Student Name:_____Student Number: _____

BCH/BIO 3170
Molecular Biology – first mid-term October 2009
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MARKING SCHEME

Part 1. Multiple choice questions (15 at 2 pts each):

OL #1:	/2
JC #2 - #15:	/28

Part 2. Short Answer Questions (6 at 5 pts each):

OL #1:	/5
JC #2 - #5:	/25

Part 2. Long answer questions (4 at 10 pts each):

OL #1:	/10
JC #2 - #4	/30

Total: /100

Instructions:
WRITE YOUR NAME and STUDENT NUMBER on the bubble sheet and on EVERY PAGE of the questionnaire.

Absolutely no books, handouts, notes or calculators are allowed.

Write your answers to the multiple choice questions (#1 to 15) on the red bubble sheet. For the multiple choice questions, select the best answer and only one answer.

Write your answers to essay questions (short and long) directly on the Questionnaire

At the end of the examination period, you must return the bubble sheet and the questionnaire.

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Part 1 – Multiple choice questions #1 to 15 (2 points each)
WRITE YOUR ANSWERS ON THE RED BUBBLE SHEET.

1. Which chemical group of the nucleotide Thymine (T) on the base has the most potential to form a hydrogen bond with an Adenine (A) on the opposite DNA strand but does not in the DNA double helix? **[Ans = C]**

A) keto group on C4
B) methyl group on C5
C) keto group on C2
D) imino group on position 3 of the ring
E) none of the above

2. Chromatin remodelling: **[Ans = E]**

A) Involves covalent modifications primarily on the N-terminal of DNA
B) Is an irreversible process that requires ATP hydrolysis
C) Reduces the overall negative charge of histones
D) Is only required for proper gene expression
E) None of the above

3. You have been given a 500kb fragment of DNA. Which of the following DNA sequences would you add to your DNA in order to generate an artificial chromosome? **[Ans = C]**

A) Telomeres, origin of replication and mitotic spindle
B) Centromeres, origin of replication and histones
C) Centromeres, telomeres and origin of replication
D) Origin of replication, centromere and kinetochore
E) Telomeres, centromere and mitotic spindle.

4. Depletion of histone H1 in a cell would: **[Ans = B]**

A) Be lethal to the organism
B) Interfere with proper cellular division
C) Prevent formation of the 10nm fiber
D) Alter gene expression since the histones could no longer be covalently modified
E) Generate an even more compact form of DNA

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5. The human genome sequencing project revealed that: **[Ans = A]**

A) Approximately one fifth of the genome is comprised of genes
B) The human genome contains twice as many genes than originally anticipated
C) The majority of the DNA is associated with histones
D) Mobile genetic elements account for 1.5% of the total genome
E) All of the above

6. Comparing prokaryotes and eukaryotes, which of the following is true? **[Ans = D]**

A) The nucleosomes of prokaryotes are significantly smaller than those of eukaryotes
B) The telomeres of both prokaryotes and eukaryotes require special telomerases to replicate
C) There are twice as many replication forks in eukaryotes than in prokaryotes
D) Replication in both prokaryotes and eukaryotes is dependent on ATP hydrolysis
E) None of the above

7. Mutations affecting Ku70 would result in: **[Ans = E]**

A) Insertion of a nucleotide
B) Altered DNA bases
C) Pyrimidine dimer formation
D) Incorporation of incorrect DNA bases
E) Double stranded DNA breaks

8. Consider one round of replication in a human somatic cell. Which one of the following statements correctly describes the status of the two daughter chromosomes relative to the parent chromosome? **[Ans = C]**

A) One daughter chromosome will be shorter at one end; the other daughter will be normal at both ends
B) One daughter chromosome will be shorter at both ends; the other chromosome will be normal at both ends
C) Both daughter chromosomes will be shorter at one end, which is the opposite end in the two chromosomes
D) Both daughter chromosomes will be shorter at one end, which is the same end in the two chromosomes
E) None of the above

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9. Initiation of replication: **[Ans = B]**

A) Begins with binding of dnaB to AT-rich sequences
B) Results in the formation of four new DNA chains at each origin of replication
C) Requires a DNA primer
D) Occurs preferentially at regions of hemi-methylated DNA
E) Occurs at least twice per cell cycle

10. Why is the uracil in RNA replaced by thymine in DNA? **[Ans = A]**

A) It is impossible to distinguish between a deaminated C and a natural U
B) Because RNA primers are required for DNA synthesis
C) There are no DNA glycosylases able to excise uracil from DNA
D) To minimize the formation of pyrimidine dimers
E) None of the above

11. Which of the following activities does *E. coli* DNA polymerase III lack? **[Ans = A]**

A) 5'—>3' exonuclease
B) 5'—>3' polymerase
C) 3'—>5' exonuclease
D) *E. coli* DNA polymerase III has ALL of the above activities.
E) *E. coli* DNA polymerase III has NONE of the above activities.

12. Which of the following statements regarding RecA is CORRECT? **[Ans = B]**

A) RecA cleaves the DNA molecules during the resolution of Holliday junctions
B) RecA is a DNA-dependent ATP-ase
C) RecA is part of the tetramic Ruv complex involved in DNA strand exchange during recombination
D) RecA induces a double-stranded break in the chromosome to initiate homologous recombination
E) All of these statements are correct

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13. Nitrogenous bases can undergo tautomeric shifts that alter their complementary base-pairing. For the sequence of double-stranded DNA given below**, identify the altered sequence** that would result from a tautomeric shift occuring in the **adenine** as indicated. The orientiation of the top strand is 5' to 3'. **[Ans = C]**

5'-CACG-3' -------> tautomeric shift in A ----------> further round of ----------> altered sequence?
3'-GTGC-5' during replication normal replication

A) CTCG
GAGC

B) CAAG
GTTC

C) CGCG
GCGC

D) CCCG
GGGC

E) CCG
GGC

14. How do some transposable elements move about the genome? (answer = c)

A) Through homologous recombination of heterochromatin
B) Via a viral intermediate and a transposase
C) Via an RNA intermediate produced by the reverse transcriptase enzyme
D) Via a DNA intermediate and base-excision repair
E) The can move by all of the mechanisms described above

15. Which of the following statements is false: **[Ans = D]**

A) Base-excision repair is a homology-dependent mechanism
B) Homologous recombination can be used to repair damaged caused by ionizing radiation
C) Site-specific recombination is guided by specific DNA sequences
D) Retroviral-like transposons require transposase for their movement in the genome
E) Gene conversion during meiosis can use either the maternal strand or the paternal strand of DNA as a template

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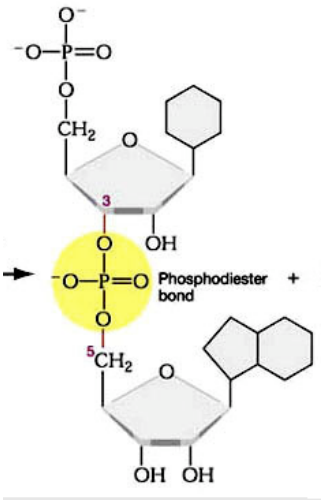
SECTION 2 – Short answer questions #1 to 6 (5 points each)

WRITE YOUR ANSWERS DIRECTLY ON THE EXAM

1. Draw a phosphodiester bond formed between 2 nucleotides of a DNA strand. Include the 5 atoms forming the phosphodiester bond and a simplified illustration of the sugar and base components of the linked nucleotides.

ANSWER: 0.75 point per atom, 0.25 point for the double bond in the phosphodiester link, 1 point for linking C3 and C5 of the riboses.



2. Describe four factors that contribute to the high-fidelity of DNA replication process.

1.25 points each

a) correct pairing of the nucleotide with its complementary base in the template strand
b) After nucleotide binding, but before covalent attachment to the growing chain, the Polymerase undergoes a conformational change which occurs more readily with correct base-pairing
c) Removal of an incorrect nucleotide at the 3' terminus of the growing chain by the 3'-to-5' proofreading exonuclease activity of the polymerase.
d) Removal of a mispaired nucleotide deeper in the growing chain by the mismatch repair system.

3. You are currently completing your Honours project in the same lab as your best friend. Yesterday your supervisor asked you both to insert a fragment containing the RFP gene (red fluorescent protein) into yeast cells. When you examine your cells the next morning, you notice that only half of your colonies are glowing red, while 100% of your friend's colonies are red. What has happened in your cells that caused only half of the colonies to be red? What did your friend do differently in order to ensure her success?

- Lack of expression is due to positional effect (*1.5 point*)
- Gene has inserted in region near heterochromatin, which has spread into the gene to silence its expression (*1 point*)
- Friend succeeded because she added barrier DNA sequences (*1.5 point*), such as HS4, on each side of her gene that protects the gene from the spread of heterochromatin (*1 point*)

4. You have spent your entire summer vacation at the beach, and unfortunately you did not wear sunscreen. Provide a brief description of the PRIMARY mechanism used to repair the pyrimidine dimers that are formed following exposure to UV rays.

- Nucleotide Excision Repair (*1 point*)

- Complex of uvrA and uvrB scans the DNA for distortions in the structure of the helix (*1 point*)

- uvrC/uvrB complex nicks **<u>one strand of the DNA</u>** on each side of the lesion (*1 point*)

- uvrD (helicase) separates the two DNA strands --> excision of lesion (*1 point*)

- DNA Polymerase and DNA Ligase repair the gap (*1 point*)

5. Describe the consequences that would arise of a eukaryotic chromosome (150 Mb in length) had one of the following features:

A) A single replication origin located in the middle of the chromosome

- It would be impossible to replicate the entire length of the chromosome in a timely fashion *(1.25 point)*
- Replication fork moves at about 200-300nt/second (would take 5-6 days)

B) A telomere at only one end of the chromosome

- Chromosome would shorten with every round of replication and eventually end up losing gene sequences (*1.25 points*)
- Protruding ends (single-stranded DNA) would be recognized as a broken chromosome and would be repaired by NHEJ (1.25 points) *award marks even if don`t mention NHEJ specifically*

C) No centromere

- Would not be able to attach to mitotic spindle and after replication, the two chromosomes would not be equally partitioned in the daughter cells. Therefore, cells could die because they do not acquire the proper number of chromosomes. (*1.25 points*)

6. Briefly describe the role of the telomerase enzyme. Why are anti-telomerase drugs considered an attractive target for cancer therapy?

- Telomere extension by the telomerase (1 point) prevents the loss of genetic information associated with shortening after every round of replication (1 point)
- Telomerase active in cancer cells but not most cell types in the body *(1 point)*
- Telomerase activity allows cancer cells to be essentially immortal, so anti-telomerase drugs would limit the number of times a cancer cell could divide *(2 points)*

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Part 3.

Essay questions – Long - 1 to 4. (10 points each)
WRITE YOUR ANSWERS DIRECTLY ON THE EXAM

1. Define the flow of genetic information in the context of the Central Dogma (no more than 3 sentences) (4 points). List 2 of the 3 reasons indicating that the Dogma is not exact and include a brief explanation. (6 points)

1. The flow of the genetic information (DNA to mRNA to protein) is based on the capacity to synthesize and to decode the sequence of complementary nucleic acids. (2 pts) Once information has passed into protein it cannot get out again. (2 pts) Segments of DNA comprise the genes that, through a series of molecular processes, give rise to each of our inherited traits. (optional)

2. Reasons indicating that the Dogma is not exact : (2 of the 3 reasons and 3 points for each)

A. There is no 1 to 1 match between the number of genes and the total number of proteins. The number of genes identified in the human genome is approximately 28 000 while the number of proteins is approximately 100 000. This is because of alternative splicing; one mRNA can generate more than one protein. Alternative splicing is the rearrangement of exons of a mRNA and is catalyzed by the splicing machinery made up of proteins.
B. The flow of genetic information is not unilateral: from DNA to proteins. Proteins can transmit genetic information to nucleic acids through the process of alternative splicing. Also enzymes catalysing the synthesis of DNA can influence the sequence of DNA: repair enzyme to correct mutations.
C. Proteins must fold to be active and folding is catalyzed by chaperones (proteins). Protein folding is not encoded in the genome but catalyzed by chaperones. Protein folding plays an essential role in the cascade of gene expression.

2. A large number of temperature sensitive replication mutants have been isolated in E. coli. These mutants are defective in DNA replication at 42°C but not at 30°C. If the temperature of the medium is raised from 30°C to 42°C, these mutants stop making DNA in one of two characteristic ways. The "quick stop" mutants halt DNA synthesis immediately, whereas the "slow-stop" mutants stop DNA synthesis only after many minutes.

Predict which of the following proteins, if temperature sensitive, would display a "quick-stop" phenotype and which would display a "slow-stop" phenotype when grown at 42°C. In each case explain your prediction.

1) DNA topoisomerase I

Fast-stop (1 point) --> inability to relieve overwinding ahead of the replication fork (1 point)

2) Single-stranded DNA binding-protein

Fast-stop (1 point) --> Inability to stabilize single-stranded DNA at the replication fork (1 point)

3) A replication initiator protein

Slow-stop (1 point) --> DNA molecules that had passed the initiation step before the temperature was increased would continue to replicate the chromosome until an initiation step was required in the next cycle (1 point)

4) DNA ligase

Slow-stop (1 point) --> the progress of the replication fork would not be stopped. Replication would cease only during the next cycle when the nicks were 'uncovered' on the template strand (1 point)

5) Helicase

Fast-stop (1 point) --> Inability to melt/unwind the DNA ahead of the replication fork (1 point)

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3. Describe how the structure known as the "Holliday Junction" is formed. How does this structure contribute to the formation of cross-over chromosomes during meiosis? (10 points)

- Endonucleases and exonucleases generate double-stranded breaks and 3' overhangs, respectively (2 points)
- The multiple binding sites on RecA allow it to bind to ssDNA and dsDNA simultaneously, forming a 3-stranded intermediate structure (2 point)
- Via a base-flipping mechanism, RecA catalyzes the identification of a homologous region (1 point)
- RecA-catalyzed branch migration extends the heteroduplex region to form the Holliday junction (1 point)

- Isomerization of the Holliday junction creates an open, symmetrical structure (2 points)

- Resolution of the open Holliday Junction structure determines whether or not cross-over will take place (controlled by enzyme expressed uniquely during meiosis – 1 point)
    Cleavage of original non-crossing strands = cross-over (0.5 point) *also accept vertical cleavage
    Cleavage of the original crossing strands = no cross-over (0.5 point) *also accept horizontal cleavage
-

4. DNA localized at chromosomal structures such as the telomere and centromere are packaged into heterochromatin. Explain how the special characteristics of each of these structures allow heterochromatin to form. (10 points)

Telomere:

- Telomere-binding proteins bind the ends of chromosomes and recruit the Sir-complex of proteins *(0.5 points)*
- Sir2 is a histone-deacetylase *(1 point)* that binds the under-acetylated N-terminal tails of histones *(1 point)*
- Sir2 deacetylates the N-terminal tails of the neighbouring nucleosome, which allows a new Sir2 protein to bind *(1 point)*
- Chromatin extends by cooperative spreading (*0.5 points* for anything that illustrates this idea)
- Deacetylation increases the overall positive charge of the histones and allows for tighter packaging *(1 point)*

Centromere:

- Histone octamers contain a variant histone H3 = CENPA (2 points)
- Nucleosomes are found at the centromere in alternating blocks containing either the normal histone H3 or the variant histone H3 (1 point)
- When folded into a higher order structure, the nucleosomes containing CENP-A are facing towards the outside of the chromosome (1 point)
- Centromere-specific proteins recognize and bind the variant histone CENP-A in order to form the kinetochore structure (1 point)