

2. a. (6 marks) Wild type *E. coli* expresses 2 units of β -galactosidase when grown with glycerol as the sole carbon source and 2,800 units when grown in glycerol medium when IPTG is also present. How much β -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.

For final; describe why I^s are dominant over I^s. (molecular explanation)

The binding site for CAP-cAMP is designated "ac").

Strain # and strain genotype	glycerol	glycerol and IPTG
1. $i^+ ac^+ p^+ o^+ z^+ y^+$	2	2,800 ✓
2. $i^- ac^+ p^+ o^+ z^+ y^+$	2800	2800 ✓
3. $i^s ac^+ p^+ o^+ z^+ y^+$	< 2	< 2 ✓
4. $i^s ac^+ p^+ o^o z^+ y^+$	2800	2800 ✓
5. $i^+ ac^- p^+ o^+ z^+ y^+$	<<< 2	~ 2 ✓
6. $i^+ ac^+ p^+ o^+ z^+ y^o$	2	2800 ✓
7. $i^+ ac^+ p^+ o^+ z^+ y^+ / i^- ac^+ p^+ o^+ z^+ y^+$	2	2800 ✓
8. $i^+ ac^+ p^+ o^+ z^+ y^+ / i^s ac^+ p^+ o^+ z^+ y^+$	< 2	< 2 ✓
9. $i^s ac^+ p^+ o^+ z^+ y^+ / i^d ac^+ p^+ o^+ z^+ y^+$	2800	2800 ✓
10. $i^+ ac^+ p^+ o^o z^+ y^+ / i^+ ac^- p^+ o^o z^+ y^+$	2	2800 X

55

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.

15
 1, 3, 6, 8, 10

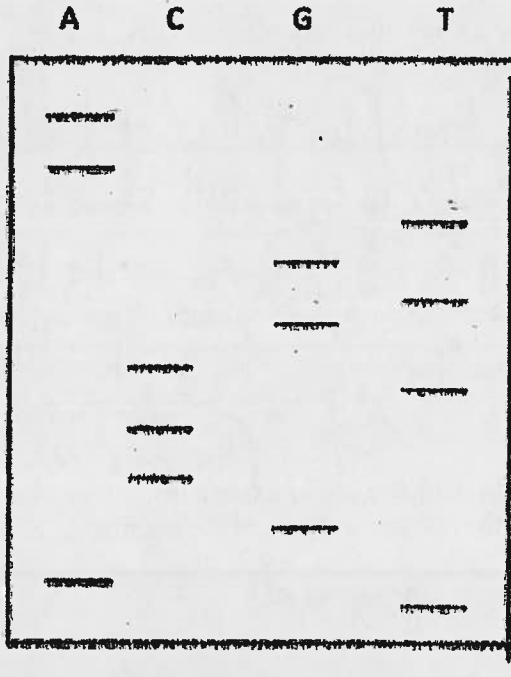
very weak growth due to I^s, but due to leakage in transcription some β -galactosidase can be made.

very weak growth (same reason as #1) has I⁻ so can't get lactose in \Rightarrow No growth on lactose

b5 ac⁻

so synthesis of transference is very low \Rightarrow very weak growth. (almost nothing)

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 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.



- 3/ b) exactly the same pattern, intensity of the bands \therefore it would be $1/10$ th as intense. Pattern is the same.
- d) stop @ 1st appearance of T
No bands are present.
- e) v. large molecules.
- f) ~~ATP~~

a. (2 marks) What is the DNA sequence as determined from the above autoradiogram? Label the 5' and 3' ends.

2/ 5'-TAGCCTCGTGTAA-3'

b. (2 marks) Suppose that you added 0.1 times as much ddATP to the reaction used in lane 1. What effect would this have on the banding pattern in lane 1? will have $1/10$ as much as original

adding a very small amount of ddATP will mean that the sequence will be less terminate by the ddATP that lacks the necessary -OH group for transcription. Thus fewer bands will maybe just one or nothing due to reduction of possibility of encountering a ddATP thus a misleading sequence.

c. (2 marks) What is the sequence of the 4 nucleotides at the 5' end of the molecule that generated the largest band in the above gel?

5'-GATC-3' \rightarrow (the first 4 of the primer) 2/

d. (2 marks) What you would expect to see if you prepared a reaction using a nucleotide mix containing only ddTTP, dATP, dCTP, dGTP (assume that one of the dNTPs was radioactive).

Since Thymine is the first base pair, and only ddTTP is found (no dTTP) and ddTTP is not labeled, then no bands show up on the gel, after autoradiography!

e. (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).

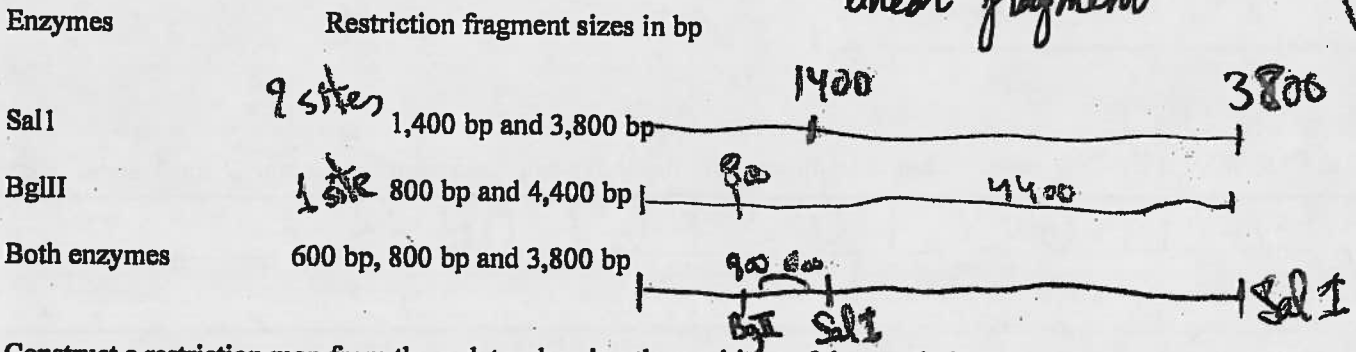
we expect to see just one band (the 3' end band) due to the lack of ddNTP which terminates the transcription at different sites randomly. One band will show because one of the dNTP is labeled. The lack of ddNTP will let RNA pol transcribe till the end without any interruption.

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

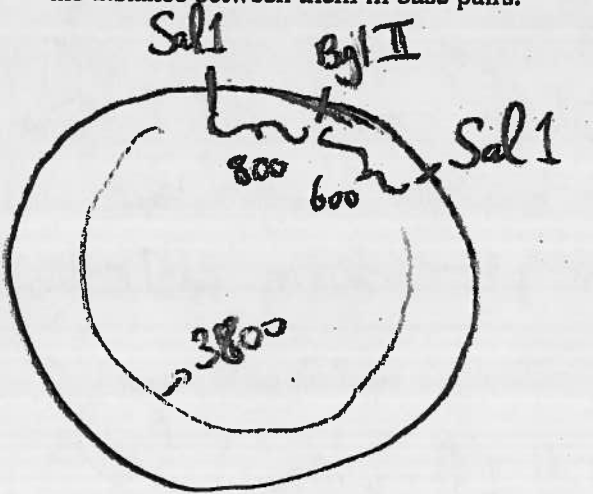
05
 (sequence of smallest band is) 5'-primer-T-3'

(Please check the back side)

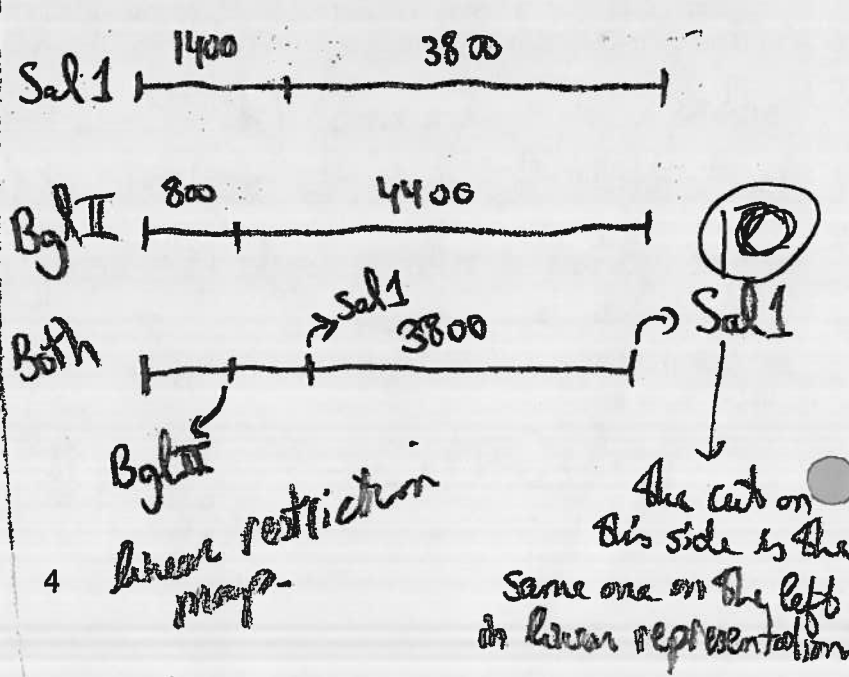
4. (10 marks) To characterize a cloned 5.2 kbp fragment you have decided to begin by constructing a restriction map. Using SalI and BglII, followed by gel electrophoresis, you determine the number and size of the fragments produced by these enzymes alone and in combination.



Construct a restriction map from these data, showing the positions of the restriction sites relative to one another and the distance between them in base pairs.



Circular restriction map



5. a. (8 Marks) Use the genetic code at the bottom of this page to complete the following table. Assume that transcription of the template strand is from left to right and that the columns represent transcriptional and translational alignments. Indicate the polarity of the DNA strands, tRNA, mRNA and protein in the blank column on the left side of the table.

C	A	C	T	A	C	T	G	A	T	T	T	polarity	DNA double helix (template strand)
G	T	G	A	T	G	A	C	T	A	A	A	5'-3'	DNA double helix (nontemplate strand)
G	U	G	A	U	G	A	C	U	A	A	A	5'-3'	mRNA
C	A	C	U	A	C	U	G	A	U	U	U	3'-5'	Appropriate tRNA anticodon
Valine	Methionine		Threonine		Lysine							Amino acids in polypeptide	

b) (2 marks) What would be the amino acid sequence in the polypeptide be if the first anticodon (the one on the left side of the table) was GAU?

anticodon = GAU ⇒ on mRNA the codon = CUA ⇒ amino acid must be Leucine

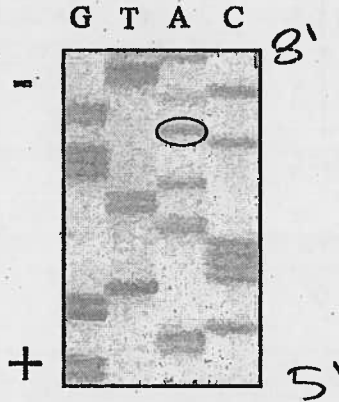
Fig. 18.9 Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display

		Second position					
		U	C	A	G		
U	UUU	Phe	UCU	Tyr	UGU	Cys	U
	UUC		UCC		UGC		C
	UUA	Leu	UCA	STOP	UGA	STOP	A
	UUG		UCG		UGG		Trp
G	CUU	Leu	CCU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC
	QUA	Pro	CCA	Gln	CGA	Arg	A
	CUG		CCG		CAG		CGG
A	AUU	Ile	ACU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC
	AUA	Met	ACA	Lys	AGA	Arg	A
	AUG		ACG		AAA		AGG
G	GUU	Val	GCU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC
	GUA	Ala	GCA	Glu	GGA	Gly	A
	GUG		GCG		GAG		GGG

TAA
WT
TAA
ATT
UAA

Name: _____ ID number: _____

1) (3 points) A double stranded DNA molecule was subjected to the dideoxy DNA sequencing method. Shown below is an autoradiogram obtained from the sequencing gel.



a) (1 point) What is the DNA sequence as determined from the above result?

5' CGAACGGTCCCAATTAGGGCAAGACTTA

b) (2 points) One of the bands in the above sequencing gel is circled. What are the five nucleotides at the 3' end of the DNA molecule making up this band?

5' AGGAC - 3'

2) (2 points) By mistake you mix together the following two strains. Describe a simple experiment that will allow you to isolate the two strains.

Strain 1 DS40 containing pBR322 having the *lacZ* and the *lacI* genes cloned in the PstI site

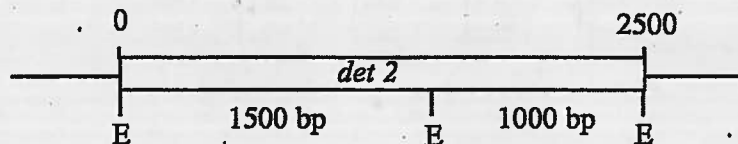
Strain 2 DS40 containing pBR322 having the *lacZ* gene cloned in the PstI site

DS40 genotype: *lacI*⁻ and *lacZ*⁻

① *lacI*⁻ *lac*⁻ *lacZ* *lacI*⁺

lac⁻ *lac*⁻ 2 *lacI*⁺
+ *lac*

3) (5 points) The figure below represents the restriction map (using EcoRI) of the gene *DET2* from *Arabidopsis thaliana*. The distance between the cut sites is presented on the figure. Describe the results of a Southern blot experiment performed with DNA samples coming from wild type *A. thaliana* and a deletion mutant lacking nucleotides 1100 to 1900 (both DNA samples were cut with EcoRI prior to electrophoresis) Your answer should include the number of bands observed on the blot as well as the size of the bands (in bp). The probe used for the experiment spans nucleotides 1300 to 2200.



	Number of band(s)	Size of band(s)
Wild type	_____	_____
Deletion mutant	_____	_____