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COURSE Biology NUMBER 367 SECTION Winter 2015/16

EXAMINATION Midterm DATE March 1, 2016 TIME 11:45 - 1:00 # OF PAGES 6

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
2=10
3=5.5
4=8
5=8
41.5

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

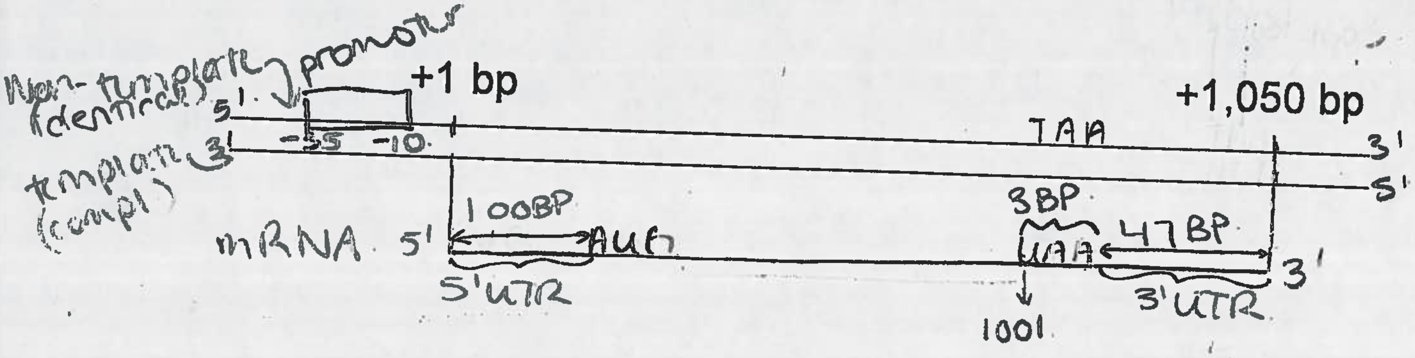
(1) **a. (1 mark)** Draw the gene's transcript just below the diagram.
Assume the following: "+1 bp" indicates the transcription start site, "+ 1,050 bp" indicates the transcription termination site, that the top strand in the diagram represents the gene's nontemplate DNA strand, and that the 5'-UTR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

↳ UAA = stop

**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.

i ✓
ii ✓
iii ✓
iv ✓
v ✓
vi ✓

(5)



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

(4)

1050BP - 100BP - 47BP = 903BP - 3BP (stop codon) = 900BP x 1 a.acid / 3BP = 300 amino acids

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10

**2. (10 marks) a. (6 marks)** Wild type *E. coli* expresses 1.0 unit of ß-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.

capcamp, RNApol, rep bound

↳ capcamp, RNA pol bound

How much ß-galactosidase would you expect the following strains to express in medium with glycerol as the sole carbon source and in medium with glycerol as the sole carbon source and IPTG? 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.

rep = 1 unit
norep = 1400

| Strain # and strain genotype | Glycerol and IPTG | Glucose | lactose |
|---|---|---|---|
| 1. $i^+ ac^+ p^+ o^+ z^+ y^+$ | 1400. | 1 ✓ | ✓ |
| 2. $i^- ac^+ p^+ o^+ z^+ y^+$ | 1400. | 1 – 3 a bit more than 1 ✓ | ✓ |
| 3. $i^s ac^+ p^+ o^+ z^+ y^+$ | ~1-3. | ~1 or <1 ✓ | X. |
| 4. $i^s ac^+ p^+ o^c z^+ y^+$ | 1400 | 1 – 3. a bit more than ✓ | ✓ |
| 5. $i^+ ac^- p^+ o^+ z^+ y^+$ can't form the open promoter complex. | ~1 or <1 | 1: ~1 ✓ | X |
| 6. $i^+ ac^+ p^+ o^+ z^+ y^-$ | 1400 | 1 ✓ | X |
| 7. $i^+ ac^+ p^+ o^+ z^+ y^+ / i^- ac^+ p^+ o^+ z^+ y^+$ | 1400 | 1 ✓ | ✓ |
| 8. $i^+ ac^+ p^+ o^+ z^+ y^+ / i^s ac^+ p^+ o^+ z^+ y^+$ | ~1-3 | ~1 or <1 ✓ | X |
| 9. $i^+ ac^+ p^+ o^+ z^+ y^+ / i^{-d} ac^+ p^+ o^+ z^+ y^+$ | 1400 | 1 – 3. ✓ | ✓ |
| 10. $i^+ ac^- p^u o^+ z^+ y^-$ $i^+ ac^- p^+ o^c$ z+ $y^+$ | 1400 | 1 – 3. ✓ | X |

$o^c > i^s$

6

$i^s > i^+$

rep   norep.

$p^u > ac$
recruit RNA pol

↳ Bad combination?

**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.

4

3, 5, 6, 8, 10 → weak growth b/c $ac^-$ and $y^-$ combination.

3 → Superrepressor

5 → no ac to bind CAP-cAMP

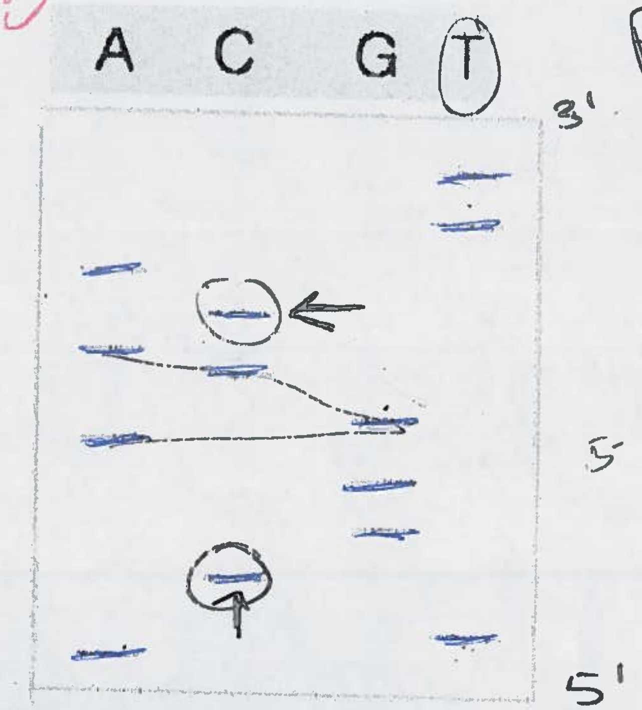
6 → no permease

8 → superrepressor

**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.

5.5

Primer 5'-GATCA-3'.



5' ATCGGAG --------

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

4

5'-ATCGGAGCACATT-3'

w/the primer = 5'-GATCAATCGGAGCACATT-3'

more ddTP.

**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?

1

more ddTTP = more chance of incorporating ddTTP = more frequent terminations and ∴ shorter bands ↳closer to 5' end of gel

Banding pattern would stay the same, the T lane would probably increase in intensity on the autoradiogram b/c there would be more fragments of each length present at each band. Autoradiogram intensity ∝ [of bands] at that spot.

**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)?

0

5'-AGCA-3'.

-5'-AGCA-3'

No ddNTP

**d. (1 marks)** What you would 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)?

0.5

without any ddNTP's present, there would not be any chain termination. One very large band would be seen at the 5' end top of the gel. It will still show on the on the gel. autoradiogram b/c 1 nucleotide was labelled ✓

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

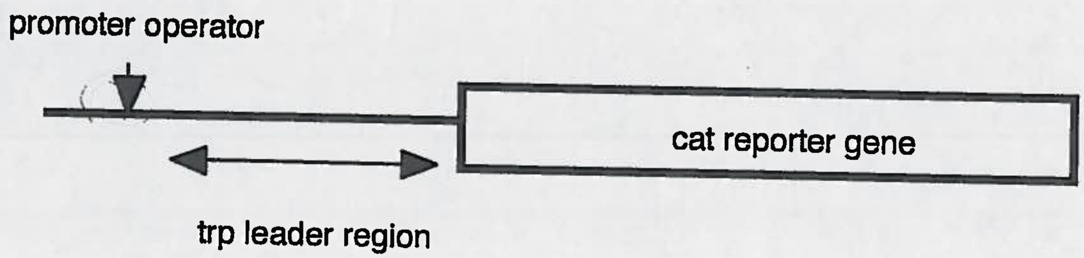
0

dTTP ✗

T is the nucleotide just before the C that generated the bottom C band

**4. (10 marks) (a) (8 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 *E. coli* strain | trp-cat 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 units ✓ |
| NO ATT Wild type | plasmid with the entire leader region deleted | 350 | 5 ✓ |
| Wild type | plasmid with region 1 deleted<br>NO Att | 350 | 5 ✓ |

- NO Att
no uuu tail.

| | | No tryp. | lots of trp. |
|---|---|---|---|
| Wild type | plasmid with the region that includes the T residues on the template strand at the end of region four deleted | 350. | 5. ✓ |
| trpR⁻ | plasmid as shown in the above figure | 350. | 35. ✓ |
| Wild type | plasmid with a trp $O^c$ mutation | 350 | 35 ✓ |
| trpR⁻ | plasmid with a trp $O^c$ mutation | 350. | 35. ✓ |
| Wild type | Plasmid with leader region 3 deleted NO Att | 350 | 5 ✓ |
| Wild type | plasmid with the start codon of the leader peptide mutated | 0.5. | 0.5. ✗ |

no repressor

repressor can't bind.

no rep.

8

rep still binds to O.

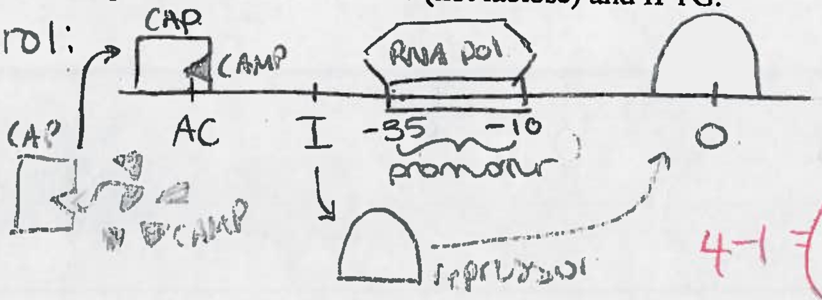
att only needs hairpin → [illegible]

no start codon = can't start anything

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.

8

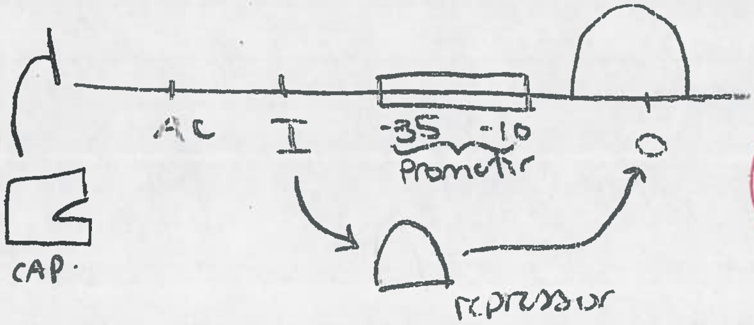
i) glycerol:



- high CAMP production.
- CAP-camp complex binds to AC → recruits RNA po
- I produces repressor and no allolactose to induce the repressor so repressor is bound to operator.

4-1 = 3

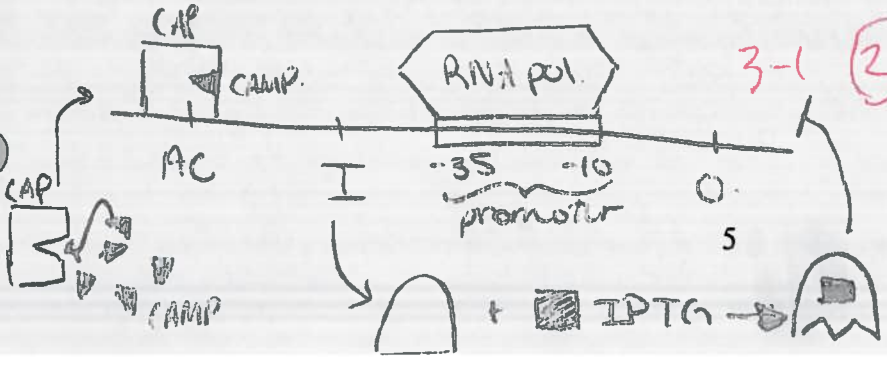
ii) glucose:



- low [CAMP] w/ high [glucose]
- no CAP-CAMP complex → do not bind to AC or recrui RNA polymerase.
- I produces repressor, which is not induced bic no allolactose present S repressor bound to operat

3

iii) glycerol + IPTG.



- high CAMP produced.
- CAP-CAMP complex formed
- I produces repressor
- IPTG present to induce the repressor = conformational change
- Repressor is not bound to operator
- CAP-CAMP complex recruit

3-1 2