Name:
Student #:

University of Toronto

Faculty of Applied Science and Engineering

Division of Engineering Science

Final Examination

BME205H1 – Cells and Biomolecules
Wednesday April 26, 2016, 9:30am – 12:00 pm
Duration: 130 minutes
Examiner: P. Gilbert

ANSWER ALL QUESTIONS ON THESE SHEETS

1. No cell phones are allowed.
2. Type A: Closed book examination, no aids permitted.
3. Calculator: Type 2 – All non-programmable calculators
4. Part 1 Multiple Choice Questions are provided in this booklet. **Your answers are to be placed on the Scantron Sheet**.
5. Part 2 Questions have the mark available in the square brackets [ ]. Each question has a strict sentence limit restriction, **each sentence written above the limit will be deducted half a mark**.
6. The final page contains two resources: (a) The genetic code table and (b) The amino acid structures

Last Name: _____

First Name: _____

Student Number: _____

Tutorial section (Failure to include correct Tutorial section will result in a **loss of 0.5 marks**):

[ ] TUT01	**Wed**	**09:00 – 10:00**	**BA2155**	**(Alex)**
[ ] TUT02	**Wed**	**09:00 – 10:00**	**GB304**	**(Stephanie)**
[ ] TUT03	**Wed**	**09:00 – 10:00**	**BA2165**	**(Michelle)**
[ ] TUT04	**Wed**	**13:00 – 14:00**	**BA2165**	**(Ben)**
[ ] TUT05	**Tues**	**13:00 – 14:00**	**BA2145**	**(Gabi)**
[ ] TUT06	**Tues**	**13:00 – 14:00**	**WB342**	**(Buddhisha)**

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PART 1: Multiple Choice Questions

PART 1 _____ out of 60

PART 2: Short Answer Questions

Question	1	2	3	4	5	6	7	8
Marks Available	5	5	5	5	5	5	5	5
Marks Achieved								

PART 2 _____ out of 40

TOTAL MARKS _____ out of 100

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PART I: Multiple Choice Questions [1 mark each]

Your answer **must** be recorded on the Scantron. Answers circled on these sheets will **not** be counted towards your grade. ***Select the answer that is most correct***.

1. Which of the following **does not** correctly compare or contrast the characteristics of gap junctions and ion channels?

	Gap junctions	Ion channels
(a)	Composed of junctional integrin proteins.	Composed of channel connexin proteins.
(b)	Transfers materials down concentration gradient.	Transfers materials down concentration gradient.
(c)	Transfers selection depends on molecular weight.	Transfer selection depends on specificity for a particular ion.
(d)	Can close in response to change in ion concentration.	Can close in response to change in chemical or electrical signals.
(e)	Transfer of materials involved in intercellular communication.	Transfer of materials occurs rapidly in an aqueous environment.

2. Water is a unique molecule and essential for supporting life on earth in part because _____.

a. two of the three atoms readily form H-bonds
b. it requires very little heat to evaporate it
c. both covalent O-H bonds are highly polarized
d. it is symmetric, with each H-atom evenly spaced around the oxygen
e. (a), (b), (c), and (d) are all features of the water molecule

3. Which of the following statements about the proton-motive force are TRUE?

a. It is a combination of a proton concentration gradient and the membrane potential
b. It is necessary for ATP generation by the ATP synthase
c. It does not require the electron transport chain
d. All of the above are correct
e. Only (a) and (b) are correct

4. RNA splicing was first discovered through hybridization experiments. These experiments supported the idea of the presence of precursor mRNA that gets modified to the mRNA found in the cytoplasm. To conduct these experiments, scientists mixed the coding strand of genomic DNA and mRNA and then visualized the DNA-RNA hybrid using electron microscopy. What result do you expect to see that supports the conclusion from these experiments?

a. Double-stranded DNA-RNA hybrid with no mismatches
b. Double-stranded DNA-RNA hybrid with loops indicative of introns on the DNA strand
c. Double-stranded DNA-RNA hybrid with loops indicative of exons on the DNA strand
d. Double-stranded DNA-RNA hybrid with loops indicative of introns on the RNA strand
e. Double-stranded DNA-RNA hybrid with loops indicative of exons on the RNA strand

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5. Myosin V is essential for:
   a. vesicle transport
   b. transcription
   c. muscle contraction
   d. actin polymerization
   e. Both (a) and (c) are correct

6. The following phases of aerobic oxidation require oxygen:
   a. ATP synthesis, citric acid cycle, electron transport chain
   b. Electron transport chain, ATP synthesis, glycolysis
   c. Glycolysis, electron transport chain, citric acid cycle
   d. Citric acid cycle, electron transport chain, glycolysis and ATP synthesis

7. Enzymes accelerate biochemical reactions by:
   a. Lowering the Gibbs free energy of the reactants
   b. Increasing the Gibbs free energy of the products
   c. Being neither destroyed or consumed
   d. Lowering the activation energy

8. Which of the following **does not** describe processes involved in exocytosis?
   a. A change in luminal-cytosolic polarity across the membrane occurs upon formation of the fusion pore.
   b. Vesicles deliver cell survival factors from the mitochondria to other organelles.
   c. The luminal phase environment of the vesicle can be changed to suit its cargo.
   d. Transport vesicles fuse with the lipid bilayer membrane to release contents.
   e. The process is triggered by a change in ion concentration balance.

9. Biomembrane microdomains consisting of cholesterol and sphingolipid clusters are:
   a. Studied using detergent extraction methods
   b. Termed 'lipid rafts'
   c. Specialized sites of signaling
   d. All of the above are correct

10. The _____ ion is found at higher concentrations in the cytoplasm than in the blood, which is an essential cellular feature to ensure proper protein synthesis.
   a. Ca^{2+}
   b. Cl-
   c. Na+
   d. K+
   e. All of the above are correct

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11. Covalent linkage to what small, highly-conserved protein marks proteins for destruction?
    a. ubiquitin
    b. phosphate
    c. actin
    d. GTP
    e. ATP

12. While RNA polymerase is a processive enzyme that remains attached to the DNA over long stretches of template, it must be associated _____ enough so that it can move from nucleotide to nucleotide along the template.
    a. loosely
    b. angularly
    c. tightly
    d. rapidly

13. During transcription, RNA polymerase II adds nucleotides at a rate of 20-50 nucleotides per second to the growing RNA chain in the 5' to 3' direction. How fast would a 10 kb gene be transcribed by RNA polymerase II?
    a. ~3 to 8 minutes
    b. ~ 3 to 8 seconds
    c. ~ 3 to 8 hours
    d. ~ 3 to 8 days

14. What aspect of the sugar molecule structure makes them so water soluble?
    a. The glycosidic bonds
    b. The branched structure
    c. The ring structure
    d. The carbonyl groups
    e. The hydroxyl groups

15. During the second step of the PCR cycle:
    a. The PCR primers anneal to the template strand
    b. DNA binds and extends the sequence
    c. DNA is denatured by high temperature
    d. Both the DNA and DNA polymerase are denatured by NaOH

16. The nucleotide at which transcription is initiated is referred to as _____.
    a. 0
    b. +1
    c. –1
    d. +2
    e. –2

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17. Where is the RNA polymerase II phosphorylated during its activation?
    a. in the carboxyl-terminal domain (CTD) of the largest RNA polymerase II subunit
    b. on the N-terminal end of the largest RNA polymerase II subunit
    c. on the central 20 amino acids of the largest RNA polymerase II subunit
    d. on the 3' end of the largest RNA polymerase II subunit
    e. on the 5' end of the largest RNA polymerase II subunit

18. RNA folding is mediated by interactions between complementary base pairs. The following RNA strand folds into a stem loop structure. How many complementary base pairs are there in the following structure?

ACGUGCCACGAUUCAACGUGGCACAGUACGU

    a. 12
    b. 6
    c. 8
    d. 10
    e. 16

19. The _____ is a network of globular proteins comprised of _____:
    a. Muscle myofibre; microtubules
    b. ATP synthase; actin and microtubules
    c. Cytoskeleton; microtubules, actin and intracellular vesicles
    d. Cytoskeleton; microtubules, intermediate filaments, and microfilaments
    e. Basement membrane; fibronectin, laminin, etc

20. The 3' end of most eukaryotic mRNAs contains a _____, while the 5' end has a _____.
    a. poly(U) tail, methylated guanosine cap
    b. methylated guanosine cap, poly(A) tail
    c. poly(A) tail, methylated guanosine cap
    d. poly(A) tail, sulfonated guanosine cap
    e. methylated guanosine cap, poly(U) tail

21. You isolate DNA from a particular organism and analyze it. The amount of cytosine was 4 µmoles and the A+T/C+G ratio is 0.25. How much thymine should be in the sample?
    a. 1 µmol
    b. 2 µmoles
    c. 3 µmoles
    d. 4 µmoles
    e. 5 µmoles

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22. If an exon is not supposed to be included in the mature mRNA, it must be excised. With what other RNA sequences is it removed together with?

a. one flanking exon and one flanking intron
b. the promoter
c. the poly(A) sequence
d. the flanking exons
e. the flanking introns

23. Translate the following mRNA sequence:
5' – GAUACUGAUGCCAUUCGGGGUAUACUAGUUGACUAG – 3'

a. (N) – Asp – Thr – Asp – Ala – Phe – Arg – Gly – Ile – Pro – Val – Asp – (C)
b. (C) – Asp – Gln – Leu – Thr – Ile – Trp – Gly – Leu – Pro – (stop) – (N)
c. (N) – Met – Pro – Phe – Gly – Val – Tyr – (stop) – (C)
d. (N) – Met – Pro – Phe – Gly – Val – Tyr – (stop & next gene) – Leu – Thr – (C)
e. (C) – Met – Gly – Leu – Thr – Val – Val – Ile – (N)

24. Which of the phenomena below is responsible for the ability of one gene to code for more than one polypeptide?

a. transcription
b. translation
c. alternative splicing
d. code degeneracy
e. wobble hypothesis

25. When the Pi (organic phosphate) is released, the myosin head:

a. Moves the actin filament to the left
b. Produces a power stroke
c. Rotates into the 'cocked' state
d. Binds to actin
e. Is released from actin

26. Integral membrane proteins have been engineered to lack the portion that normally projects into the extracellular space. What happens to the mobility of this engineered protein in the plasma membrane of cells?:

a. They do not move at all
b. They move at a much greater rate than the wild-type protein
c. They are not inserted into the membrane so nothing can be learned about their mobility
d. They move at a much slower rate than the intact protein

27. What is appropriate clothing for laboratory work in the MB325 teaching lab?

a. Old clothing
b. Comfortable shoes, loose clothing
c. Long pants, long hair tied back, close-toed shoes
d. Sandals, shorts, cool clothing
e. Any clothing is appropriate for the lab

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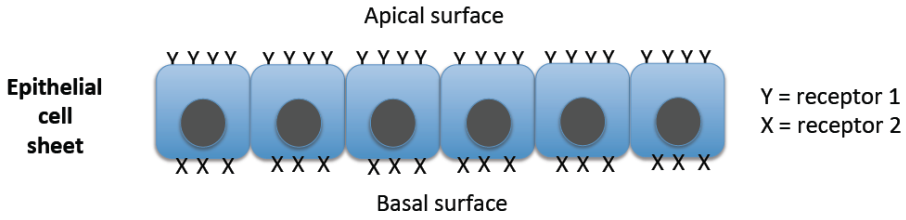
28. The activation of a membrane integrin by the binding of its cytoplasmic portion to molecules in the cytoplasm and the resultant increase in its affinity for an extracellular ligand is called _____.

a. integration
b. inside-out signaling
c. outside-in signaling
d. right-side-out signaling
e. simple signaling

29. Cell surface receptors are integral membrane proteins embedded within the plasma membrane. Like the lipids that make up the plasma membrane, receptors diffuse laterally in the membrane. Therefore, in theory, we might expect a given receptor to be equally distributed around the entire cell. However, in epithelial cell sheets it is common to find a specific receptor restricted to the apical or the basal surface of the cell. What cellular structure is involved in **maintaining** polarized distribution of receptors?



a. Cadherins
b. Gap junctions
c. Hemidesmosomes
d. Tight junctions
e. Focal adhesions

30. Which of the following represents an important function of the extracellular matrix?

a. Cell signaling
b. Structural support
c. Tissue organization
d. Tissue strength
e. All of these choices are important functions of the extracellular matrix

31. Which pH below would be most likely to favor the enzymatic function of a lysosomal enzyme?

a. 4.6
b. 6.5
c. 7.6
d. 8.5
e. 11.3

32. The building blocks of a nucleotide are _____.

a. a phosphate group and a nitrogenous base
b. a pentose sugar, a phosphate group and an amino acid
c. a pentose sugar and a phosphate group
d. a pentose sugar, a phosphate group and a nitrogenous base
e. a pentose sugar and a nitrogenous base

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33. In glycolysis, _____ is converted to pyruvate and ATP is generated by _____ in the _____ of oxygen.

a. Lipids, proton motive force, absence
b. Glucose, substrate level phosphorylation, presence
c. Glucose, substrate level phosphorylation, absence
d. Glucose, proton motive force, absence
e. Glucose, proton motive force, presence

34. Which molecule below is a GTP-binding protein that is required for the release of a clathrin-coated vesicle from the membrane on which it was formed?

a. dynamin
b. AP2
c. actin
d. clathrin
e. triskelion

35. Which of the models below suggests that the Golgi cisternae are transient structures that form at the cis face of the stack by fusion of membranous carriers from the ER and ERGIC and that each cisterna travels through the Golgi complex from the cis to the trans end of the stack, changing in composition as it progresses?

a. the cargo carrying model
b. the vesicular transport model
c. the secretory transport model
d. the cisternal maturation model
e. the chemiosmotic model

36. Restriction endonucleases:

a. Recognize specific symmetric (palindrome) DNA sequences
b. Have been isolated as molecular biology tools from bacteria.
c. Can create sticky- or blunt-ended fragments
d. All of above are correct
e. Only (a) and (b) are correct

37. Rigor mortis takes place after death of an organism and is characterized by the stiffening of muscles. This happens because:

1. the body is depleted of ADP and therefore unable to break the actin-myosin bond required for muscle relaxation
2. the body is depleted of ATP and therefore unable to rotate the myosin head required for muscle relaxation
3. the actin filaments begin rapidly polymerizing which then stiffen the muscles
4. the body is depleted of ATP and therefore unable to break the actin-myosin bond required for muscle relaxation

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38. Which of the following point mutation(s) will, by definition, result in no change in the amino acid sequence?
    a. Synonymous DNA mutation
    b. Non-synonymous DNA mutation
    c. Nonsense DNA mutation
    d. Frameshift DNA mutation

39. Which of the following is a function of membranes?
    a. compartmentalization
    b. selectively permeable barrier
    c. mediates intercellular interactions
    d. helps cells respond to external stimuli
    e. All of these are correct.

40. _____ takes up large particles to form a phagosome, which then fuses with a lysosome to digest the particles. _____ is responsible for uptake of materials that will be used by the cells and for dampening a signal transduction event.
    a. Bulk phase endocytosis, Receptor-mediated endocytosis
    b. Phagocytosis; Bulk phase endocytosis
    c. Phagocytosis; Receptor-mediated endocytosis
    d. Pinocytosis; Phagocytosis
    e. Receptor-mediated endocytosis; Pinocytosis

41. The RGD (arginine-glycine-aspartic acid) peptide can function as a clot-busting agent because:
    a. It antagonizes the binding of fibrinogen to integrin receptors found on platelets.
    b. It induces the breaking down of fibrin into fibrinogen.
    c. It leads to platelet death.
    d. a, b, and c are all correct explanations of how RGD peptides fights clots.

42. You are given 2 different strands of DNA. Strand 1 has a 40% GC content, while strand 2 has a 60% GC content. Which of the following is true regarding the melting temperatures of strands 1 and 2?
    a. The melting temperature of strand 2 will be higher, because G and C are connected with more hydrogen bonds than A and T.
    b. The melting temperature of strand 2 will be higher, because A and T are connected with more hydrogen bonds than G and C.
    c. The melting temperature of strand 1 will be higher, because A and T are connected with more hydrogen bonds than G and C.
    d. The melting temperature of strand 1 will be higher, because G and C are connected with ionic bonds which are hard to break.
    e. The melting temperatures will be identical because they depend on the physicochemical properties of DNA and not its GC content.

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43. Signaling receptors internalized via _____ serves to dampen signal transduction if they are then transported to the _____ for destruction.

a. endocytosis/lysosome
b. endocytosis/proteasome
c. exocytosis /lysosome
d. exocytosis/proteasome

44. Scientists have been able to modify yeast such that their ribosomes incorporate alternative amino acids that are not in the usual set of 20. They do this by designing new tRNAs that target one or more of the stop codons, replacing it's normal function. This concept is called the "expanded genetic code." To honour your favorite child-star turned pop-icon, you want to design a tRNA to incorporate Selenocysteine. Your goal is to replace two of the three stop codons with a single new tRNA. Which tRNA anticodon sequence would you design that could potentially replace 2 stop codons?

a. 5' CUA 3'
b. 5' UCA 3'
c. 5' UGA 3'
d. 5' UAG 3'
e. A single tRNA will not be sufficient. You need two tRNAs to target two different sequences.

45. The regulatory region of gene X is located at position –23. This means that:

a. the gene is actively being transcribed.
b. the regulatory sequence is upstream from the transcription start site of the gene.
c. the regulatory sequence is downstream from the transcription start site of the gene.
d. The gene starts at locus –23 of the genome.
e. The gene cannot be transcribed, because it regulatory sequence is not at +1.

46. You mix a cell sample with genetically engineered cells that express n-cadherin, e-cadherin, or p-cadherin. You then observe that your sample adheres preferentially to cells expressing n-cadherin. Your sample most likely expresses:

a. n-cadherin
b. e-cadherin
c. p-cadherin
d. no cadherin
e. both n-cadherin and e-cadherin

47. If you look at a culture dish of cells under a microscope and you see that all the cells are small, round, and floating this could mean:

a. The cells are dead.
b. The cells are contaminated and what you are seeing is floating bacteria.
c. The cells are fine; it is a non-adherent cell line.
d. (a), (b), and (c) could explain why the cells are small, round, and floating.

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48. Which of the following single-stranded and double-stranded nucleotide sequences could correspond to a portion of mRNA sequence?

a. 5' – TGCGATAGGAACCT – 3'
b. 5' – TGCGATAGGAACCT – 3'
   3' – AGGCTATCCTTGGA – 5'
c. 5' – UGCGAUAGGAACCU – 3'
   3' – ACGCUAUCCUUGGA – 5'
d. 5' – UGCGAUAGGAACCT – 3'
e. 5' – UGCGAUAGGAACCU – 3'

49. siRNA is a technique to control gene expression at the _____ level.

a. Post-Translational
b. Translational
c. Transcriptional
d. Processing

50. Investigating the effects of a compound, *THT-PST*, on cells which can grow on minimal media. You apply the chemical onto cells, and grow them on a complete growth media for a few days to remove any trace of the chemical from the cells. After verifying that they can no longer grow on minimal media, you find that the cells can grow on minimal media supplemented with Riboflavin. What lasting effect did *THT-PST* have on the cells?

a. It inhibited the enzyme activity somewhere in the Riboflavin synthetic pathway.
b. It damaged the DNA of a gene somewhere in the Riboflavin synthetic pathway.
c. It damaged the RNA of a gene somewhere in the Riboflavin synthetic pathway.
d. It breaks down Riboflavin, not allowing the cell to make use of it.

51. Which of the following correctly describes the mechanism involved in CRISPR/Cas9?

a. It is a mechanism naturally present in mammalian cells
b. The Cas9 is guided by a reference RNA sequence
c. The Cas9 endonuclease can be delivered via injection in saline solution
d. The DNA sequence that is carried can target any sequence/gene of interest
e. The CRSPR/Cas9 needs to be active in the cytoplasm

52. Iron binds to the transferrin receptor on the surface of cells and the receptor / ligand complex enters cells via receptor-mediated endocytosis. You are studying fibroblasts from a patient with abnormally high levels of extracellular iron and abnormally low levels of intracellular iron. Interestingly, iron is seen to accumulate on the extracellular side of the cell, but fails to enter the cell. Which of the following provides the best explain this observation?

a. a mutation in the transferrin receptor prevents the transferrin receptor from binding to the adaptor protein complex
b. a mutation in the adaptor protein complex prevents the adaptor protein complex from interacting with clathrin
c. a mutation in the transferrin receptor prevents iron from binding the transferrin receptor
d. both (a) and (b) can explain the observation
e. both (a) and (c) can explain the observation

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53. What statement below explains the uniform width of the DNA molecule along its entire width?
    a. Repulsion between phosphate groups keeps the strands a uniform distance apart
    b. Attraction between phosphate groups keeps the strands a uniform distance apart
    c. A purine nitrogenous base always pairs with anther purine nitrogenous base
    d. A pyrimidine nitrogenous base always pairs with another pyrimidine nitrogenous base
    e. A pyrimidine nitrogenous base always pairs with a purine nitrogenous base

54. Which of the following correctly describes the characteristics of collagen 1 and IV?

	Collagen I	Collagen IV
(a)	Has a globular structure.	Has a fribrillar structure.
(b)	Found in abundance in tendons and ligaments.	Found in abundance in scar tissue.
(c)	Provides an adaptive structural network that changes solubility according to environmental changes.	Provides an insoluble network and determines mechanical properties.
(d)	Forms flat collagen lattices.	Forms tight collagen helices.
(e)	Found in the extracellular matrix of most tissues.	Found in the basal lamina of the basement membrane.

55. What drives the rotation of the F_1 head of ATP synthase?
    a. proton movement from the matrix to the intermembrane space
    b. proton movement from intermembrane space to the matrix
    c. proton movement from the cytoplasm to the intermembrane space
    d. ATP hydrolysis
    e. ATP condensation

56. Once the sigma (σ) factor leaves the core enzyme, what happens?
    a. Transcription begins.
    b. Transcription terminates.
    c. The core enzyme continues synthesis.
    d. The core enzyme discontinues synthesis.
    e. The core enzyme backs up 25 nucleotides.

57. What is always the first amino acid incorporated at the N-terminus of a nascent polypeptide chain?
    a. cysteine
    b. leucine
    c. methionine
    d. asparagine
    e. glycine

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58. Which of the following statements regarding general transcription factors (GTFs) is **False**?

a. General transcription factors form the pre-initiation complex.
b. In eukaryotes the preinitiation complex assembles at the TATA box.
c. As long as TATA-binding protein (TBP) remains bound to the promoter, additional rounds of transcription will occur.
d. TFIIH has helicase and RNA editing enzymatic activities.

59. Fill in the blanks with a letter from below. mRNA concentration is a function of its rate of _____ and rate of _____.

a. synthesis; nuclear export
b. synthesis; degradation
c. splicing; degradation
d. splicing; nuclear export
e. degradation; nuclear export

60. Proteins are described by their structure. The sequence of amino acids that make up a protein describes which level of protein structure?

a. primary
b. secondary
c. tertiary
d. quaternary

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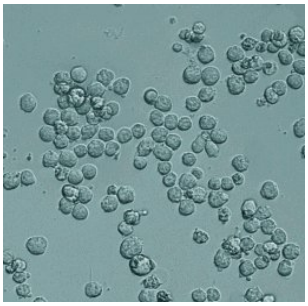
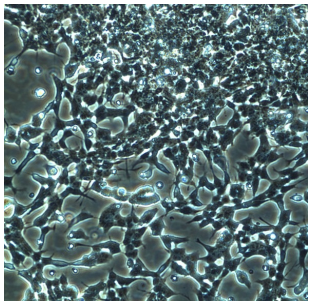
PART 2: Short Answer Questions

Q1. [**5 marks**] Your Lab TA hands you two flasks each with 10 mL of culture media containing cells. She tells you to transfer each flask of cells into a 10 cm tissue culture plastic dish, to place the dishes in the incubator overnight, and to acquire representative images the next day. Below are the light microscope images you captured.



Flask 1



Flask 2

Q1a. Based on the images you took, which flask contained non-adherent cells and which contained adherent cell [0.5 mark]? How do you know [0.5 mark]? Flask 1 = non-adherent; Flask 2 = adherent [0.5 mark]. The cells from flask 1 are very round in morphology, while the cells from flask 2 appear to have attached to the tissue culture plate and spread out [0.5 mark].

Q1b. Your TA then asks you to quantify the viability of the cells you plated. Before she tells you what to do, you suggest a method you learned in the BME205 Microscopy lab. What dye do you suggest staining the cells with [0.5 mark]? How does this dye allow you to assess viability [1 mark]? Tryan blue dye [0.5 mark]. The plasma membrane of live cells excludes the dye, but the membrane of dead cells is permeable allowing the dye to enter the cell and stain the cell a blue colour [1 mark].

Q1c. Ultimately to assess viability you will need to calculate the % total cells that are alive in each field of view. You find that it rather difficult to discern how many cells are in Flask 2, but you remember another dye that you used in BME205 microscopy lab that could help out. What is the dye [0.5 mark]? How will it help you to count the total number of cells [1 mark]. Fast Blast DNA stain [0.5 marks] will allow you to easily visualize the circular nucleus of each cell in the Flask 2 images [1 mark].

Q1d. Within the Flask 2 field of view you count 22 blue cells using the method in **Q1b** and 236 cells using the method in **Q1c**. What % of the cells in your Flask 2 field of view are alive [1 mark]?
236–22=214 live cells
(214/236)*100 = 91% of the cells are alive

Q2. [5 marks] Consider the following questions relating to restriction digest.

Q2a. For your summer research project you will perform a restriction digest experiment on the *Denimin* gene from DNA isolated from volunteer blood donors. Unfortunately, it seems that your DNA isolation method was not efficient and you have very little DNA to work with. Your supervisor tells you to amplify the DNA using PCR, since your lab is equipped with machinery and reagents to perform PCR, but your lab does not have any reagents specific for the *Denimin* gene. How many and which *Denimin* specific reagents do you need to order? Be specific, but limit your response to 1 sentence [2 marks].

Solution: You need 2 primers. [1 mark] for using the word "primer" and [1 mark] for indicating that two primers are needed. (Writing that both a forward and reverse primer are needed is also acceptable)

Q2b. You fully digest the DNA fragment pictured below (top portion of image) with the restriction enzymes EcoRI, HindII NotI, and XbaI and then run the sample on an agarose gel. In the white box, which represents your agarose gel (bottom right), draw the positions of the horizontal bands that will appear on your agarose gel based on the DNA ladder image (bottom left) [2 marks].

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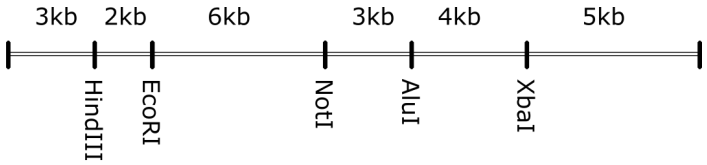

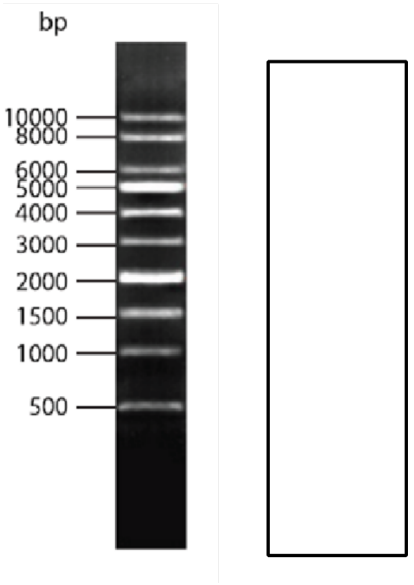

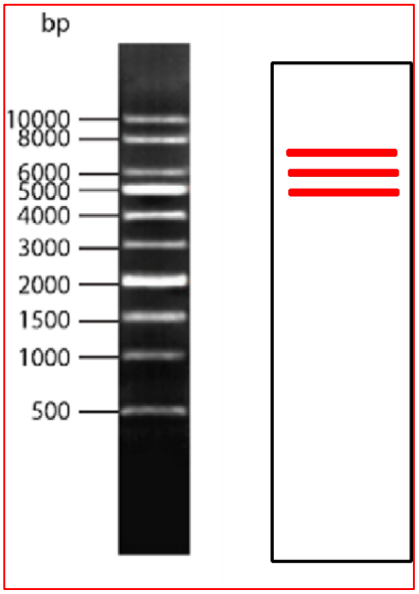

Marking Scheme: [1 mark] for correct number of bands and [1 mark] for correct placement of bands

Q2c. Unhappy with the quantity of your largest fragment on your gel you decide to perform PCR with your digested sample. Though your PCR worked with an undigested sample, it did not work with the digest under identical conditions. Explain the most likely reason why the PCR reaction failed, taking into account the mechanism of PCR [1 mark].
Marking scheme: [1 mark] for indicating that the primers are on different DNA molecules, or that breaks in the amplified strand would prevent the primers from amplifying exponentially.

Q3. **[5 marks]** As a budding scientist, you are interested in the role of SMH on the migration of endothelial cells. To study its importance, you decide to employ siRNA technology to knock-down the SMH gene in cultured cells. The following is your mRNA target sequence of interest:

5' – CCG AUC AAU CGC UUU GGU AUC CGG GAA ACC – 3'

Q3a. Design an <u>siRNA</u> to knockdown expression of the gene above [2 marks]
Marking Scheme:

+0.5 mark: contains an RNA sequence with perfect complementary to the above target
+0.5 mark: it is 21-23 nucleotides in length
+1 mark: double stranded (sense and anti-sense components)

Should contain 21-23 base pairs of the following:
5' - CCG AUC AAU CGC UUU GGU AUC CGG GAA ACC – 3' (Sense)
3' - GGC UAG UUA GCG AAA CCA UAG GCC CUU UGG – 5' (Anti-sense) or written 5-3',

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5' – GGU UUC CCG GAU ACC AAA GCG AUU GAU CGG - 3'

Q3b. In two sentences or fewer, name [0.5 marks] and describe [0.5 marks] a method to deliver your newly designed siRNA sequence into cells.

Any one of the following two part answers will suffice for full marks:

- Lipofection- encapsulate in liposome and deliver to cells (Transfection)
- Electroporation – brief powerful electric shock is introduced to cells, causing temporary loss of semipermeability and allowing to introduce siRNA
- Viral transfection – introduce into cells via a viral vector

Q3c. Will the siRNA permanently silence the SMH transcript? Explain your answer in 2 or fewer sentences [1 mark].

No. siRNA provides transient knockdown [0.5 mark] of a single gene since knockdown occurs at the mRNA level in the cytoplasm [0.5 mark] rather than by permanently altering the genome sequence.

Q3d. Describe one difference between siRNA and miRNA in animals. Limit your answer to 2 sentences or less [1 mark].

Any of the following differences may be described:

1. siRNA is synthetic and used as a research tool in the laboratory [0.5 mark], while miRNA is naturally occurring [0.5 mark].
2. siRNA is double stranded [0.5 mark], while miRNA is derived from a single-stranded precursor that folds upon itself [0.5 mark].
3. siRNA are typically designed to have high specificity to the target sequence [0.5 mark]. However, because miRNAs are not perfectly matched to a target sequence, they can regulate the expression of more than one gene [0.5 mark].

Q4. [5 marks] As we age our tissues lose their innate ability to repair themselves. You hypothesize that one explanation for this might be that the aged tissue environment is missing the soluble proteins that normally tell immune cells to migrate towards a region of injury. You plan out a quick and dirty experiment. You will culture young macrophages, a type of immune cell, on one side of a culture dish. On the other side of the culture dish you will place porous beads that have been soaked in skeletal muscle tissue extract created from either young mice (Condition 1) OR from very old mice (Condition 2). The tissue extract slowly leaks out of the porous beads generating a concentration gradient of the young or old skeletal muscle soluble proteins. You acquire microscope images of your macrophage cells every 3 minutes for 12hrs and then analyze the resulting time-lapse videos.

Q4a. [1 mark] Just as you predicted, the macrophages exposed to young skeletal muscle extract exhibit directed migration towards the beads (where the extract concentration is highest), while the macrophages exposed to old skeletal muscle extract migrate randomly. Below are the steps involved in migration. Indicate what order each step of migration occurs by labeling within the chart (e.g. Step 1, Step 2, Step 3 or Step 4).

Order	Steps in cell locomotion
Step 3	The nucleus, organelles and cytoplasm are pushed toward the front of the cell in a myosin-dependent manner
Step 1	Lamellipodia at the front of the cell extends forward (extension)
Step 4	The rear of the cell detaches from the substrate and adhesion receptors are recycled to the front of the cell
Step 2	A new focal adhesion at the front of the cell is formed

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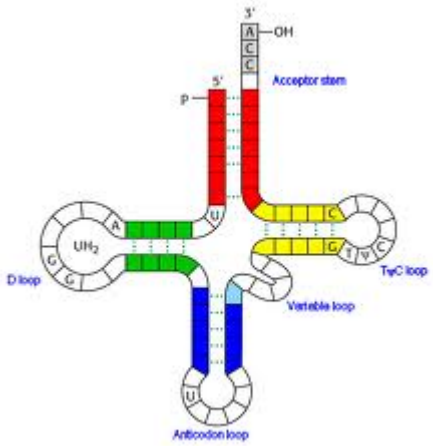
Q4b. [2 marks] In two sentences or less, describe the mechanism that drives the extension step of migration.

The extension step of migration is induced by a rapid burst of actin polymerization at the leading edge of the cell that pushes the cell membrane forward [1 mark]. Critical to this polymerization burst is the generation of branched actin structures [0.5 marks], which requires a nucleation event [0.5 marks] AND/OR is an Arp2/3-dependent process [0.5 marks]. **For the final +0.5 mark** one **must indicate that the generation of branched actin structures requires a nucleation event AND/OR Arp2/3.**

Q4c. [2 marks] Actin monomers are bound to ATP during the polymerization process, however, ATP hydrolysis is not thought to be required to polymerize an actin filament. What is a role that ATP hydrolysis is thought to serve in actin filaments (note: discussed in class). Limit your answer to 2 sentences. Actin monomers bound to ATP will be concentrated at the plus end of the fiber [0.5 marks]. As time passes, ATP in actin monomers close to the minus end hydrolyze to ADP + pi [0.5 marks] causing a conformational change [0.5 marks] that may be detected by proteins such as Arp2/3 and serve as a landmark for branch sites [0.5 marks].

Q5 [5 marks]. The anticodon sequence of a tRNA is indicated just below the tRNA image. Write the codon that this tRNA pairs perfectly with [1 mark] and the amino acid that can associate with the tRNA [1 mark]. What other codon(s) would be recognized by this tRNA [2 marks]? What is the term used to describe this indiscriminate binding [1 mark]?



Solution:

mRNA codon- 5'- ___ ___ ___ -3' (CCG)

Amino Acid(s) - _____

Codon(s):

Anticodon = 5'-CGG-3'

Name of the interaction:

Amino Acid = Proline
Codons = CCC, CCA, CCG, and CCU [1 mark for partially correct, 2 marks for listing all four sequences]
Name of interaction = wobble hypothesis / wobble interaction

Q6. [5 marks] During your summer research project, you are tasked with conjugating (a.k.a. 'attaching') thiolated DNA to gold nanoparticles. You synthesize DNA-conjugated gold nanoparticles and you then try to quantify the number of DNA strands on the particles. You first strip the DNA from 100 particles using 10% of 1 M DTT. You then use SYBR Gold, a nucleic acid stain, to stain the stripped DNA and visualize the fluorescence using a microplate reader. Alongside your sample, you run a standard curve with known DNA quantities to determine the concentration of your stripped DNA sample.

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The following table indicates the DNA standards and corresponding fluorescence intensity obtained for the standard curve.

Number of DNA strands	Fluorescence intensity (AU)
5.500×10^7	5.495×10^5
3.025×10^7	3.020×10^5
5.500×10^6	5.45×10^4
3.025×10^6	2.975×10^4
5.500×10^5	5.000×10^3

If you obtain a fluorescence intensity of 4×10^5 AU for your stripped DNA, how many strands of DNA were attached **per particle** [5 marks]? Show all work and reasoning and include subheadings as a guide for all your steps. **1 mark will be deducted if subheadings are not included.**

Step 1. Determining the relationship between number of DNA and fluorescence using standard curve [2 marks]

In a standard curve, number of DNA strands will be the independent variable (x-axis) and fluorescence will be the dependent variable (y-axis).

Slope = $(\text{fluorescence}_2 - \text{fluorescence}_1)/ (\text{Number of DNA}_2 - \text{Number of DNA}_1)$

Slope = $(5.495 \times 10^5 - 5.000 \times 10^3) / (5.500 \times 10^7 - 5.500 \times 10^5)$

$= 0.01$

Intercept: $5.495 \times 10^5 = 5.500 \times 10^7 (0.01) + b$

$-b = 5.500 \times 10^7 (0.01) - 5.495 \times 10^5$

$b = -500$

Fluorescence = 0.01 * Number of DNA strands - 500

No part marks given for attempts that do not provide the correct numbers.

2 marks for

1) Correct relationship (including exact numbers where slope = 0.01 and intercept = -500) between fluorescence and DNA

Step 2. Determining number of stripped DNA strands [1 mark] using above relationship [1 mark]

$4 \times 10^5 = 0.01(\text{Number of DNA}) - 500$

Number of DNA = 4.01×10^7

1 mark for using above relationship to calculate number of strands – will not be penalized if step 1 is incorrect

Step 3. Determining number of strands per particle [2 marks]

DNA per particle = 4.01×10^7 DNA / 100 particles = 4.01×10^5 DNA per particle

1 mark for dividing step 2 result by 100 particles

1 mark for correct answer

Q7. [5 marks] Cells require a substrate to grow on. Correct binding between the extracellular matrix (ECM) and integrins promotes growth and proliferation. Answer the following questions regarding this area of study.

Q7a. What is the name of the technique you learned in tutorials which allows you to quantify the force of cells binding and pulling on the ECM [1 mark]?

Solution: Traction force microscopy [1 mark]

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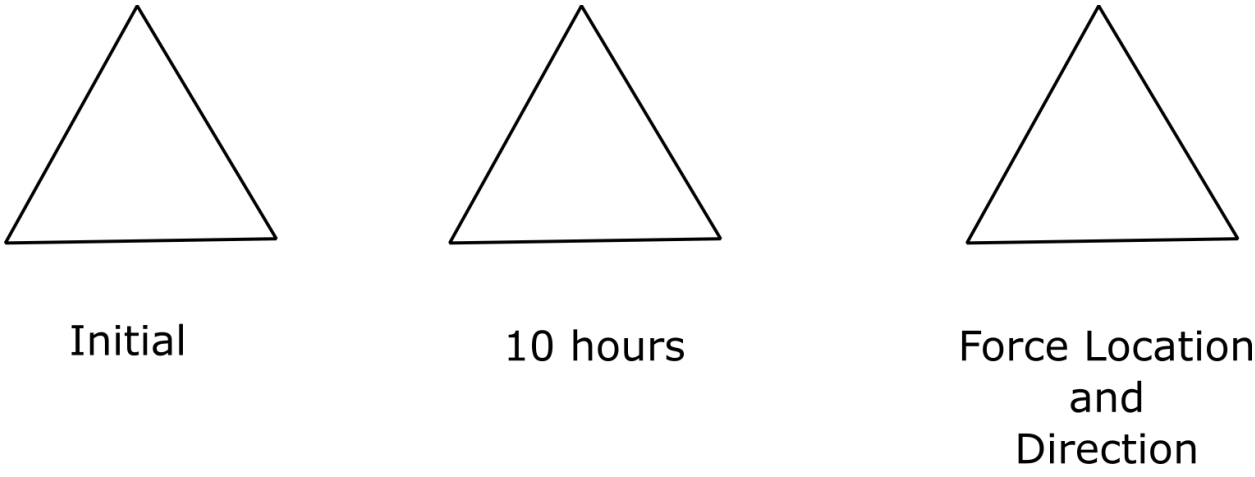
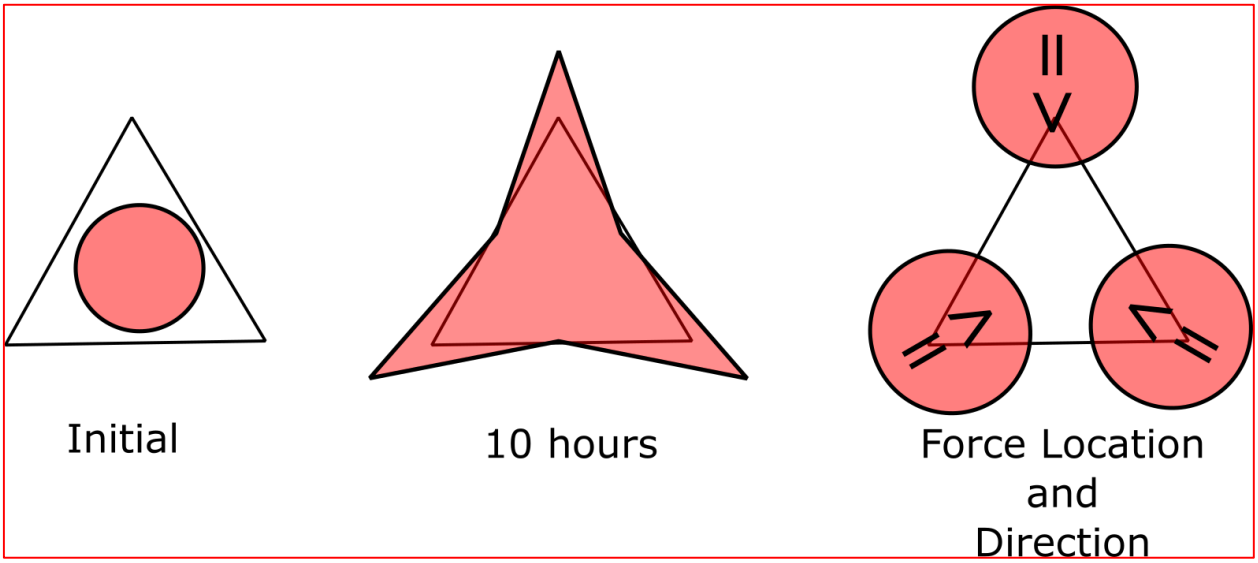
Q7b. You pipette a single cell onto a surface covered with a micro-patterned triangular coating of fibronectin. On the left, draw the initial shape of the cell just after pipetting [1 mark]. In the middle, draw the shape of the cell after 10 hours of incubation [1 mark]. On the right, circle the areas on the surface which experience the most force [1 mark], and for each area, draw an arrow indicating the direction the surface experiences the force at the 10 hour time-point [1 mark].





Solution: Initial should be rounded [1 mark], 10 hours should protrude from the 3 corners of the shape and the shape should be generally similar to the fibronectin patterning [1 mark]. Highest forces will be at the tips of the micropatterned shape [1 mark] and the force directions would pull towards the center of the cell [1 mark].

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Q8. [5 marks] Cells have developed a number of different mechanisms that allow them to translate extracellular cues into intracellular changes in gene expression. Recent studies revealed a clever association between extracellular environmental stiffness, actin polymerization state, and activity of Serum Response Factor (SRF) – a transcription factor. In four or fewer sentences, describe SRF activity is modulated by environmental stiffness [5 marks]? SRF translocation to the nucleus requires binding to its co-factor MAL [1 mark]. MAL also binds to actin monomers [1 mark]. In soft environments, actin monomers are available for MAL to bind to (since there is little actin polymerization [0.5 marks]), so SRF is stuck in the cytoplasm [1 mark]. In stiff environments, cells polymerize actin filaments [0.5 marks], thereby reducing the free actin monomer pool, which frees MAL to instead bind and shuttle SRF where SRF can modify gene expression [1 mark].

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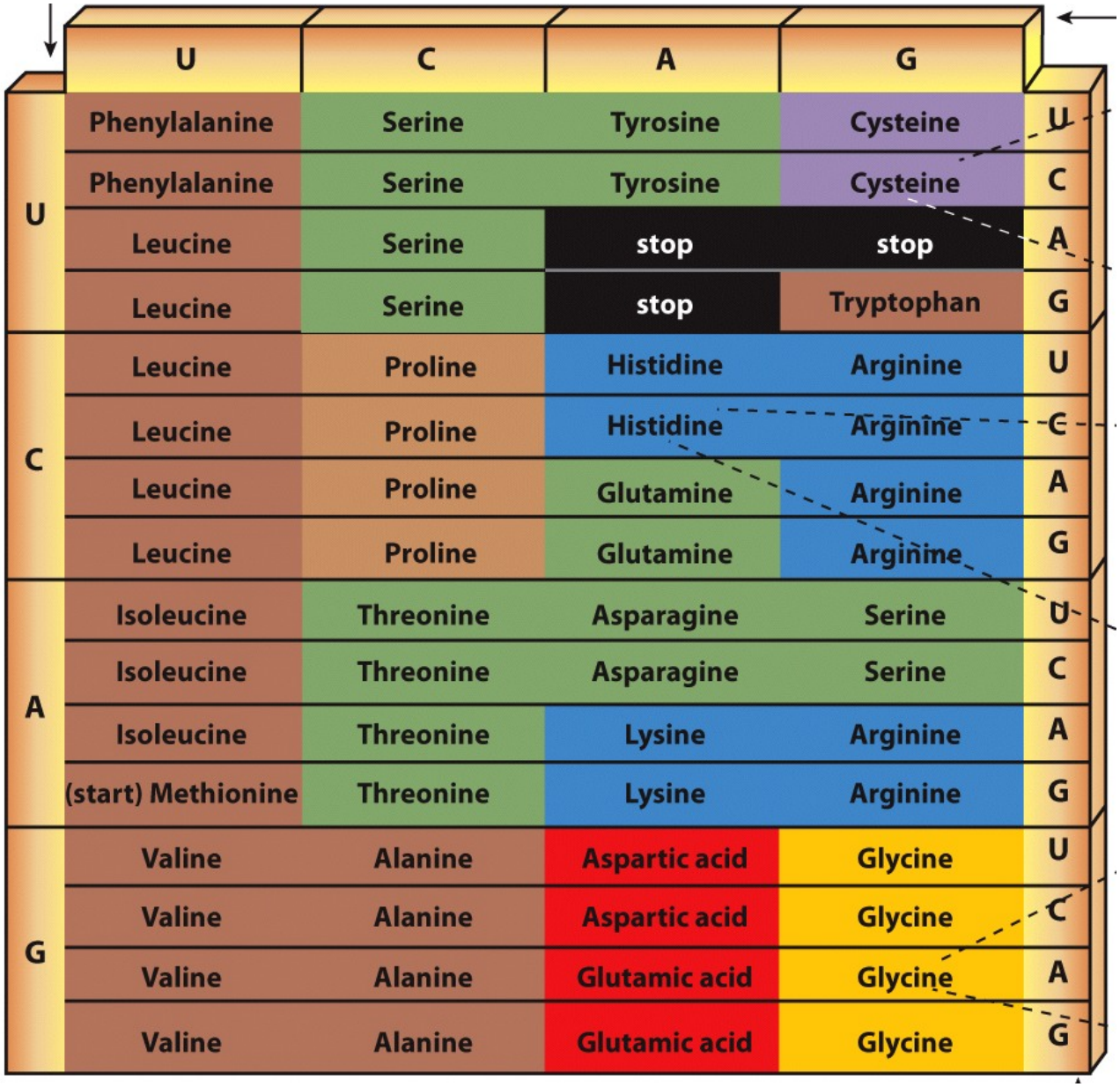
RESOURCES

Resource 1: The Genetic Code Table



	U	C	A	G	
U	Phenylalanine	Serine	Tyrosine	Cysteine	U
	Phenylalanine	Serine	Tyrosine	Cysteine	C
	Leucine	Serine	stop	stop	A
	Leucine	Serine	stop	Tryptophan	G
C	Leucine	Proline	Histidine	Arginine	U
	Leucine	Proline	Histidine	Arginine	C
	Leucine	Proline	Glutamine	Arginine	A
	Leucine	Proline	Glutamine	Arginine	G
A	Isoleucine	Threonine	Asparagine	Serine	U
	Isoleucine	Threonine	Asparagine	Serine	C
	Isoleucine	Threonine	Lysine	Arginine	A
	(start) Methionine	Threonine	Lysine	Arginine	G
G	Valine	Alanine	Aspartic acid	Glycine	U
	Valine	Alanine	Aspartic acid	Glycine	C
	Valine	Alanine	Glutamic acid	Glycine	A
	Valine	Alanine	Glutamic acid	Glycine	G

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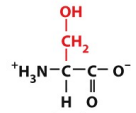
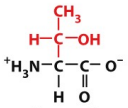
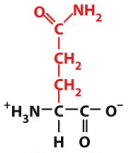
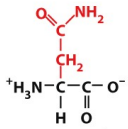
Resource 2: The Amino Acids

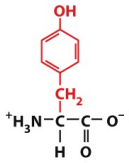
Polar uncharged


Serine
(Ser or S)

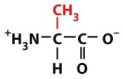

Threonine
(Thr or T)

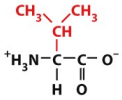

Glutamine
(Gln or Q)

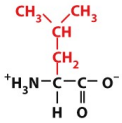

Asparagine
(Asn or N)

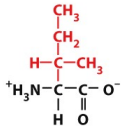

Tyrosine
(Tyr or Y)

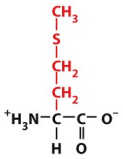
Nonpolar

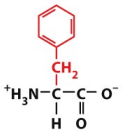

Alanine
(Ala or A)

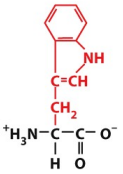

Valine
(Val or V)


Leucine
(Leu or L)

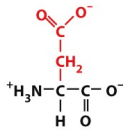

Isoleucine
(Ile or I)

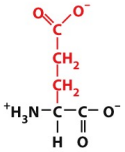

Methionine
(Met or M)

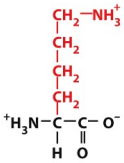

Phenylalanine
(Phe or F)

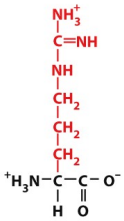

Tryptophan
(Trp or W)

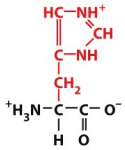
Polar charged


Aspartic acid
(Asp or D)

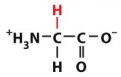

Glutamic acid
(Glu or E)

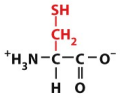

Lysine
(Lys or K)

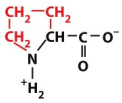

Arginine
(Arg or R)


Histidine
(His or H)

Side chains with unique properties


Glycine
(Gly or G)


Cysteine
(Cys or C)


Proline
(Pro or P)

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Student #:

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