

## PRACTICE PRACTICE PRACTICE PRACTICE PRACTICE

Name: \_\_\_\_\_ Student #: \_\_\_\_\_

BTEC8002 Molecular Biotechnology

Mini Test 1 (Lectures 1-6)

March 2016

Time allotted – 1hour 30minutes, Total Marks – 90

The number of marks for each question is shown in brackets after the question

Read each question carefully and if you are not sure of the answer, move onto the next question then come back to missed questions at the end.

### Question 1

#### a. What are the components that make up a deoxyribonucleotide? Name an example (8)

A **deoxyribonucleotide** is the monomer, or single unit, of DNA, or deoxyribonucleic acid. Each deoxyribonucleotide comprises three parts: a nitrogenous base, a deoxyribose sugar, and one phosphate group.

The nucleobase can either be adenine, guanine, cytosine, or uracil. Without the phosphate group, the composition of the nucleobase and sugar is known as a nucleoside.

**Deoxyadenosine monophosphate**, also known as **deoxyadenylate**, or **dAMP**

**Deoxyguanosine monophosphate**, also known as **deoxyguanylate**, or **dGMP**

**Thymidine monophosphate**, also known as **5'-thymidylate**, **thymidylate**, **TMP**, or **dTMP**

**Deoxycytidine monophosphate**, also known as **deoxycytidylate**, or **dCMP**

#### b. What do the numbers 3' and 5' refer to? Where do they come from? (5)

The 5' and 3' mean "five prime" and "three prime", which indicate the carbon numbers in the DNA's sugar backbone. The five carbons in the ribose backbone are numbered starting from the O in clockwise direction - therefore the carbon which has the base (A, T, C, G) attached is called 1'. The 2' carbon does nothing special, the 3' carbon has an OH group, the 4' carbon connects the ribose ring to 1' via the O bond, and the 5' carbon is outside of the ring, attached to the 4' carbon on one side and phosphate on the other. The 5' carbon has a phosphate group attached to it and the 3' carbon a hydroxyl group. This asymmetry gives a DNA strand a "direction". For example, DNA polymerase works in a 5' -> 3' direction, that is, it adds nucleotides to the 3' end of the molecule (the -OH group), thus advancing to that direction.

#### c. List 3 differences between DNA and RNA (5)

1. DNA contains the sugar deoxyribose, while RNA contains the sugar ribose. The only difference between ribose and deoxyribose is that ribose has one more -OH group than deoxyribose, which has -H attached to the second (2') carbon in the ring.
2. DNA is a double stranded molecule while RNA is a single stranded molecule.
3. DNA is stable under alkaline conditions while RNA is not stable.
4. DNA and RNA perform different functions in humans. DNA is responsible for storing and transferring genetic information while RNA directly codes for amino acids and as acts as a messenger between DNA and ribosomes to make proteins.

## Question 2

### a. Define the following terms related to DNA replication:

#### i. Primer (2)

A **primer** is a strand of short nucleic acid sequences (generally about 10 base pairs) that serves as a starting point for [DNA synthesis](#). It is required for DNA replication because the [enzymes](#) that catalyze this process, [DNA polymerases](#), can only add new [nucleotides](#) to an existing strand of DNA. The polymerase starts replication at the [3'-end](#) of the primer, and copies the [opposite strand](#).

#### ii. Nucleosome (2)

A **nucleosome** is a basic unit of [DNA](#) packaging in [eukaryotes](#), consisting of a segment of DNA wound in sequence around eight<sup>[1]</sup> [histone protein](#) cores

#### iii. Lagging Strand (2)

The lagging strand is the strand of nascent DNA whose direction of synthesis is opposite to the direction of the growing replication fork.

The lagging strand is synthesized in short, separated segments. On the lagging strand template, a [primase](#) "reads" the template DNA and initiates synthesis of a short complementary [RNA](#) primer. A DNA polymerase extends the primed segments, forming [Okazaki fragments](#). The RNA primers are then removed and replaced with DNA, and the fragments of DNA are joined together by [DNA ligase](#).

#### iv. Origin of replication (2)

An origin of replication is a sequence of DNA at which replication is initiated on a chromosome, plasmid or virus. For small DNAs, including bacterial plasmids and small viruses, a single origin is sufficient. Larger DNAs have many origins, and DNA replication is initiated at all of them; otherwise, if all replication had to proceed from a single origin, it would take too long to replicate the entire DNA mass.

### b. Define the following terms related to DNA transcription

#### i. Promoter (2)

**promoter** is a region of [DNA](#) that initiates [transcription](#) of a particular [gene](#). Promoters are located near the transcription start sites of genes, on the same strand and [upstream](#) on the DNA (towards the [5'](#) region of the [sense strand](#)). Promoters can be about 100–1000 [base pairs](#) long

#### ii. Transcription factors (2)

**transcription factor** (sometimes called a sequence-specific [DNA](#)-binding factor) is a [protein](#) that binds to specific [DNA sequences](#), thereby controlling the rate of [transcription](#) of genetic information from DNA to [messenger RNA](#).<sup>[1][2]</sup> Transcription factors perform this function alone or with other proteins in a complex, by promoting (as an [activator](#)), or blocking (as a [repressor](#)) the recruitment of [RNA polymerase](#) (the enzyme that performs the [transcription](#) of genetic information from DNA to RNA) to specific genes

**iii. Upstream (2)**

*Upstream is toward the 5' end of the RNA molecule and downstream is toward the 3' end. When considering double-stranded DNA, upstream is toward the 5' end of the [coding strand](#) for the gene in question*

**iv. Downstream (2)**

*Each strand of DNA or RNA has a [5' end](#) and a [3' end](#), so named for the carbon position on the [deoxyribose](#) (or [ribose](#)) ring. By convention, upstream and downstream relate to the 5' to 3' direction in which RNA [transcription](#) takes place. **downstream** is toward the 3' end. Due to the anti-parallel nature of DNA, this means the 5' end is downstream.*

**Question 3**

**a. Name the types of RNA polymerase present in a eukaryotic cell? Which type of RNA do they transcribe? (6)**

*RNA polymerase I – rRNA*

*RNA Polymerase II – mRNA*

*RNA polymerase III - tRNA*

*The RNA polymerase binds, unwinds and copies specific DNA sequences into an RNA strand as it moves along the DNA strand*

**b. What is alternative splicing? (8)**

**Alternative splicing** is a regulated process during [gene expression](#) that results in a single [gene](#) coding for multiple [proteins](#). In this process, particular [exons](#) of a gene may be included within or excluded from the final, processed [messenger RNA](#) (mRNA) produced from that gene.<sup>[1]</sup> Consequently, the proteins [translated](#) from alternatively spliced mRNAs will contain differences in their amino acid sequence and, often, in their biological functions (see Figure). Notably, alternative splicing allows the [human genome](#) to direct the synthesis of many more proteins than would be expected from its 20,000 protein-coding genes. Alternative splicing is sometimes termed differential splicing.

**c. What is the function of enhancer sequences in gene expression? (3)**

an **enhancer** is a short (50-1500 bp) region of [DNA](#) that can be bound by [proteins](#) ([activators](#)) to activate [transcription](#) of a [gene](#).<sup>[1][2]</sup> These proteins are usually referred to as [transcription factors](#). Enhancers are generally [cis-acting](#), located up to 1 Mbp (1,000,000 bp) away from the gene and can be upstream or downstream from the start site, and either in the forward or backward direction.<sup>[2][3]</sup> There are hundreds of thousands of enhancers in the human genome.<sup>[2]</sup>

In [eukaryotic](#) cells the structure of the [chromatin](#) complex of DNA is folded in a way that functionally mimics the supercoiled state characteristic of [prokaryotic](#) DNA, so although the enhancer DNA may be far from the gene in a linear way, it is spatially close to the [promoter](#) and gene. This allows it to interact with the [general transcription factors](#) and [RNA polymerase II](#).<sup>[4]</sup> The same mechanism holds true for [silencers](#) in the eukaryotic genome.

Silencers are antagonists of enhancers that, when bound to its proper transcription factors called [repressors](#), repress the transcription of the gene. Silencers and enhancers may be in close proximity to each other or may even be the same region only differentiated by the transcription factor the region binds to.

**d. What are introns and exons? (4)**

Introns and exons are parts of genes. Exons code for proteins, whereas introns do not.

**Question 4**

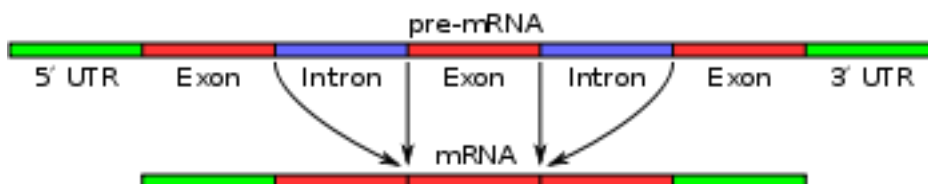
**a. What does spliceosome do? (5)**

A **spliceosome** is a large and complex [molecular machine](#) found primarily within the [splicing speckles](#) of the cell nucleus of [eukaryotic cells](#). The spliceosome is assembled from [snRNAs](#) and [protein complexes](#). The spliceosome removes [introns](#) from a [transcribed pre-mRNA](#), a kind of [primary transcript](#). This process is generally referred to as [splicing](#).<sup>[1]</sup> Only [eukaryotes](#) have spliceosomes and [metazoans](#) have a second spliceosome, the [minor spliceosome](#).<sup>[2]</sup>

**b. What modifications are necessary for mRNA to be translated into protein (=bind to ribosome)? (5)**

These include 5' capping, which is set of enzymatic reactions that add [7-methylguanosine](#) ( $m^7G$ ) to the 5' end of pre-mRNA and thus protect the RNA from degradation by [exonucleases](#). The  $m^7G$  cap is then bound by [cap binding complex](#) heterodimer (CBC20/CBC80), which aids in mRNA export to cytoplasm and also protect the RNA from decapping.

Another modification is 3' cleavage and polyadenylation. They occur if polyadenylation signal sequence (5'- AAUAAA-3') is present in pre-mRNA, which is usually between protein-coding sequence and terminator. The pre-mRNA is first cleaved and then a series of ~200 adenines (A) are added to form poly(A) tail, which protects the RNA from degradation. Poly(A) tail is bound by multiple [poly\(A\)-binding proteins](#) (PABP) necessary for mRNA export and translation re-initiation.



Simple illustration of exons and introns in pre-mRNA and the formation of mature mRNA by splicing. The UTRs are non-coding parts of exons at the ends of the mRNA.

A very important modification of eukaryotic pre-mRNA is [RNA splicing](#). The majority of eukaryotic pre-mRNAs consist of alternating segments called [exons](#) and [introns](#). During the process of splicing, an RNA-protein catalytical complex known as [spliceosome](#) catalyzes two transesterification reactions, which remove an intron and release it in form of lariat structure, and then splice neighbouring exons together. In certain cases, some introns or exons can be either removed or retained in mature mRNA. This so-called

alternative splicing creates series of different transcripts originating from a single gene. Because these transcripts can be potentially translated into different proteins, splicing extends the complexity of eukaryotic gene expression.

Extensive RNA processing may be an evolutionary advantage made possible by the nucleus of eukaryotes. In prokaryotes, transcription and translation happen together, whilst in eukaryotes, the nuclear membrane separates the two processes, giving time for RNA processing to occur.

### Question 5 (10)

Choose the five correct statements in the list of statements given below. Indicate the correct statements by circling the corresponding letter for the statement.

Note: **Only FIVE** statements should be chosen. Zero marks will be awarded if **more** than five statements are chosen.

- A. Genetic information flows from DNA to protein to RNA
- B. Nucleotides are nucleosides with a phosphate group**
- C. Transcription is the process by which the information in an mRNA molecule is used to make proteins with specific amino acid sequences
- D. Ribosomes are made of rRNA and proteins
- E. Eukaryotic chromosomes are linear**
- F. DNA is synthesized in 5' -> 3' direction**
- G. In complementary base pairing C always forms hydrogen bonds with A
- H. Different cell types have different DNA sequences
- I. DNA primase makes an DNA primer**
- J. RNA is always single-stranded**

Correct statements: ...**b**..... .....**e**..... .....**f**..... .....**i**.....  
.....**j**.....

### Question 6

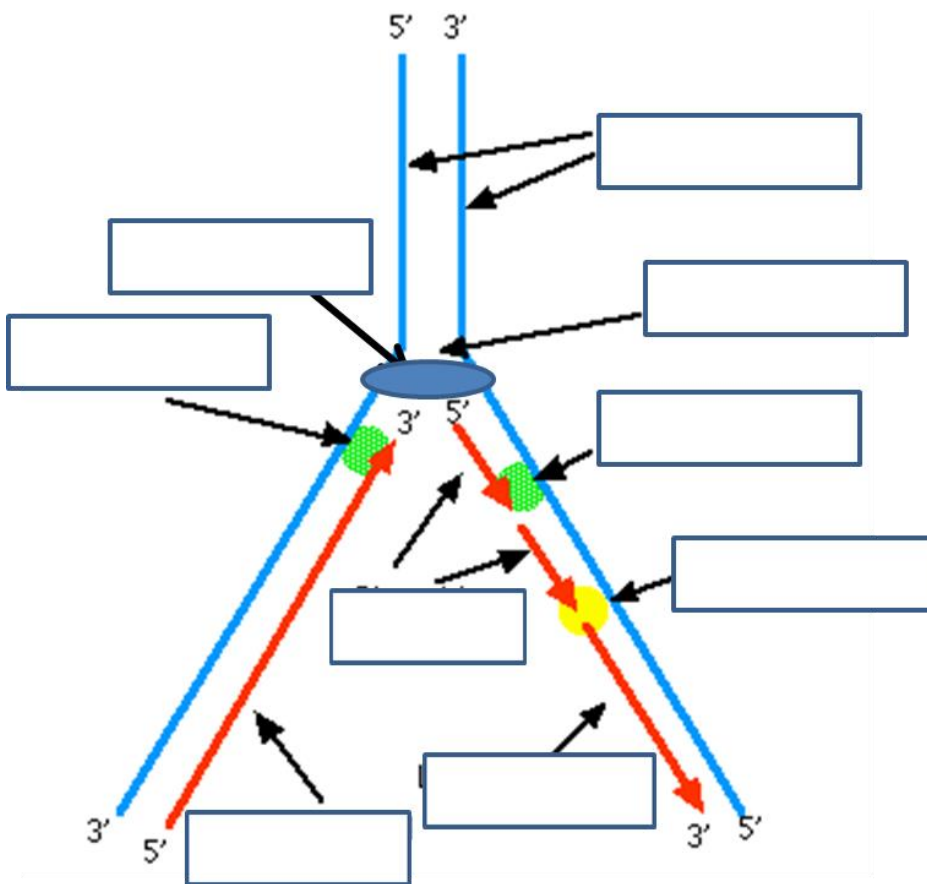
#### a. Name and briefly describe the three stages of transcription (6)

**Initiation** – In this stage the transcription machinery binds to the DNA sequence that is to be copied

**Elongation** – Once the transcription machinery has bound correctly, the process of transcription begins and a new RNA strand is created

**Termination** – Once the gene has been copied completely, transcription stops and the transcription machinery and the newly created RNA strand dissociate from the DNA strand

- b. Identify the structures present in the figure provided below using the letters taken from the code below the figure. (8)



Use the following labels (some can be used more than once):

- A. Replication fork
- B. Helicase/primase
- C. Leading strand
- D. Lagging strand
- E. Okazaki fragment
- F. DNA polymerase
- G. Template strands

The figure is not drawn to scale.

**END of practice MINI-TEST**