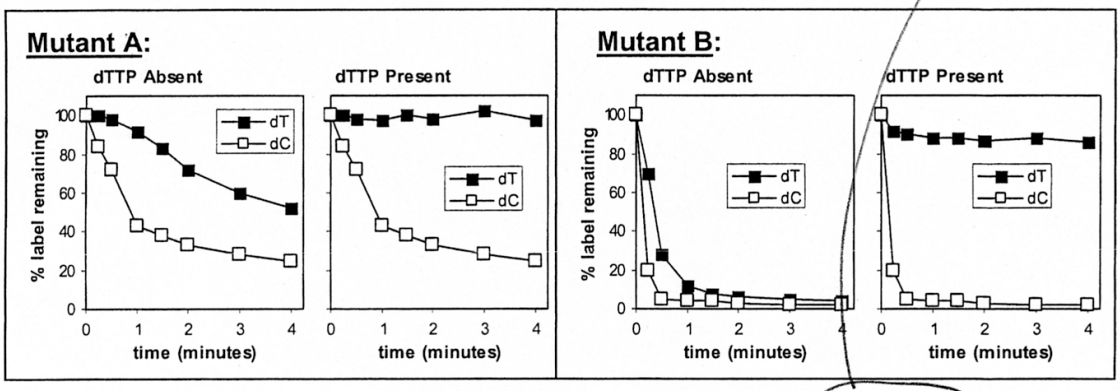


You measure the loss of labeled dT and dC residues over time either without any dTTP present (no DNA synthesis is possible), or with dTTP present (DNA synthesis can occur). Enzyme purified from wild-type cells yields the following results:



Remember to keep your answers short.

Enzymes purified from two different mutant strains, however, yield these results:



a. What activity is affected in mutant A and how is it affected? (≤10 words)

DNA pol I "proofreading" ("or 3'→5' exonuclease") not as efficient as wild type

b. What activity is affected in mutant B and how is it affected? (≤10 words)

DNA pol I proofreading is more (too?) efficient (removes bases more efficiently, even correct ones).

c. What growth phenotype would you predict for mutant B? (≤10 words)

Slower growth due to time/energy used in "excessive" proofreading