

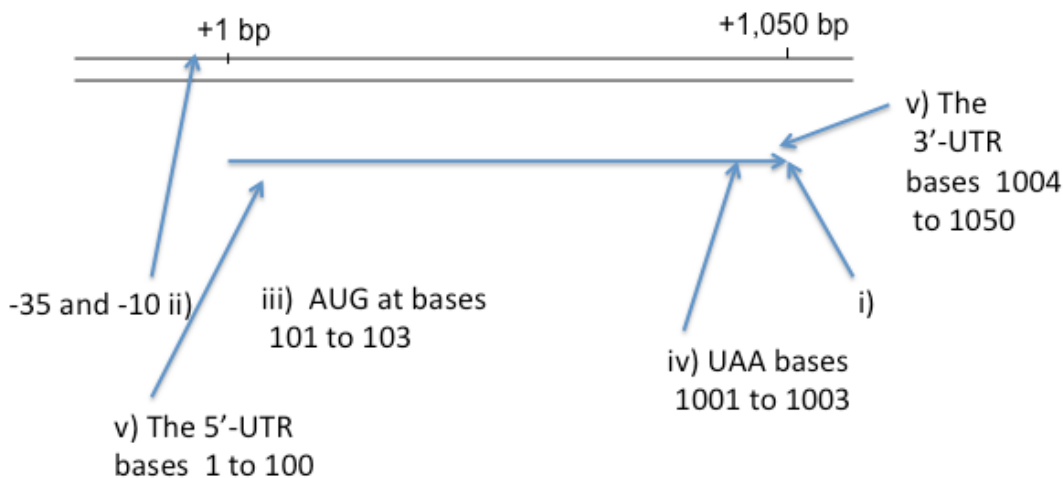
STUDENT NAME _____		STUDENT ID# _____					
COURSE	Biology	NUMBER	367	SECTION	Winter 2015/16		
EXAMINATION	Midterm	DATE	March 1, 2016	TIME	11:45 - 1:00	# OF PAGES	5
INSTRUCTOR		Reg Storms					
MATERIALS ALLOWED		Yes, notes and textbooks					
CALCULATORS ALLOWED		Yes					
SPECIAL INSTRUCTIONS: Answer all questions on the exam sheet. Total marks 50. Materials allowed (notes, textbooks and calculator). Number of pages 5.							

1. (10 marks) The diagram below represents a region of double stranded DNA that harbours an *Escherichia coli* gene.

a. (1 mark) Draw the gene's transcript just below the diagram.

Assume the following: "+1 bp" indicates the transcription start site, "+1,050 bp" indicates the transcription termination site, that the top strand in the diagram represents the gene's nontemplate DNA strand, and that the 5'-UTR (untranslated region) is 100 bases and that the first residue of the stop codon TAA is at bp 1,001.

b. (5 marks) Please label your diagram indicating the following information. **i)** The 3' end of the transcript. **ii)** The promoter elements making sure you indicate their location on the nontemplate strand. **iii)** The position of the translation start site (AUG). **iv)** The position of the translation stop site (UAA). **v)** The two UTRs on the mRNA. **vi)** The 3' end of the template strand.



b. (4 marks) How long in amino acid residues is the protein expressed from this gene? **Answer: 300 amino acids**

2. (10 marks) a. (6 marks) Wild type *E. coli* expresses 1.0 unit of β -galactosidase when grown with glycerol as the sole carbon source. Also, an *E. coli* strain with an i^- mutation expressed about 1,400 units when grown with glycerol as the carbon source.

How much β -galactosidase would you expect the following strains to express in medium with glycerol as the sole carbon source and IPTG and in medium with glucose as the sole carbon source? Note: IPTG acts as an inducer of the lac operon but does not require the lac permease for entry into cells and is not metabolized.

The binding site for CAP-cAMP is designated "ac". The lac operon promoter sequence is designated "p". The transcription activator CAP-cAMP is essential for the recruitment of RNA polymerase to the lac promoter.

Strain # and strain genotype	Glycerol and IPTG	Glucose
1. $i^+ ac^+ p^+ o^+ z^+ y^+$	1400 units	< 1.0 units probably about 0.05
2. $i^- ac^+ p^+ o^+ z^+ y^+$	1400 units	< 2 units
3. $i^s ac^+ p^+ o^+ z^+ y^+$	1.0 units	< 1.0 units
4. $i^s ac^+ p^+ o^c z^+ y^+$	1400 units	< 2 units
5. $i^d ac^- p^+ o^+ z^+ y^+$	< 30 units	< 30 units
6. $i^+ ac^+ p^+ o^+ z^+ y^-$	1400 units	< 1.0 units
7. $i^+ ac^+ p^+ o^+ z^+ y^+ / i^- ac^+ p^+ o^+ z^+ y^+$	1400 to 2800 units	< 2.0 units
8. $i^+ ac^+ p^+ o^+ z^+ y^+ / i^s ac^+ p^+ o^+ z^+ y^+$	1.0 to 2 units	< 2 units
9. $i^+ ac^+ p^+ o^+ z^+ y^+ / i^d ac^+ p^+ o^+ z^+ y^+$	1400 to 2800 units	< 4 units
10. $i^+ ac^- p^u o^+ z^+ y^- / i^+ ac^- p^+ o^c z^+ y^+$	1400 units	< 4 units

b. (4 marks) Which of the above strains would not be able to grow with lactose as the sole carbon source? Indicate the strains using the strain numbers from the left column.

Answer: Strain 3, 5, 6, 8, and 10

3. (10 marks) You decide to perform dideoxy sequencing on a PCR product. You add the appropriate primer (5'-GATCA-3'), DNA polymerase, DNA template, buffer, dNTPs (one of them, dGTP, radioactive), and a small amount of one of the four ddNTPs to each of four reaction tubes. After separating the reaction products by running a polyacrylamide gel and performing autoradiography, the following image was obtained. Lanes are labelled according to the ddNTPs added. When answering this question assume that the smallest fragment seen on the gel is 100 nucleotides long and contains about 25 A, C, G and T residues. Also assume that the fragments that generated the autoradiogram bands go from largest to smallest as you go from the top to the bottom of the figure.



a. (4 marks) What is the DNA sequence as determined from the above autoradiogram? Label the 5' and 3' ends.

Answer: 5' -ATCGGAGCACATT- 3'

b. (2 marks) Suppose that you added 10 times as much ddTTP to the reaction used for lane 4 (the "T" lane). What effect would this have on the banding pattern?

Answer: The "T" lane bands (banding pattern) would be the same but the bands themselves would be much weaker because the DNA polymerization reactions (elongation) would have terminated at a much higher frequency during synthesis/replication of the 100 bases between the primer and the sequences that appeared on the gel. Also, if bands still appeared on the autoradiogram the bands corresponding to the shorter DNA molecules on the gel would be more intense (same specific activity/per base of synthesis but more molecules due to more early termination events) relative to the longer molecules.

c. (2 marks) What is the sequence of the 4 nucleotides at the 3' end of the molecule that generated the fourth band from the top of the gel (the band that migrated the fourth shortest distance noting that during electrophoresis the positive electrode was at the bottom of the gel used to generate the above image)?

Answer: 5'- GCAC-3' where the 3' residues is a dideoxy CMP

d. (1 marks) What would you expect to see if you prepared a reaction using a nucleotide mix containing only dATP, dCTP, dGTP, dTTP (assume that one of the dNTPs was radioactive)?

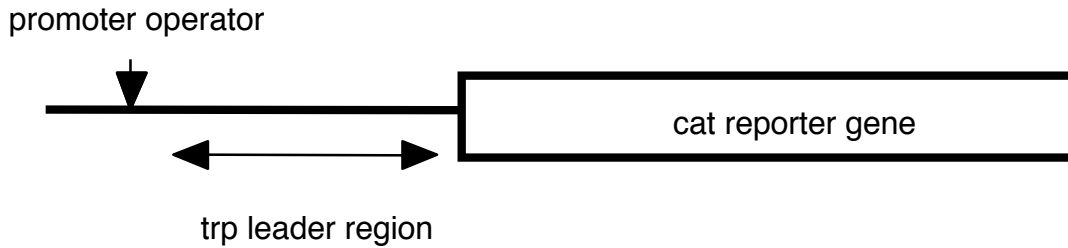
Answer: A dark band at the top of the autoradiogram. It would be generated by large extension product that would correspond approximately to the product of the synthesis rate of the DNA polymerase and the extension time of the reaction.

f. (1 marks) What is the nucleotide at the 3' end of the DNA molecule that generated the bottom band in the C lane?

Answer: Dideoxy CMP

4. (10 marks) (10 marks) The tryptophan operon of *Escherichia coli* is subject to regulation by attenuation (10-fold) and transcriptional control (70-fold). The chloramphenicol acetyl transferase (*cat*) reporter gene shown in the figure below was cloned into a plasmid vector and the plasmid transformed into the recently discovered *E. coli* strain. The transformed strain expresses about 350 units of chloramphenicol acetyl transferase during growth in medium without tryptophan.

Figure of reporter gene:



How much *cat* activity will the combinations of *E. coli* strains and mutated reporter genes express under the growth conditions indicated?

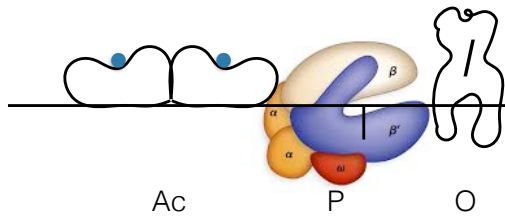
Genotype of <i>E. coli</i> strain	trp- <i>cat</i> reporter gene plasmid	Relative levels of chloramphenicol acetyl transferase activity	
		Minimal medium with no tryptophan	Minimal medium with lots of tryptophan
Wild type	plasmid as shown (see the above figure)	350 (0.5 X 10 X 70) 10 times for overriding of attenuation and 70 time for lack of repression	0.5 units
Wild type	plasmid with the entire leader region deleted	350	5 (0.5 X 10 because no attenuation)
Wild type	plasmid with region 1 deleted	350	5 (0.5 X 10 because attenuation will not occur since region 2 will hybridize with region 3 therefore formation of the 3-4 stem loop followed by a series of U residues signalling transcription termination will not occur)
Wild type	plasmid with the region that includes the T residues at the end of region four deleted	350	5 (0.5 X 10 because attenuation will not occur)
trpR ⁻	plasmid as shown in the above figure	350	35 (0.5 X 70 because repressor cannot bind)
Wild type	plasmid with a trp O ^c mutation	350	35 (0.5 X 70 because repressor cannot bind)

trpR ⁻	plasmid with a trp O ^c mutation	350	35 (0.5 X 70 because repressor cannot bind)
Wild type	Plasmid with leader region 3 deleted	350	5 (0.5 X 10 because attenuation will not occur)
Wild type	plasmid with the start codon of the leader peptide mutated	35 (350/10 because it always attenuates transcription)	0.5 (as per row one both repression and attenuation)

5. (10 marks) In the space below draw a diagram of the lac operon control region (closed promoter complex) including the bound proteins in a wild type strain growing with the following; i) a carbon source that is not a preferred carbon source (not lactose), ii) a preferred carbon source and iii) a carbon source that is not a preferred carbon source (not lactose) and IPTG.

Answers:

i)



ii)



iii)

