UNIVERSITY OF JOHANNESBURG

FACULTY	: Science
DEPARTMENT	: Biotechnology and Food Technology
CAMPUS	: DFC
MODULE	: BTN1GB1 Fundamental Genetics
SEMESTER	: Second
EXAM	: Final Exam 2020

DATE	: 9 November 2020		
ASSESSOR(S)	: DR MH Serepa-Dlamini		
MODERATOR	: D AM Abrahams		
DURATION	: 24 Hours	MARKS	: 100

NUMBER OF PAGES: 11 PAGES

INSTRUCTIONS:

1. Number your answers clearly.
2. Please submit a typed exam.
3. You have 24 hours to write this exam.
4. Submit on 10 November 2020 9am 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.**

SECTION A

Question 1

a. Describe the roles of CRISPR/Cas9 and sgRNA in the nuclease system.

The CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/ CRISPR associated protein9) (√) is prokaryotic immune system which confers resistance to foreign genetic elements such as double stranded DNA material in plasmids and phages and provide acquired immunity (√). The CRISPR sequences (protospacer) (√) are derived from DNA fragments of phages that had previously infected the prokaryote (√). The Protospacer gets inserted into the CRIPR locus(√) and transcribed to pre-crRNA then to mature crRNA (√) which will pair with Cas9 (√). Cas enzyme cuts the foreign DNA material(√) through endonuclease activity. The sgRNA is required to pair (√) (using the crRNA as a guide) the Cas9 [forming effector CRISPR/Cas9 system] with its intended target site in the DNA and to activate the CRISPR/Cas nuclease domains for cleavage (Cas9) (√).

[10]

SECTION B

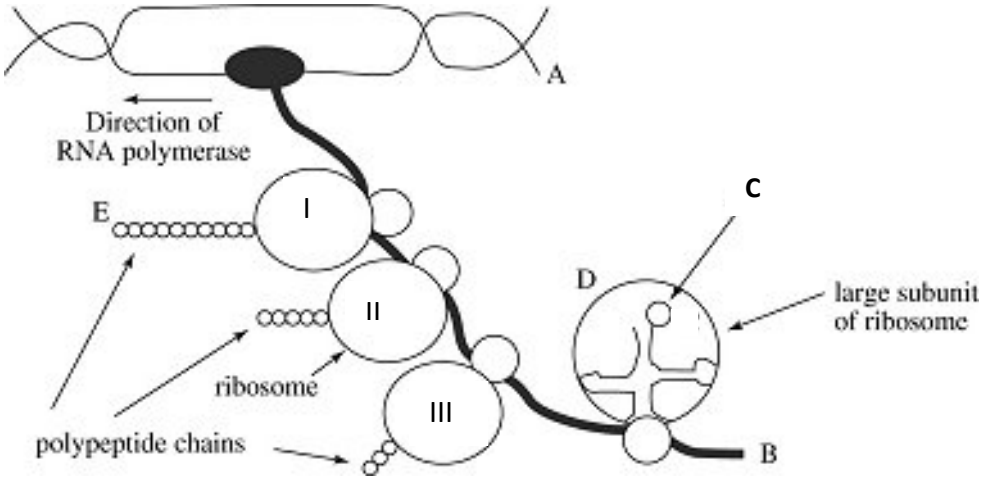
Question 1

1) Define the following terms as they are used in molecular biology:
   i. **Genomics-The study of the structure and functions of the whole genome.**
   ii. **Proteomics-Characterization of all proteins encoded by a genome.**
   iii. **Structural genomics-The study of the sequence of the genome.**
   iv. **Functional genomics-The study of the functions of genetic information contained within a genome.**
   v. **Deoxyribonucleic acid-polymers consisting of fewer than a hundred to millions or even billions of monomeric units called nucleotides - hereditary unit**

   [2 Marks each]
2) **The promoter is a site for RNA polymerase loading and initiation of transcription. To accomplish this, the RNA polymerase must form an open complex in which the two DNA strands are separated over a short distance. Due to the effect of AT-rich versus GC-rich DNA on the overall thermal stability of DNA, the strand separation is more readily accomplished in sequences that are are AT-rich.** (2 Marks)
3) **Assuming the two genes use similar transcription initiation modes, tesA will be more efficiently transcribed. The tesB -10 sequence deviates more from the consensus sequence, and its higher G≡C content will be more difficult to to melt.** (5 Marks)
4) **The sequence reported for a gene is, by convention, that of a coding strand, and the sequence are always written in the 5′to 3′ direction. In the nucleotide sequence of the RNA, U replaces T, so the mRNA for amino acids is; 5'-AUGACCAUGAUUACG-3'** ; **amino acids are met thr met Ile thr** (8 Marks) **[25]**

Question 2

1) The drawing below represents a simultaneous transcription in bacteria. Answer the questions that follow, the direction of RNA pol is given by the arrow.



a) The letter B is nearest to which end of which molecule? (2)

5' of mRNA

b) Which end of the polypeptide chain is near to the letter E? (1)

N-terminal/terminus

c) Which ribosome began translation first? (1)

Ribosome I

d) What type of RNA is within the large ribosomal subunit? (1)

rRNA molecule(s)

e) What is the size of the large ribosomal subunit? (1)

50S subunit

f) Which subunit of the ribosome initiates translation? (1)

16S rRNA

g) The letter A is next to which end of which molecule? (2)

5' of DNA

h) What does the letter C represent? (1)

Amino acid **[10]**

Question 3

1) Translate the following mRNA into protein, starting from the first initiation codon. 5′CCGAUGGCCAUGGCAGCUCGGUGUUACAAGGCUUGCAUCAGUACCAGUUU GAAUCC-3′ (10 Marks)

N-Met-Ala-Met-Ala-Ala-Arg-Cys-Tyr-Lys-Ala-Cys-Ile-Ser-Thr-Ser-Leu-Asn-C

2) Name and discuss the three steps involved in translation in prokaryotes (15 Marks) **[25]**

Translation occurs in the cytoplasm of both prokaryotic (Pr) cells. In prokaryotes, ribosomes can begin translating the mRNA even before RNA polymerase completes its transcription. In eukaryotes, translation and transcription are completely separated in time and space with transcription in the nucleus and translation in the cytoplasm. The process of Protein synthesis occurs in three stages: initiation, elongation, and termination.

Initiation

The small ribosomal subunit binds to the mRNA. In prokaryotes, the 16S rRNA of the small

subunit binds to the Shine-Dalgarno sequence in the 5' untranslated region of the mRNA. In eukaryotes, the small subunit binds to the 5' cap structure and slides down the message to the first AUG. The charged initiator tRNA becomes bound to the AUG start codon on the message through base pairing with its anticodon. The initiator tRNA in prokaryotes carries fmet, whereas the initiator tRNA in eukaryotes carries Met.

The large subunit binds to the small subunit, forming the completed initiation complex. There are two important binding sites on the ribosome called the P site and the A site. The peptidyl site (P site) is the site on the ribosome where (f) met-tRNA initially binds. After formation of the first peptide bond, the P site is a binding site for the growing peptide chain. The aminoacyl site (A site) binds each new incoming tRNA molecule carrying an activated amino acid.

Elongation

Elongation is a three-step cycle that is repeated for each amino acid added to the protein after the initiator methionine. Each cycle uses four high-energy bonds (two from the ATP used in amino acid activation to charge the tRNA, and two from GTP). During elongation, the ribosome

moves in the 5' to 3' direction along the mRNA, synthesizing the protein from amino to carboxyl terminus. The three steps are:

- **A charged tRNA binds in the A site. The particular aminoacyl-tRNA is determined by the mRNA codon aligned with the A site.**
- **Peptidyl transferase, an enzyme that is part of the large subunit, forms the peptide bond between the new amino acid and the carboxyl end of the growing polypeptide chain. The bond linking the growing peptide to the tRNA in the P site is broken, and the growing peptide attaches to the tRNA located in the A site.**
- **In the translocation step, the ribosome moves exactly three nucleotides (one codon) along the message. This moves the growing peptidyl-tRNA into the P site and aligns the next codon to be translated with the empty A site.**

Termination

When any of the three stop (termination or nonsense) codons moves into the A site, peptidyl transferase (with the help of release factor) hydrolyzes the completed protein from the final tRNA in the P site. The mRNA, ribosome, tRNA, and factors can all be reused for additional protein synthesis.

Question 4

1) During mismatch repair, why is it necessary to distinguish between the template strand and the newly made daughter strand? How is this accomplished? (10 **marks)**

Mismatch repair is aimed at eliminating mismatches that may have occurred during DNA replication. In this case, the wrong base is in the

newly made strand. The binding of MutH, which occurs on a hemimethylated sequence, provides a sensing mechanism to distinguish between the unmethylated and methylated strands. In other words, MutH binds to the hemimethylated DNA in a way that allows the mismatch repair system to distinguish which strand is methylated and which is not.

2) A double stranded DNA contains 10% guanine, what is the percentage of adenine. Show all your calculations (4 marks)

In a dsDNA (or dsRNA) (ds=double-stranded)

% A = % T (% U), %G = %C

% purines = % pyrimidines

A sample of DNA has 10% G; what is the % T

10%G+ 10%C= 20%, therefore,% A +% T must total 80%

40% A and 40% T

Therefore the answer is 40% A.

3) A tRNA has the anticodon sequence 3´–CAG–5´. What amino acid does it carry? (3 Marks)

Because the anticodon is 3´–CAG–5´, it would be complementary to a codon with the sequence 5´–GUC–3´. According to the genetic code, this codon specifies the amino acid valine. Therefore, this tRNA must carry valine at its acceptor stem.

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

```
   1 atggcaactg gaaagattgt ccagattatc ggcgccgttg ttgacgtcga attccctcag
  61 gacgcggtac cgcaagtgta cagcgccctc gaggttatga atggtgatgc gcgtctggtg
 121 ctggaagttc agcagcagct cggcggcggc gtagtacgta ccatcgcaat gggtacgtct
 181 gacggcctga agcgtggtct gagcgtcaac gacctgcaga aaccgattca ggtacccgtc
 241 ggtaaagcga ccctgggccg tatcatgaac gttctcggcg agccaatcga tatgaaaggc
 301 gagctgaaag aagaagatgg cagcgcagta gagatcgcct ctattcaccg cgcagcccct
 361 tcttatgaag atcagtctaa ctcgcaggaa ctgctggaaa ccggcatcaa ggttatcgac
 421 ctgatgtgtc cgtttgctaa aggcggtaaa gtcggtctgt tcggtggtgc gggtgtaggt
 481 aaaaccgtca acatgatgga actgatccgt aacatcgcgg ctgaacactc aggttactca
 541 gtgtttgccg gtgtgggtga gcgtactcgt gagggtaacg acttctacca cgaaatgact
 601 gactctaacg ttatcgataa agttgcactg gtctatggcc agatgaacga gccgccgggt
 661 aaccgtctgc gcgtagcact gaccggtctg accatggcgg aaaaattccg tgatgaaggc
 721 cgcgacgttc tgctgttcat cgataacatc taccgttata ccctggccgg tacagaagtt
 781 tctgcactgc tgggtcgtat gccatctgcg gtaggttacc agccaacgct ggcagaagag
 841 atgggtgtgt tgcaggagcg tattacctcc accaagaccg gttcaatcac ctccgtacag
 901 gccgtttacg tccctgcgga tgacctgact gacccatcac cagcaactac ctttgcgcac
 961 ttagactcaa cggtaacgct gagccgtcag atcgcctctc tgggtatcta cccggccgtt
1021 gacccgctgg actctaccag ccgtcagctg gatccgctgg ttgtcggtca ggagcactat
1081 gatgttgcac gtggcgttca gtcactgctg cagcgttatc aggaactgaa agacatcatc
1141 gccatcctcg gtatggatga gctgtctgaa gaagacaaac tgctggtggc acgtgcgcgt
1201 aagattcagc gcttcctgtc tcagccgttc ttcgttgcag aagtattcac cggttcaccg
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1261 ggcaaatacg tgacgctgaa agacactatc cgtggcttta aaggcatcat ggaaggtgag
1321 tttgaccacc tgccagagca ggccttctac atggttggcg ccatcgaaga agccgtggaa
1381 aaagcgaaga aactgtaa
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1. Design primers to amplify the gene. (6)

   FP: 5' ATGGCAACTGGAAAGATTGTC 3' (3)

   RP: 5' TTACAGTTTCTTCGCTTTTTC 3' (3)

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

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

   FP Tm=60 °C (1)

   RP Tm=56 °C (1)

3. Determine the suitable annealing temperature. (2)

   60-5=55°C (2)

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)

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

   1398 (2)

[30]