UNIVERSITY OF JOHANNESBURG

FACULTY	: Science	
DEPARTMENT	: Biochemistry	
CAMPUS	: APK	
MODULE	: BIC3A10/BIC03A3	MOLECULAR BIOLOGY
SEMESTER	: First	
EXAM	: May 2019	

DATE	: 25 May 2019	**SESSION**	: 08:30-11:30
ASSESSOR(S)	: PROF LA PIATER DR F ALLIE		
MODERATOR	: DR S HUSSEY		
DURATION	: 3 HOURS	**MARKS**	: 100

NUMBER OF PAGES: 10 PAGES

INSTRUCTIONS:

1. Answer ALL THE QUESTIONS.
2. Number your answers clearly
3. Appendix 1 – A codon table has been included on the last page of the exam question paper.

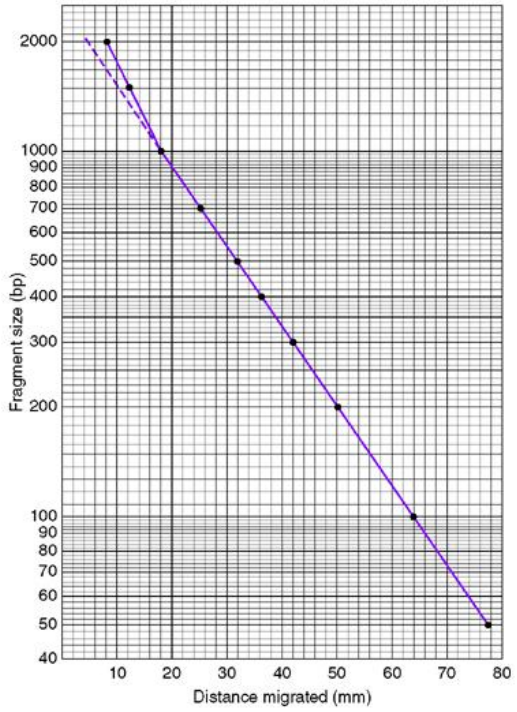
SECTION A [50]

QUESTION 1 [17]

1.1. An example of a restriction enzyme is *Nco*I which recognizes and cuts as shown: C↓CATGG. For the following DNA sequence, draw both strands of the product molecules following *Nco*I digestion: (3)

5' GGAATTCCATGGAATTTAAGTCCATGGGGACCTAATTCC 3'

1.2. What principle/phenomenon is depicted in the following figure: (2)



(b)

1.3. Manual Sanger sequencing was the method of choice prior to automated DNA sequencing.

1.3.1 Discuss the manual technique, including the principle and important reagents used in the reaction. (10)

1.3.2 Draw the banding pattern you would expect to see on a DNA sequencing gel if you annealed the primer 5' ATGAG 3' to the following single-stranded DNA fragment and carried out a DNA sequencing experiment (lanes are **electrophoresed from left to right in the order A, C, G, T**): (2)

GCTAATAGCCTCAT

QUESTION 2 [8]

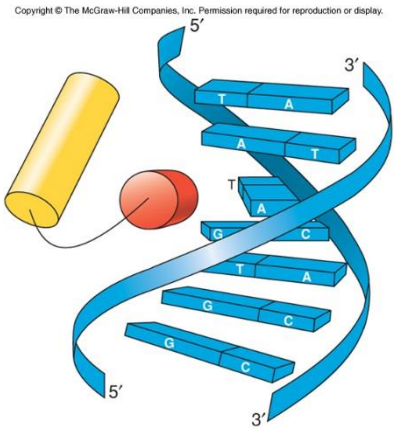
2.1. In terms of forces that stabilise nucleic acid, what do you understand by the term:

2.1.1 Base stacking (2)

2.1.2 Hydrophobic forces (2)

2.1.3 Ionic interactions (2)

2.2. The following diagram represents a protein-DNA interaction. Name the motif and function of such an association. (2)



QUESTION 3 [16]

3.1. Complete (high/moderate/low/yes/no) the table below which compares the properties of prokaryotic DNA polymerases (redraw in your answer book). (12 x ½ = 6)

Property	Pol I	Pol II	Pol III
Processivity/turnover number*	High/Moderate/Low	High/Moderate/Low	High/Moderate/Low
Lethal mutant	Yes or No	Yes or No	Yes or No
3' → 5' Exonuclease	Yes or No	Yes or No	Yes or No
5' → 3' Exonuclease	Yes or No	Yes or No	Yes or No

* nucleotides polymerized min^{-1}.$molecule^{-1}$

3.2 Discuss how DNA replication is terminated and RNA primers are removed for (i) prokaryotes and (ii) eukaryotes. (10)

QUESTION 4 [9]

4.1 You are given the information below. Based on this, discuss the strength of the given promoter in detail using the information. (4)

Alternative Sigma Factors of *E. coli*					
Sigma Factor	Name		Consensus sequences		
			-35	Spacing	-10
Housekeeping	σ^{70}	RpoD	TTGACA	16-18	TATAAT
Stationary phase	$\boldsymbol{\sigma^{38}}$	RpoS	CCGGCG	16-18	CTATACT
Nitrogen control	$\boldsymbol{\sigma^{54}}$	RpoN	TTGGNA	6	TTGCA
Flagellar motion	$\boldsymbol{\sigma^{28}}$	FliA	CTAAA	15	GCCGATAA
Heat shock	$\boldsymbol{\sigma^{32}}$	RpoH	CTTGAA	13-15	CCCCATNT
Extracytoplasmic heatshock	$\boldsymbol{\sigma^{24}}$	RpoE	GAACTT	16	TCTGAT

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source          1..1310
                /organism="Escherichia coli"
                /mol_type="genomic DNA"
                /strain="EJ500"
                /db_xref="taxon:562"
                /map="35.4 min"
     regulatory     69..72
                /regulatory_class="minus_35_signal"
                /note="FliA-dependent promoter activity"
     regulatory     89..96
                /regulatory_class="minus_10_signal"
                /note="FliA-dependent promoter activity"
     regulatory     119..123
                /regulatory_class="ribosome_binding_site"
     gene           130..462
                /gene="flxA"
     CDS            130..462
                /gene="flxA"
                /codon_start=1
                /transl_table=11
                /product="FlxA"
                /protein_id="BAA14048.1"
                /translation="MSVTIQGNTSTVISNNSAPEGTSEIAKITRQIQVLTEKLGKISS
```

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                     EEGMTTQQKKEMAALVQKQIESLWAQLEQLLRQQAEKKNEDATVQPDKK
                     EEKKDDTNT
                    AGTIDIYV"
   regulatory     477..499
            /regulatory_class="terminator"
ORIGIN

        1 aagctttact ttctgaggcg cgccagccgc gaggaaaaca atctgaacat caaacaatta
       61 atgacacatt aaatacgatt ttgtgcatgc cgatagtgct tttttaaaag aaagattttt
      121 gagaaatcta tgtctgtcac aattcaggga aatacctcaa ccgttatttc aaacaactcc
      181 gccccggaag gaacatcaga aatagccaaa atcacaagac aaattcaggt gctgactgaa
      241 aagcttggga aaatctcatc ggaagagggg atgacgacac agcagaaaaa agaaatggct
      301 gcattggtac agaagcaaat tgaaagcctc tgggctcaac tggagcagtt gttaaggcag
      361 caggcagaga aaaagaatga agacgcgaca gttcagcctg ataaaaaaga agagaaaaaa
      421 gacgatacaa ataccgctgg caccattgat atttacgtct aagtgacagc cgtattgtgg
      481 ccctcatcgg gccacttttc gccatcagcc ttttctttaa agacatatta tctttgtatc
      541 atttctgata gttaacatta caagatataa gtaatggacg actcccaatt agtctattta
      601 aatcgcacga gtttaactga caacccatga tcaattatga attgcaacta tttctgtagt
      661 cacttttgtg gggacagtcc acaaaactgc caacttccgc ttcttgctct tagcggacat
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4.2 Clustal Ω is used for alignment of e.g. protein sequences which will allow inferring of a function. In terms of the output, what is the significance of symbols? (3)

4.3 Interpret the output below following retrieval of a eukaryotic sequence. (2)

CDS join (49...112,153...392)

SECTION B [50]

QUESTION 1 [10]

All questions must be answered in the answer booklet provided. Choose the correct answer and use a cross to indicate your choice at the back of the answer booklet. Multiple choice questions answered on the question paper will not be assessed. Multiple choice questions must be marked clearly with a pen and not pencil. 1.1 - 1.10 only have ONE correct answer.

1.1 The nucleotide sequence listed below represents the transcriptional template strand of a gene.

Template DNA strand 3' TACAGAAGTTGATGCATC 5'

Which of the following is the non-template DNA strand complementary to the template strand?

A. 5' CTACGTAGTTGAAGACAT 3'
B. 3' CTACGTAGTTGAAGACAT 5'
C. 5' AUGUCUUCAACUACGUAG 3'

D. 5' ATGTCTTCAACTACGTAG 3'
E. 3' ATGTCTTCAACTACGTAG 5'

1.2 Which of the following is the mRNA transcribed from the template DNA in 1.1 (assume that there is no intron splicing or other processing)?

A. 3' AUGUCUUCAACUACGUAG 5'
B. 5' AUGUCUUCAACUACGUAG 3'
C. 3' UACAGAAGUUGAUGCAUC 5'
D. 5' UACAGAAGUUGAUGCAUC 3'
E. 5' GAUUCUACUUCAGACGAU 3'

1.3 Which of the following is the peptide that is produced when the mRNA (from question 1.2) is translated?

A. (Amino end) Tyr Arg Ser (Carboxyl end)
B. (Amino end) Asp Ser Thr Ser Asp Asp (Carboxyl end)
C. (Carboxyl end) Asp Ser Thr Ser Asp Asp (Amino end)
D. (Carboxyl end) Met Ser Ser Thr Thr (Amino end)
E. (Amino end) Met Ser Ser Thr Thr (Carboxyl end)

1.4 Enhancers are used by ____ to regulate ____.

A. eukaryotes, transcription.
B. eukaryotes, translation.
C. eukaryotes, mRNA splicing.
D. prokaryotes, transcription.
E. prokaryotes, translation

1.5 The conversion of a closed promoter complex to an open promoter complex in bacteria requires _____.

A. the activity of alternative promoters
B. hydrogen bond breakage of base pairs around the initiation site
C. a G-C rich sequence adjacent to +1
D. strong interaction between the core enzyme and the -10 box
E. The CTD of the polymerase needs to be phosphorylated

1.6 During the activation of a promoter recognized by RNA polymerase II, which is an acceptable order of binding to the core promoter?

A. TFIIA, TFIIB, TFIID, and then RNA polymerase and TFIIF
B. TFIID, TFIIA, TFIIB, and then TFIIF and RNA polymerase
C. TFIIB, TFIID, RNA polymerase, TFIIF, and then TFIIA
D. TFIID, RNA polymerase and TFIIF, TFIIA, and then TFIIB
E. RNA polymerase and TFIIF, TFIID, TFIIA, and then TFIIB

1.7 In the formation of an aminoacyl-tRNA

A. the amino terminus of the amino acid is directly attached to the 5' end of the tRNA.
B. the carboxyl terminus of the amino acid is directly attached to the 5' end of the tRNA.
C. the amino terminus of the amino acid is directly attached to the 3' end of tRNA.
D. the carboxyl terminus of the amino acid is directly attached to the 3' end of the tRNA.
E. the side chain group of the amino acid is directly attached to the anticodon loop of the tRNA

1.8 Which of the following processes occurs to eukaryotic mRNA during post-transcriptional processing?

A. addition of a 5' methylated guanosine cap
B. addition of a 3' poly-A tail
C. splicing of RNA segments
D. all of the above
E. none of the above

1.9 The role of a metabolite that controls a repressible operon is to:

A. Bind to the promoter region and decrease the affinity of RNA polymerase for the promoter.
B. Bind to the operator region and block the attachment of RNA polymerase to the promoter.
C. Increase the production of inactive repressor proteins.
D. Bind to the repressor protein and inactivate it.
E. Bind to the repressor protein and activate it.

1.10 Old and new strands of DNA in bacteria can be distinguished by

A. DNA glycosylases
B. Methylation patterns
C. 3' → 5' exonuclease activity
D. AP endonucleases
E. RNA polymerase II

Question 2 **[8]**

2.1 *Briefly* describe the Genetic code. (3)

2.2 Many antibiotics inhibit bacterial protein synthesis. For example, tetracyclines are inhibitors of growth (bacteriostatic) and block the A site on the bacterial ribosome,

while chloramphenicol “kills” bacteria (bacteriocidal) and blocks peptidyl transfer. Briefly discuss the specific effects that you would expect each of these antibiotics to have on protein synthesis. (5)

QUESTION 3 **[16]**

3.1 “O6 alkylations on guanine residues can be directly reversed by an enzyme encoded *by the MGMT gene.”*

3.1.1 What is the full name of this enzyme? (1)

3.1.2 What type of DNA repair mechanism is this system? (1)

3.1.3 Why is the enzyme you named in 3.1.1 referred to as a “suicide enzyme”? (2)

3.2 Which DNA bases can form cyclobutane pyrimidine dimers (CPDs)? Which bases are found in the most common type of CPD? (2)

3.3 Which pathway(s) is/are responsible for repairing CPDs? (2)

3.4. In two brief sentences describe how the pathway(s) you mentioned in 3.3 works. (2)

3.5 dsDNA breaks in eukaryotes are probably the most dangerous form of DNA damage. Discuss the Model for Non-homologous End-Joining for repair of dsDNA breaks. (6)

QUESTION 4 **[16]**

4.1 Define the term “operon”. (2)

4.2 Mutations in the genes of the *lac* operon might affect the regulation of β-galactosidase synthesis. Redraw the table below and complete the chart for each mutation by indicating whether β-galactosidase would be regulated normally (**R**), always (**ON**) or always (**OFF**). (10)

mutation	β-galactosidase regulation	Brief Explanation
mutation in operator site prevents repressor from binding		
mutation in *lacI* gene prevents repressor from binding operator		
mutation in *lacI* gene prevents repressor from binding lactose		
mutation in -10 region of *lacZ* promoter prevents sigma factor from binding		
nonsense mutation in *lacZ* gene		

4.3 Each of the mutations listed in the table below would affect the function of the *lac* operon in *E. coli*. Redraw the table and indicate for each mutation whether β-galactosidase would be produced (at a high level) in the presence or absence of the molecules shown. The results for an *unmutated* operon are given as an example in the first row. (4)

	β-galactosidase produced at <u>high level</u> when:	
	lactose absent (-) glucose absent (-)	lactose present (+) glucose absent (+)
no mutation (control)	**No**	**Yes**
mutation in repressor gene: prevents repressor from binding operator		
mutation in CAP binding site: prevents CAP from binding DNA		

_____END_____

Appendix 1

First Base of mRNA Codon	Second Base of mRNA Codon: U	C	A	G	Third Base of mRNA Codon
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	GGU 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