UNIVERSITY OF JOHANNESBURG

FACULTY	: Science
DEPARTMENT	: Biotechnology and Food Technology
CAMPUS	: DFC
MODULE	: BTN7X05/BTN1YD4/MCB41-1 Advanced Molecular Biotechnology
SEMESTER	: First & Second

START DATE	27 October 2020 :8am	**DUE DATE**	: 29 October 4pm
ASSESSOR(S)	: DR MH Serepa-Dlamini		
EXTERNAL MODERATOR	: Prof A Samie		
DURATION	: 3 Days	**MARKS**	: 100

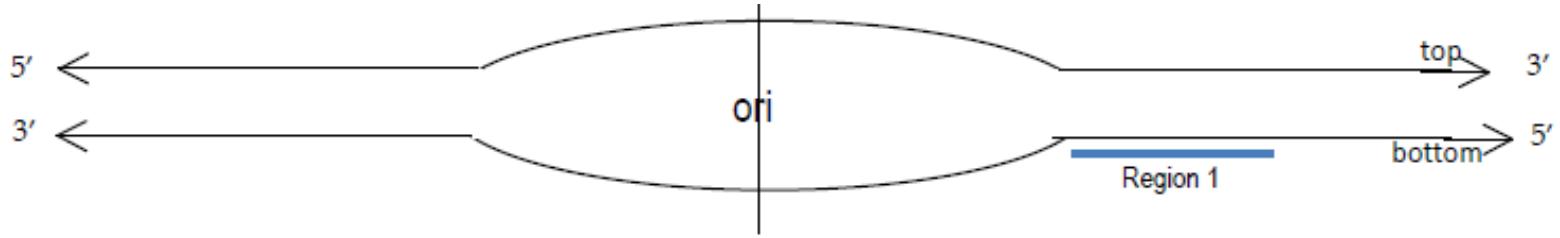
NUMBER OF PAGES: 11 PAGES

INSTRUCTIONS:

1. Number your answers clearly.
2. Please submit a typed exam.
3. You have 3 days to write this exam.
4. Submit on 29 October 2020 4pm through email to Dr MH Serepa-Dlamini and post a copy of your submission on Turn it in earlier or 30 minutes before due time.
5. All the Best.
6. **ANSWERS IN BOLD.**

Question 1

1.1. Consider the following origin of replication that is found on a chromosome. The sequence of region 1 is shown below.



Region 1: 5′…CTGACTGACA…3′
3′…GACTGACTGT…5′

a) Within Region 1, which strand will be the template for leading strand synthesis, the top or the bottom? (2)

The bottom strand will be the template for leading strand synthesis.

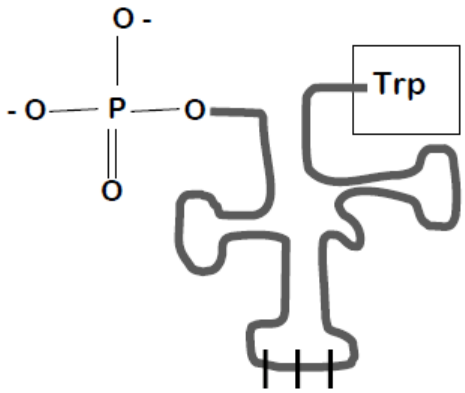
b) If we assume that a lagging strand fragment is made from region 1, what will be its sequence? (5)

5' T G T C A G T C A G 3'

c) You examine DNA replication in an *Escherichia coli* mutant, which has a partially defective DNA polymerase. In vitro experiments using the mutant DNA polymerase gives an error rate of 10^{-3}, as compared to the expected error rate of 10^{-6}. Which of the following activities is the mutant polymerase likely to be missing, as compared to the normal polymerase? Write down all that apply. (2)

3'→5' exonuclease

d) Below is a schematic of the molecule that inserts the fourth amino acid **W** into the mutant polymerase.



i. This schematic represents a ___ **tRNA**_____. (2)

ii. What is the anticodon for this diagram? (2)

CCA

iii. What is the nucleotide attached to Trp (2) [**15**]

A

Question 2

2.1. Below are 210 consecutive base pairs of DNA that includes only the beginning of the sequence of gene X. The underlined sequence (from position 20-54) represents the promoter for gene X and the underlined and italicized sequence (from position 71-90) encodes the gene X ribosome binding (RBS) site. Transcription begins at and includes the T/A base pair at position 60 (bold).

```
   1         10         20         30         40         50         60         70
   I---------I---------I---------I---------I---------I---------I---------I
5' ATCGGTCTCGGCTACTACATAAACGCGCGCATATATCGATATCTAGCTAGCTATCGGTCTAGGCTACTAC
3' TAGCCAGAGCCGATGATGTATTTGCGCGCGTATATAGCTATAGATCGATCGATAGCCAGATCCGATGATG
                                  Promoter

           80         90         100        110        120        130        140
   ---------I---------I---------I---------I---------I---------I---------I
5' CAGGTATCGGTCTGATCTAGCTAGCTTCTCTTCTCTCTCTCCCCCGCGGGGGCTGTACTATCATGCGTCG
3' GTCCATAGCCAGACTAGATCGATCGAAGAGAAGAGAGAGGGGGCGCCCCCGACATGATAGTACGCAGC
       RBS

           150        160        170        180        190        200        210
   ---------I---------I---------I---------I---------I---------I---------I
5' TCTCGGCTACTACGTAAACGCGCGCATATATCGATATCTAGCTAGCTATCGGTCTCGGCTACTACGTAAA
3' AGAGCCGATGATGCATTTGCGCGCGTATATAGCTATAGATCGATCGATAGCCAGAGCCGATGATGCATTT
```

a) What are the first 6 nucleotides of the mRNA from gene X? (6)

5' U A G G C U 3'

b) What are the first 4 amino acids encoded by gene X? Indicate the N and C terminus. (6)

N met arg arg leu C

c) In mutation 1, there is an insertion of the following three base pairs immediately after the C/G base pair at position 100 (shown in bold).

```
5′ TGT 3′
3′ ACA 5′
```

i. Would the mRNA expressed from this version of gene X be longer, shorter, or the same as that produced from the normal gene X? Explain and if longer or shorter, indicate by how many in bases.(2)

The insertion is after the promoter so the mRNA would be longer by 3 nucleotides.

ii. If the mRNA can be translated,

1) Would you expect the protein to be longer, shorter, or the same as that produced from the normal gene X? If longer or shorter, indicate by how many in amino acids.(2)

The protein would be the same as the normal protein because the insertion is before the start codon.

2) Do you expect that the protein produced will have the same function as the normal protein X? Explain your thinking. (2)

The protein produced is identical to the normal protein, so it will have the same structure and function.

d) In mutation 2, there is an insertion of the following four base pairs immediately after the A/T base pair at position 130 (shown in bold).

```
5′ ATGT 3′
3′ TACA 5′
```

i. Would the mRNA expressed from this version of gene X be longer, shorter, or the same as that produced from the normal gene X? Explain and if longer or shorter, indicate by how many in bases.(2)

The insertion is after the promoter so the mRNA would be longer by 4 nucleotides.

ii. If the mRNA can be translated,

1) What are the first four amino acids produced?Indicate the N and C terminus. (6)

N met phe met arg C

2) Would you expect the protein to be longer, shorter, or the same as that produced from the normal gene X? If longer or shorter, indicate by how many in amino acids.. (2)

The protein encoded by mutant 2 will be longer by 2 amino acids.

3) Do you expect that the protein produced will have the same function as the normal protein X? Explain your thinking. (4) [32]

This mutant protein is nearly identical to the normal protein. It is different in that it has two additional amino acids at the very N terminus, but these two terminal amino acids are unlikely to change the overall tertiary structure of the protein, so it is unlikely that this insertion will change the function of the protein.

Question 3

The gene sequence below runs in the 5'---3' direction and codes for the bacterial ATP synthase subunit beta AtpD protein.

```
   1 gtgtacagcg ctcttgaggt taagaatggt gatgctcgtc tggtgcttga agttcagcag
  61 cagctgggtg gtggcgtagt gcgtactatc gccatgggta cttctgacgg cctgaagcgc
 121 ggtctggaag ttgccgacct gaaaaaaccg atccaggtac cggttggtaa agcaaccctc
 181 ggccgtatca tgaacgtgct gggcgagcct atcgacatga aaggcgacct gaaagaagaa
 241 gatggcagtg cagtagaggt ttcctctatt caccgccctg cgccttctta tgaagagcag
 301 tctaactcgc aggaactgct ggaaaccggc atcaaggtta tcgacctgat gtgtccgttc
 361 gcgaagggcg gtaaagtcgg tctgttcggt ggtgcgggtg tgggtaaaac cgtaaacatg
 421 atggagctga tccgtaacat tgcggctgag cactcaggtt actcggtatt tgccggcgtg
 481 ggtgagcgta ctcgtgaggg taacgacttc taccacgaaa tgactgactc caacgttatc
 541 gacaaagttg cgctggtgta tggccagatg aacgagccgc cgggtaaccg tctgcgcgtt
 601 gcactgaccg gtctgaccat ggcggagaaa ttccgtgatg aaggccgtga cgttctgctg
 661 ttcatcgaca acatctaccg ttataccctg gccggtacag aagtctctgc actgctgggt
 721 cgtatgccat ctgcggtagg ttatcagcca acgctggcag aagagatggg tgtgttgcag
 781 gagcgtatta cctccaccaa aaccggttca atcacctccg tacaggccgt ttacgtccct
 841 gcggatgacc tgactgaccc gtcaccggca accacctttg ctcacttaga ctcaacagtc
 901 accctgagcc gtcagatcgc ctctctgggt atctacccag ccgttgatcc gctggactca
 961 accagccgtc agctggatcc actggttgtg ggtcaggagc actacgatgt tgcacgtggc
1021 gtacagtcac tgctgcagcg ttatcaggaa
```

3.1. Design primers to amplify the gene. (6)

FP: 5' GTGTACAGCGCTCTTGCG 3' (3)

RP: 5' TTCCTGATAACGCTGCAGC 3' (3)

3.2. Calculate the Tm for each primer. (3)

Tm = 2(A+T) + 4 (G+C) (1)

FP Tm=58 °C (1)

RP Tm=58 °C (1)

3.3. Determine the suitable annealing temperature. (2)

58-5=53°C (2)

3.4. Why do we subtract 5 °C when for determining the annealing temperature? (2)

The melting temperatures of the primers have to be within 5 °C of each other, when determining the annealing temperature we subtract 5 °C from the primer with the highest melting temperature so that both primers will have an annealing temperature that is below their melting temperature. This is to ensure that primers do not melt before annealing to the gene of interest. (2)

3.5. What is the expected PCR product length in bp? (2)

1050 bp (2)

6. Which step follows annealing during PCR? (2)

Extension/Elongation (2) (17)

Question 4

The following questions are about BLAST which can be found on the NCBI website.

4.1. You have a DNA sequence and you wish to search for other DNA sequences to find one that encodes the same or similar protein. Which of the four Basic Blast programs should you use? (2)

tblastx

4.2. You have a protein sequence and you wish to know what other proteins look like it. Which of the four Basic Blast programs should you use? (2)

protein blast (also called blastp)

4.3. You have DNA and you wish to find other DNA sequences that look like it. Which of the four Basic Blast programs should you use? (2)

nucleotide blast or blastn

4.4. You have protein sequence and you wish to search DNA databases to find genes that encode a similar protein. Which of the four Basic Blast programs should you use? (2)

tblastn

4.5. BLAST search this accession number [DQ859805] and state which gene it is and from what species. (4) [12]

ATP synthase beta subunit (atpD) gene; *Pantoea ananatis* strain ICMP 1850 species.

Question 5

5.1. The following DNA template strand was utilized in a Sanger sequencing experiment, 5` AATTGCGTCAGTCGTA 3`. Using the technique behind Sanger sequencing:

a) Write down the ALL the fragments which will result from the tube with ddGTP. (3)

3' TTAACGCAGTCAGCAT 5' (Complementary strand of the above)

3' TTAACG 5' Fragment 1

3' TTAACGCAG 5' Fragment 2

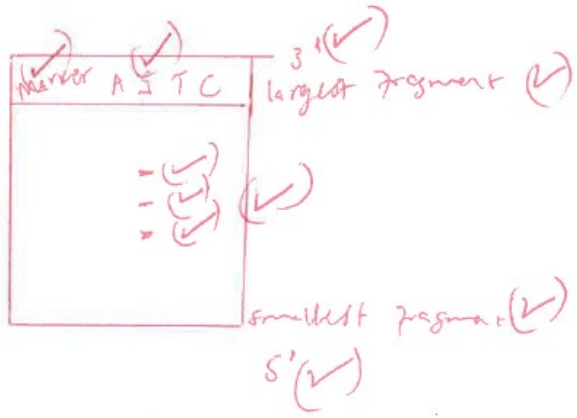
3' TTAACGCAGTCAG 5' Fragment 3

b) Write down the sequence of the larger fragment from the answer in a). (1)

TTAACGCAGTCAG

c) Indicate all the fragments from the experiment on a gel drawing, provide accurate illustrations with labels and indicate the reading of the bands from the 5` to 3`. (8)

ONLY THE BANDS FOR ddGTP are necessary



d) Where does the primer anneal on the above strand and why? (2)

At the 3' end of DNA and direction of synthesis will be from 5' to 3' end of the primer.

e) Outline the steps of PCR and briefly explain each step. (10) [24]

The main PCR steps are Denaturing, Annealing and Extension:

<u>Denaturation</u> (96 °C): As in DNA replication, the two strands in the DNA double helix need to be separated. The separation happens by raising the temperature of the mixture, causing the hydrogen bonds between the complementary DNA strands to break. Heat the reaction strongly to separate, or denature, the DNA strands. This provides single-stranded template for the next step.

1. **<u>Annealing</u> (50- 65°C): Primers (forward and reverse) bind to the target DNA sequences and initiate polymerisation. This can only occur once the temperature of the solution has been lowered. One primer binds to each strand. Cool the reaction so the primers can bind to their complementary sequences on the single-stranded template DNA.**
2. **<u>Extension</u> (72 °C): New strands of DNA are made using the original strands as templates. A DNA polymerase enzyme joins free DNA nucleotides together. This enzyme is often *Taq* polymerase, an enzyme originally isolated from a thermophilic bacteria called *Thermus aquaticus*. The order in which the free nucleotides are added is determined by the sequence of nucleotides in the original (template) DNA strand.**

The result of one cycle of PCR is two double-stranded sequences of target DNA, each containing one newly made strand and one original strand.

The cycle is repeated many times (usually 20–35) as most processes using PCR need large quantities of DNA. It only takes 2–3 hours to get a billion or so copies. Raise the reaction temperatures so Taq polymerase extends the primers, synthesizing new strands of DNA.