Name: _____
Student #: _____

University of Toronto
Faculty of Applied Science and Engineering
Division of Engineering Science
BME205S FINAL Examination
Tues April 17, 2018: 9:30 – 12:00 pm
Duration: 150 minutes
Examiner: P. Gilbert

1. No cell phones are allowed. A calculator is permitted (**Type 2**).
2. **Type C**: Closed book examination. A single, double-sided aid sheet is permitted.
3. Part 1 Multiple Choice Questions. ***Mark multiple choice answers on Scantron***. Each is worth 1 mark.
4. Part 2 Questions have the mark available in the square brackets [ ]; each portion of a question also shows how many marks are allocated to it. ***Each question has a strict sentence limit restriction, each sentence written above the limit will be deducted half a mark.***
5. There is a blank page at the end of the test for rough work.
6. Write name on each page of exam booklet = **1 mark** and Scantron = **1 mark**; Bubble in Student # on Scantron = **1 mark**; Proper bubble etiquette on Scantron = **2 marks**

Last Name: _____

First Name: _____

Student Number: _____

Tutorial section:

[ ]	TUT 01	Tu	13:00	14:00	BA2139
[ ]	TUT 02	Tu	13:00	14:00	RS310
[ ]	TUT 03	Wd	13:00	14:00	BA2165
[ ]	TUT 04	Wd	10:00	11:00	BA3008
[ ]	TUT 05	Wd	10:00	11:00	BA2175
[ ]	TUT 06	Wd	10:00	11:00	BA3012

PART I: Multiple Choice _____ **out of 45**

PART 2: Short Answer Questions

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

PART 2 _____ **out of 50**
TOTAL MARKS _____ **out of 100**

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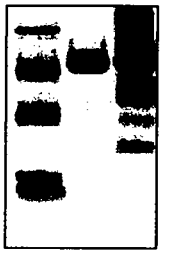
PART 1: Multiple Choice
ANSWERS MUST BE FILLED OUT ON SCANTRON SHEET

1. In your thesis studies you are tasked with uncovering the function of YOY, a mysterious protein. You construct a plasmid with the *YOY* gene and introduce the plasmid into cells to overexpress the protein. Next, you collect the cells, split the cells evenly into two tubes, centrifuge both tubes, and remove the supernatant leaving a cell pellet at the bottom of the tube. To one tube you add a lysis buffer containing DTT and to the other tube a lysis buffer lacking DTT. You run both samples (middle & right columns) on an SDS PAGE gel alongside the molecular weight ladder (left column), and using the western blot technique, you probe the membrane with an antibody that specifically recognizes YOY. With results in hand, you realize you did not keep track of which sample you loaded into each well. Based on your understanding of DTT treatment, which well was DTT treated? How do you know?



a. the middle column because DTT degrades proteins
b. the middle column because DTT breaks disulfide bonds
c. the right column because DTT degrades proteins
d. the right column because DTT breaks disulfide bonds
e. None of the samples appear to have been treated with DTT.

2. Which of the following stimuli commonly activates channels that sense and respond to touch?
a. A localized physical stretch in response to touching can activate neurons that respond to touch.
b. Bacteria transferred from one person to another in the course of touching acts as a ligand for sensory neuron channels.
c. An intracellular phosphorylation event most commonly activates channels in sensory nerves.
d. A rapid change in membrane potential most commonly activates channels in sensory nerves.

3. Ionic bonds are _____.
a. strengthened in the presence of water
b. weak bonds in biological systems that are broken and reformed
c. a type of covalent bond
d. involved in maintaining the tertiary structure of proteins
e. (b) and (d) are accurate statements about ionic bonds

4. Which of the following amino acids is most likely to be found on the outer surface of an ion channel that is interacting with lipids in the biomembrane?
a. cysteine
b. tyrosine
c. aspartate
d. arginine
e. glutamic acid

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5. Which of the following is a mechanism by which different cell types are 'born' during the process of development from a single fertilized embryo?
   a. Growth factors produced by cells diffuse and create concentration gradients that pattern different cell types in the development embryo.
   b. Differential mRNA splicing drives the birth of different cell types in the developing embryo
   c. The different cell types of an embryo arise via a spontaneous and random process
   d. Neither (a), (b), or (c) describes a mechanism by which different cell types are born during development

6. Which of the following is the BEST explanation for why animal fat is a solid at room temperature while vegetable oils are liquid?
   a. Animal fats have more double bonds than do vegetable oils.
   b. Animal fats have no amphipathic character.
   c. Animal fats have fewer double bonds than vegetable oils.
   d. Animal fats have longer fatty-acid tails than do vegetable oils.

7. Which of the following statements about enzymes is CORRECT?
   a. A single enzyme typically reacts with many different substrates.
   b. They lower the activation energy of a reaction.
   c. They cannot function in an aqueous environment.
   d. They slow down the rate of a reaction so that it proceeds more accurately.

8. Which codon serves as the start codon in mRNA for translation?
   a. AGU (arginine)
   b. UGA (a stop codon)
   c. UGG (tryptophan)
   d. AUG (methionine)
   e. UUU (phenylalanine)

9. The K_m for an enzyme-catalyzed reaction:
   a. Is a measure of the affinity of the substrate for the enzyme.
   b. Determines the shape of the kinetics curve.
   c. Is a measure of the rate of the reaction.
   d. Determines the V_{max} for the reaction.

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10. Which of the following statements about neurons is CORRECT?
    a. All neurons possess a single axon and a single dendrite
    b. All neurons possess a single axon, but can have many dendrites
    c. All neurons possess many axons, but only a single dendrite
    d. All neurons possess many axons, and can have many dendrites

11. In Lab #4 (Nanotechnology), you used centrifugation to pellet a bacterial sample and then resuspended the bacterial cell pellet in 100ul of 'Lysozyme Solution'. What was the purpose of exposing the bacterial cell pellet to lysozyme?
    a. To protect the bacterial genetic material from degradation
    b. To separate the bacterial genetic material from proteins
    c. To break down the bacterial cell wall
    d. To release the genetic material from the bacterial nucleus

12. Which of the following statement(s) about cells is/are CORRECT?
    a. Cells are the basic building block of tissues and organs
    b. Cells contain DNA, RNA, and proteins
    c. All cells possess a nucleus where the genetic material is contained
    d. (a), (b), and (c) are all correct statements about cells
    e. (a), and (b) are correct statements about cells

13. You isolate DNA from a particular organism and analyze it. The amount of adenine was 16 μmoles and the A+T/G+C ratio is 4.0. How much guanine should be in the sample?
    a. 1.5 μmoles
    b. 6 μmoles
    c. 3 μmoles
    d. 12 μmoles
    e. 4 μmoles

14. All the following statements describe biomembranes EXCEPT:
    a. Different biomembranes may contain different proportions of the same phospholipids.
    b. The two leaflets of a biomembrane always contain the same phospholipids.
    c. Biomembranes never have free edges.
    d. Cholesterol can make some regions of a biomembrane thicker by binding to phospholipids and extending the lipid tails.

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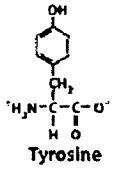
15. Which of the following is a basic property of adult neural stem cells?
    a. They remain unspecialized
    b. They divide and produce daughter cells that differentiate into all cell types of the brain.
    c. They divide and produce at least one daughter cell that remains unspecialized (self-renew)
    d. (a), (b), and (c) are all basic properties of neural stem cells

16. What kind of inhibitor(s) binds loosely to an enzyme and can be overcome with high substrate to inhibitor ratios?
    a. Irreversible inhibitor
    b. Competitive inhibitor
    c. Non-competitive inhibitor
    d. Both reversible and irreversible
    e. None of these

17. In Lab #5 (Restriction Digest) you prepared an agarose gel to separate your restriction digest fragments. Why did your DNA samples migrate towards the cathode?
    a. Due to the net negative charge caused by the SDS treatment
    b. Due to the net positive charge caused by the SDS treatment
    c. DNA has a net positive charge due to the A/T and C/G base-pairing
    d. DNA has a net negative charge due to the phosphate backbone

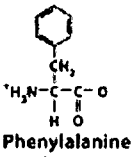
18. Kinases, which are responsible for the activation or inactivation of a number of proteins, add phosphate groups onto which of the following amino acids?

a.



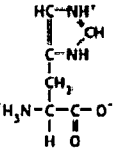
Tyrosine
(Tyr or Y)

b.



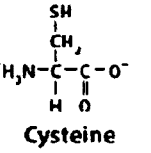
Phenylalanine
(Phe or F)

c.



Histidine
(His or H)

d.



Cysteine
(Cys or C)

19. _____ can be used to extract proteins from biomembranes in their folded and active form.
    a. Ionic detergents
    b. Non-ionic detergents
    c. High ionic strength salts
    d. Neither (a), (b), nor (c) can be used to extract folded, active proteins from biomembranes

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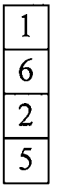
20. Pepsin is an enzyme that is made in the stomach and helps to digest proteins. For your summer research project, you've been tasked with purifying the pepsin protein. After months of hard work, you believe you have the purified protein in your flask. However, when you perform a functional test, the purified enzyme fails to digest protein. Which of the following might explain your disappointing result?
   a. The solution you used to perform your assay was pH 4
   b. You performed your experiment at 37C
   c. You forgot to add substrate to the reaction flask
   d. Answers a, b, and c might all explain your negative result.

21. What is the volume dispensed from a P200 pipette given the following numbers displayed:
   a. 1.625 µl
   b. 16.25 µl
   c. 162.5 µl
   d. 1625 µl



22. Why do large diameter neurons convey information more quickly than small diameter neurons?
   a. This is an incorrect statement. Instead, it is small diameter neurons that convey information more quickly than large diameter neurons.
   b. There is lower axial resistance to the flow of current down large diameter axons due to a greater number of ions per unit length
   c. Small diameter axons are often myelinated, which reduces the speed of the action potential
   d. The intensity of the action potential signal increases with increasing axon size

23. Which of the following point mutation(s) will, by definition, result in no change to the primary structure of the encoded protein?
   a. Synonymous DNA mutation
   b. Non-synonymous DNA mutation
   c. Nonsense DNA mutation
   d. Frameshift DNA mutation

24. A weak acid with a pKa of 5.5 will likely buffer (slow changes in pH due to the addition of OH^- or H^+ ions) in the pH range between:
   a. 4.5 and 6.5
   b. 5.5 and 8.5
   c. 6.0 and 7.0
   d. 6.5 and 8.5

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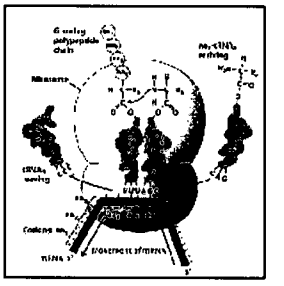
25. What process is pictured to the right?
    a. Degradation via the proteasome
    b. Translation
    c. Transcription
    d. Phosphorylation
    e. Cell division



26. Neurotransmitters such as dopamine, GABA, and serotonin exert potent effects on human physiology. What determines whether a neurotransmitter is excitatory or inhibitory?
    a. Neurotransmitters cannot be both excitatory or inhibitory.
    b. Once a neurotransmitter exits the blood stream and enters a specific tissue, tissue resident enzymes modify the neurotransmitter to be either excitatory or inhibitory.
    c. Whether a neurotransmitter is excitatory or inhibitory depends on the specific channel to which it binds.
    d. The magnitude of the action potential determines whether a neurotransmitter is excitatory or inhibitory.

27. What must you do each time you enter the MB325 teaching laboratory?
    a. Wash your hands
    b. Ask your TA what to do.
    c. Leave the MB325 door propped open so that others can enter easily.
    d. Remove your winter coat and hang it with the lab coats
    e. Put on the appropriate personal protective equipment (PPE)

28. Which of the following is a covalent interaction?
    a. The interactions between adjacent base pairs in the DNA double helix structure
    b. The interactions between amino acids in integral membrane proteins and lipid tails in the biomembrane
    c. The interaction between water molecules and salt ions
    d. Phosphorylation (i.e. modification of an amino acid with a phosphate group)

29. Which of the following amino acids can change the activity of proteins when the protein experiences a shift in environmental acidity (i.e. change in pH)?
    a. histidine
    b. glycine
    c. proline
    d. cysteine
    e. lysine

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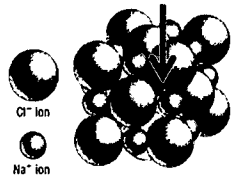
30. Sterols, such as cholesterol, are found in biomembranes though they are not classified as phospholipids. Sterols consist primarily of a long chain of hydrocarbons, but are considered amphipathic due to:
    a. A two-pronged fatty-acyl tail
    b. The presence of a phosphate-based head group
    c. A hydroxyl substituent on one ring
    d. The carbon based ring groups
    e. Neither (a), (b), (c), nor (d) explains why sterols are considered amphipathic

31. Which of the following statements about electromyography (EMG) are TRUE?
    a. EMG signals, like all bipotential signals, require pre-processing to remove noise.
    b. EMG electrodes convert electrical signals to mechanical signals.
    c. Typically, an EMG signals has components between 5-200 Hz.
    d. EMG records electrical activity produced by tendons
    e. (a) and (c) are both TRUE statements about EMG.

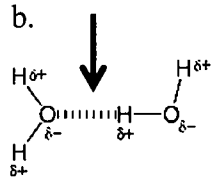
32. In _____ the genome is organized such that genes encoding for enzymes that are all involved in a single process, such as breaking down lactose, are arranged on different chromosomes.
    a. prokaryotes
    b. eukaryotes
    c. all organisms
    d. no organism is

33. In which of the following images does the arrow point at a hydrogen bond?

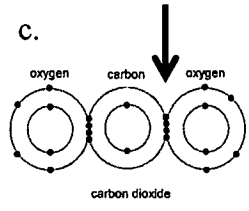
a.



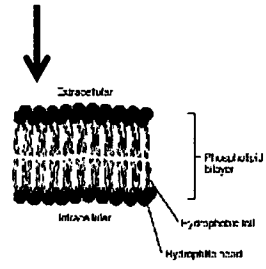
b.



c.



d.



34. Which of the following cellular organelles is/are enclosed by two cellular membranes?
    a. Mitochondria
    b. Endoplasmic reticulum
    c. Lysosome
    d. All organelles are enclosed by two cellular membranes
    e. No organelles are enclosed by two cellular membranes

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35. In the process of translation, tRNAs coupled with a specific amino acid contribute their amino acid to the growing polypeptide chain based on the genetic code. Where do the amino acids coupled to the tRNAs come from?

a. Amino acids are made by cells through a series of chemical reactions.
b. The proteins we eat are broken down into the individual amino acids for use in the cell.
c. The proteasome breaks proteins down into individual amino acids for use in the cell.
d. The gene encoding for a specific tRNA includes the sequence for its attached amino acid. The two entities are translated together and the final folded protein is the tRNA coupled to its appropriate amino acid.
e. (a), (b), and (c) are all ways that amino acids can end up in cells.

36. Why does DNA carry the genetic code rather than RNA?

a. RNA possesses a 2'-hydroxyl group that can act as a nucleophile and break the RNA strand
b. DNA is double stranded while RNA is single stranded
c. Chromosomes (DNA) are easier to pass to offspring than RNAs
d. Neither (a), (b), or (c) are reasons that DNA carries the genetic code rather than RNA
e. (a), (b), and (c) are all reasons that DNA carries the genetic code rather than RNA

37. When a membrane bound vesicle fuses with a cell, the portion of an integral membrane protein that was facing the inside of the vesicle will:

a. Face the exoplasmic side of the cell membrane
b. Face the cytoplasmic side of the cell membrane
c. Typically be cleaved and released into the cytoplasm
d. Be degraded to prevent foreign proteins from entering the cell

38. In eukaryotic cells, protein synthesis in the cytoplasm utilizes three types of RNA molecules. Which of the following can act as a ribozyme to catalyze peptide bond formation?

a. mRNA
b. tRNA
c. rRNA
d. (a), (b), and (c) can all act as ribozymes to catalyze peptide bond formation
e. Neither (a), (b), nor (c) can all act as ribozymes to catalyze peptide bond formation

39. Degradation via the proteasome is one of the ways that protein life span is regulated (no protein = no activity). Which of the following mutations might you expect to prevent or delay proteasome-mediated protein degradation?

a. A UGU codon, which is normally targeted by the E3 ligase for ubiquitin addition, is mutated to a UGC
b. A mutation that sequesters the protein in the cytoplasm

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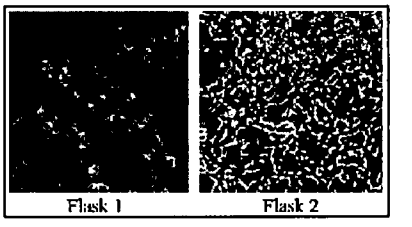
c. A mutation in the E3 ligase that prevents addition of more than a single ubiquitin
d. (a), (b), and (c) are all mutations that might prevent or delay proteasome-mediated protein degradation.

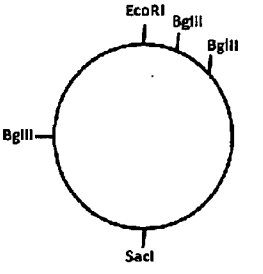
40. You capture light microscope images of two flasks (see below). Based on your images, which flask do you think contained non-adherent cells and how do you know?

a. Flask 1, because the cells are rounded in morphology
b. Flask 1, because the cells are flattened and spread out
c. Flask 2, because the cells are rounded in morphology
d. Flask 2, because the cells are flattened and spread out



41. The circular plasmid to the right was fully digested with the restriction enzymes PstI, EcoRI, and BglII. How many bands will appear on an agarose gel electrophoresis?

a. 1
b. 2
c. 3
d. 4
e. 5



42. Some RNAs serve to encode proteins, while other RNAs carry out enzymatic functions in cells. What allows RNA, but not DNA, to play such versatile roles in cells?

a. Single stranded RNAs can fold into tertiary structures that possess catalytic properties like proteins
b. The uracil found in RNA, but not DNA, is highly reactive
c. DNA is restricted to the nucleus, so its function is inherently limited
d. (a), (b), and (c) are reasons that RNA, and not DNA, can take on versatile roles in the cell
e. Neither (a), (b), or (c) are reasons that RNA, and not DNA, can take on versatile roles in the cell

43. You disrupt all hydrophobic interactions in a protein. What level of structure will be preserved?

a. primary structure
b. secondary structure
c. tertiary structure
d. quaternary structure
e. primary and secondary

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44. Which of the following statements about the Na+-K+ pump is INCORRECT?
    a. It requires ATP (energy) for its activity
    b. It pumps 3 Na+ ions out of the cells and 2 K+ ions into the cell
    c. It is important for maintaining the resting potential of a biomembrane
    d. It shuts down when an action potential is initiated

45. Based on your understanding of synapse function, neurodegenerative disease might be caused by which of the following?
    a. Mutations that impair the release of a neurotransmitter
    b. Mutations that impair the production of a neurotransmitter
    c. Mutations that impair Ca2+ influx
    d. Mutations in channels that prevent or impair ligand binding
    e. (a), (b), (c), and (d) are all mutations that might cause neurodegenerative disease

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PART 2: SHORT ANSWER QUESTIONS

1. [8 marks] You differentiate pluripotent stem cells into neurons and want to characterize them according to their membrane potential. You already know the different concentrations of most ions (see the table) and calculated their equilibrium potential. However, there are a few questions left to answer:

Table 1: Ion Concentrations

Ionic Species	Intracellular Concentration	Extracellular Concentration	Equilibrium Potential
Sodium (Na^+)	15 mM	145 mM	V_{Na} = +60.59 mV
Potassium (K^+)	150 mM	4 mM	V_K = −96.80 mV
Calcium (Ca^{2+})	0.00007 mM	2 mM	
Chloride (Cl^-)	10 mM	110 mM	V_{Cl} = −64.05 mV

Nernst equation: $V_m = \frac{61.5mV}{z} \cdot \log_{10} \frac{[X]_o}{[X]_i}$

Goldman-Hodgkin-Katz equation: $V_m = 61.5mV \cdot \log_{10} \frac{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}$

z = the valence of the ion in question
o = outside (extracellular)
i = inside (intracellular)
P_x = permeability for ion X

a. Assuming the membrane is at rest, name the two different gradients across the membrane that affect ions [**2 marks**]. Which gradient typically moves the potassium ions out of a cell [**1 mark**]?

b. The hypothetical ion X is negatively charged and more concentrated inside than outside of the cell. Is the equilibrium potential negative, positive or zero [**1 mark**]?

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c. The value for the equilibrium potential of **calcium** is missing in the table, calculate it using the values from the table and the Nernst equation [**2 marks**]. ***You must show your work for full marks.***

d. You were able to figure out the permeability of the membrane for potassium, sodium, and chloride ions at rest ($P_K : P_{Na} : P_{Cl} = 1 : 0.05 : 0.45$). Calculate the resting membrane potential using the Goldman-Hodgkin-Katz equation. ***You must show your work for full marks.***

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2. [8 marks] Following your basic characterization of neurons differentiated from pluripotent stem cells in Short Answer #1, you now stimulate the neurons to elicit action potentials.

a. Assume that the plasma membrane permeability changes following stimulation with values for potassium, sodium, and chloride as the following: $P_K : P_{Na} : P_{Cl} = 1 : 12 : 0.5$. How does this affect the membrane permeability compared to the neurons at rest **[1 mark]**? Does the membrane potential become more positive or more negative **[1 mark]**?

b. A neuron receives an input that opens potassium channels ($P_K : 10$), what happens to the membrane potential **[1 mark]**? How does it affect the ability of the neuron to fire an action potential **[1 mark]**?

c. Tetrodotoxin is a neurotoxin found in pufferfish. It blocks the voltage-gated sodium channels. How does this affect the action potential **[1 mark]**? How does it affect the muscle **[1 mark]**?

d. You use genetic engineering techniques to culture neuronal cells with sodium-potassium pumps that transport 2 sodium ions for every 3 potassium ions (rather than 3 sodium ions for every 2 potassium ions); assuming that all other aspects are held equal, how does this affect the diffusion force (chemical gradient) on potassium ions? Is the force larger or smaller **[1 marks]**? In which direction does it point to **[1 mark]**?

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3. [**8 marks**] You are interested in using CRISPR/Cas9 to edit and correct the *Hbb* gene in patients with sickle cell disease for a 4th year genetic engineering group design project. The *Hbb* gene encodes for haemoglobin beta, a protein unit of haemoglobin responsible for oxygen transport in red blood cells. Sickle cell disease is caused by a single nucleotide point mutation in *Hbb*, causing the replacement of a hydrophilic amino acid glutamic acid with the hydrophobic amino acid valine.

The beginning of the coding strand of the diseased *Hbb* gene is given below in 5' to 3' orientation, and the codon with the point mutation is bolded and underlined.

ACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGCATCTGACTCCT**GTG**GAGAAGTCTGCCG
TTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACAGGT
TTAAGGAGACCAATAGAAACTGGGCATGTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTCTGCCTATTG
GTCTATTTTCCCACCCTTAGGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCT
GATGCTGTTATGGGCAACCCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGGAC
AACCTCAAGGGCACCTTTGCCACACTGAGTGAGCTGCACTGTGACAAGCTGCACGTGGATCCTGAGAACTTCAGGGTGAGTCTA
TGGGACGCTTGATGTTTTCTTTCCCCTTCTTTTCTATGGTTAAGTTCATGTCATAGGAAGGGGATAAGTAACAGGGTACAGTTT
AGAATGGGAAACAGACGAATGATTGCATCAGTGTGGAAGTCTCAGGATCGTTTTAGTTTCTTTTATTTGCTGTTCATAACAATT
GTTTTCTTTTGTTTAATTCTTGCTTTCTTTTTTTTTCTTCTCCGCAATTTTTACTATTATACTTAATGCCTTAAC...

a. The Cas9 protein your group has been asked to use is a modified Cas9 called *yCas9*, which recognizes **an alternative PAM sequence**: 5'-YGG-3', where "Y" represents any pyrimidine nucleotide. Circle all regions in the below excerpt of the *Hbb* gene where you expect *yCas9* can recognize [**2 marks**].

5'ATGGTGCATCTGACTCCT**GTG**GAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGAT3'

b. Your group uses the CRISPRdirect online resource (as demonstrated to you in Tutorials 8 and 10) to identify possible target sequence positions for CRISPR/yCas9 guide RNA. Your group members debate choosing between the following three sequences:

	position	sequence information				number of target sites
Sequence	start - end	GC% of 20mer	T_m of 20mer	TTTT in 20mer	restriction sites	20mer +PAM
A	32 - 54	50.00 %	72.23 °C	-	BtgI NcoI	2
B	76 - 98	60.00 %	78.50 °C	-		1
C	169 - 191	35.00 %	64.37 °C	-		1

Name: _____
Student #: _____

Which of the three proposed sequences in the table (A, B, or C) would be the most useful in targeting the mutation of interest? In 2 sentences or less, explain your reasoning by describing why the other 2 designs are not as useful. Then, write out the 20mer+PAM sequence of your chosen guide RNA design and indicate the 5' to 3' orientation [**4 marks**].

c. Since sickle cell disease primarily affects blood cells, you group needs a method to deliver the *yCas9* protein, guide RNA, and repair template DNA to a patient's blood stem cells. Propose a possible delivery method to these cells and draw a schematic to explain the method [**2 marks**].

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4. [8 marks] Your lab is interested in cloning out a truncation of GFP contained within the following sequence (Hint: The sequence has been translated for you in all three translation frames. Use the bold/underline to help understand the sequence alignment):

```
5'gcttcgggcgccacgatggtgagcaagggcgaggagctgttcaccggggtggtgccgcagcctggtcgagctggacggcgacgtaaacgg
  A  S  G  A  T  M  V  S  K  G  E  E  L  F  T  G  V  V  P  Q  P  G  R  A  G  R  R  R  K  R
   L  R  A  P  R  W  *  A  R  A  R  S  C  S  P  G  W  C  R  S  L  V  E  L  D  G  D  V  N  G
    F  G  R  H  D  G  E  Q  G  R  G  A  V  H  R  G  G  A  A  A  W  S  S  W  T  A  T  *  T  A
3'cgaagcccgcggtgctaccactcgttcccgctcctcgacaagtggccccaccacggcgtcggaccagctcgacctgccgctgcatttgcc
          10        20        30        40        50        60        70        80


  ccacaagttcagcgtgtccggcgacccttatctgcaccaccggcaagctgcccgtgccctggcccaccctcgtgaccacctgataaggcc
  P  Q  V  Q  R  V  R  R  P  L  S  A  P  P  A  S  C  P  C  P  G  P  P  S  *  P  P  D  K  A
   H  K  F  S  V  S  G  D  P  Y  L  H  H  R  Q  A  A  R  A  L  A  H  P  R  D  H  L  I  R  X
    T  S  S  A  C  P  A  T  L  I  C  T  T  G  K  L  P  V  P  W  P  T  L  V  T  T  *  *  G  X
  ggtgttcaagtcgcacaggccgctgggaatagacgtggtggccgttcgacgggcacgggaccgggtgggagcactggtggactattccgg
          100       110       120       130       140       150       160       170
```

a. Design Primers (20bps) to amplify the target protein present in the above sequence [**2 marks**].

b. Determine the melting temperatures (T_m) of each primer and choose an appropriate annealing temperature for your reaction (Hint: Tm = 2(A+T) + 4(G+C)). Are these two primers well matched? Explain your answer in one sentence or less [**4 marks**].

c. Mutations can be introduced into amplified sequence by changing one base pair in an otherwise perfectly matched primer. Design new primers such that the third amino acid becomes Arginine (R), highlighting any changes. How will this affect the annealing temperature that you choose and why [**2 marks**]?

Name: _____
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5. [6 marks] You've been invited to brainstorm in a research group on different nanoparticle designs for drug delivery to kill cancer cells.

a. Two of the design criteria you are considering are how you will get your nanoparticles to your cancer cells for (i) targeted delivery and (ii) triggered release. Describe three different designs for targeted delivery and/or triggered release (**in a maximum** of **one sentence each**) [**3 marks**].

b. Name two organelles that are important for nanoparticle drug delivery and use one sentence to explain why [**2 marks**].

c. Name two reasons why you want targeted delivery of your cancer cells [**1 mark**].

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6. [6 marks] Consider the following questions related to restriction digest and gel electrophoresis.

a. You begin by creating an agarose gel to perform a restriction fragment analysis. You've chosen to use the following recipe: 2% agarose powder by weight (adds 2mL of volume when brought into solution), 88mL distilled water, 10mL 10X TBE buffer. However, a postdoc informs you that it is bad technique to keep TBE stocks at a 10x concentration as this can form a precipitate, and that you should use 5X TBE. In an effort to save the lab money, how much distilled water must you add to your 346mL stock 10x TBE to bring it in line with the postdoc's suggestion [**1 mark**].

b. Using your new 5x TBE, rewrite the above recipe to produce the same gel [**2 marks**].

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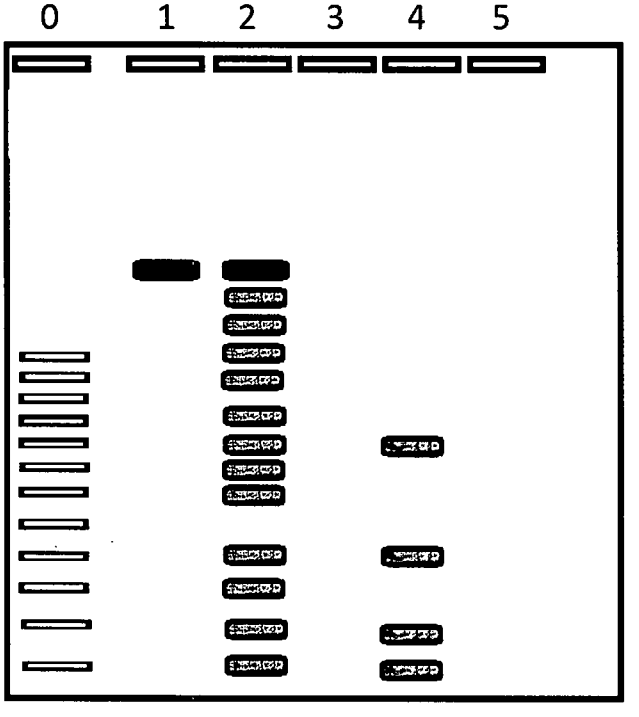
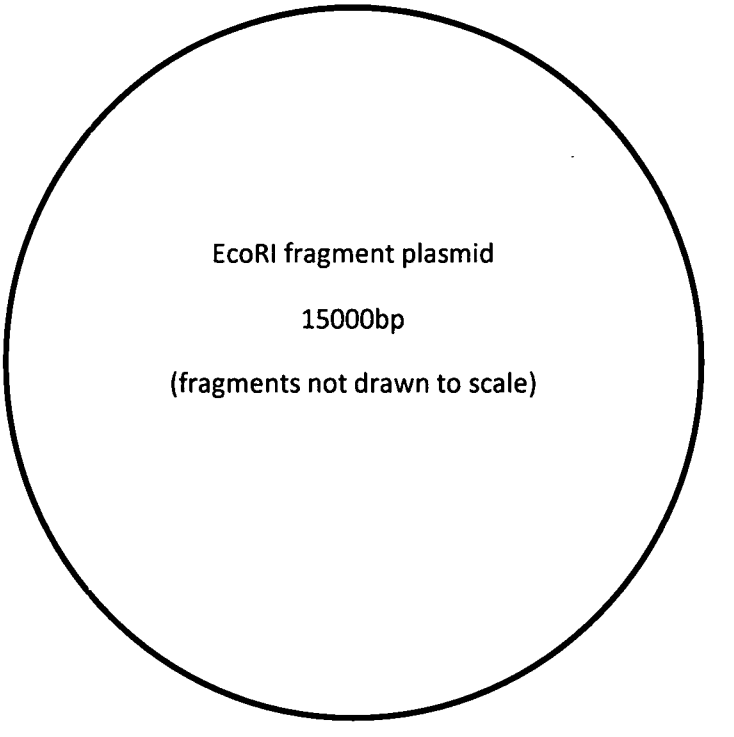
c. You perform a restriction digest of a 15000 bp plasmid with the restriction enzyme EcoRI. You take out a small sample after 1 minute and stop its digestion, and then let the reminder of the reaction digest for 4 additional hours before stopping that sample as well. You then run your samples on an agarose gel and stain for dsDNA. As a ladder, you use a mix with markers at 1000bp increments from 1000bp to 12000bp inclusive. Lane 0 is your ladder, Lane 1 is undigested sample, Lane 2 is the 1-minute digestion, and Lane 4 is the 4-hour digestion sample.

Label your ladder [**1 mark**]. Draw a possible plasmid map showing the correct fragment sizes [**1 mark**], and a possible order for the fragments [**1 mark**]





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7. [**6 marks**] You are studying a previously undiscovered parasite known for infiltrating the brainstem of its host organism and can significantly alter the behavior of the host. You know that it secretes a 10 amino acid peptide which reduces the host's immune response and allows the parasite to thrive. Knowing that this could be a powerful immune modulating drug, you seek to understand the peptide. You perform an EcoRI digestion of the parasite's genome and by doing molecular cloning, you confirm the following dsDNA sequence will encode for the peptide.

```
Peptide reference fragment (PRF-1)
5′ AATTCTTAATCAATATGGGTAATCAGCAGAATATACATG----- 3′
3′ ----GAATTAGTTATACCCATTAGTCGTCTTATATGTACTTAA- 5′
```

a. Circle all of the possible start codon(s) present in this sequence [**1 mark**].

b. What is the expected sequence of the peptide [**2 marks**]?

c. In your investigations, you identified four mutant sequences (I, II, III, IV). Each sequence contains only 1 of the 4 base pair mutations seen below and underlined, with the remaining base pairs the same as the reference sequence above.

```
5′ AATTCTCAATCAATAGGGGTGATCAGCAGAATACACATG----- 3′
3′ ----GAGTTAGTTATCCCCACTAGTCGTCTTATGTGTACTTAA- 5′
 Mutant  ^I       ^II  ^III         ^IV
```

You express these four mutants and purify the proteins fragments. When you analyze by mass spectroscopy, you find that one of these mutants creates a peptide of about double the mass of the non-mutated peptide! Which mutant sequence is most likely responsible for this double-mass peptide [**1 mark**] and what property of the mutant enabled the double-mass peptide [**2 marks**]**?**

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Resource #1

Amino Acid Structures

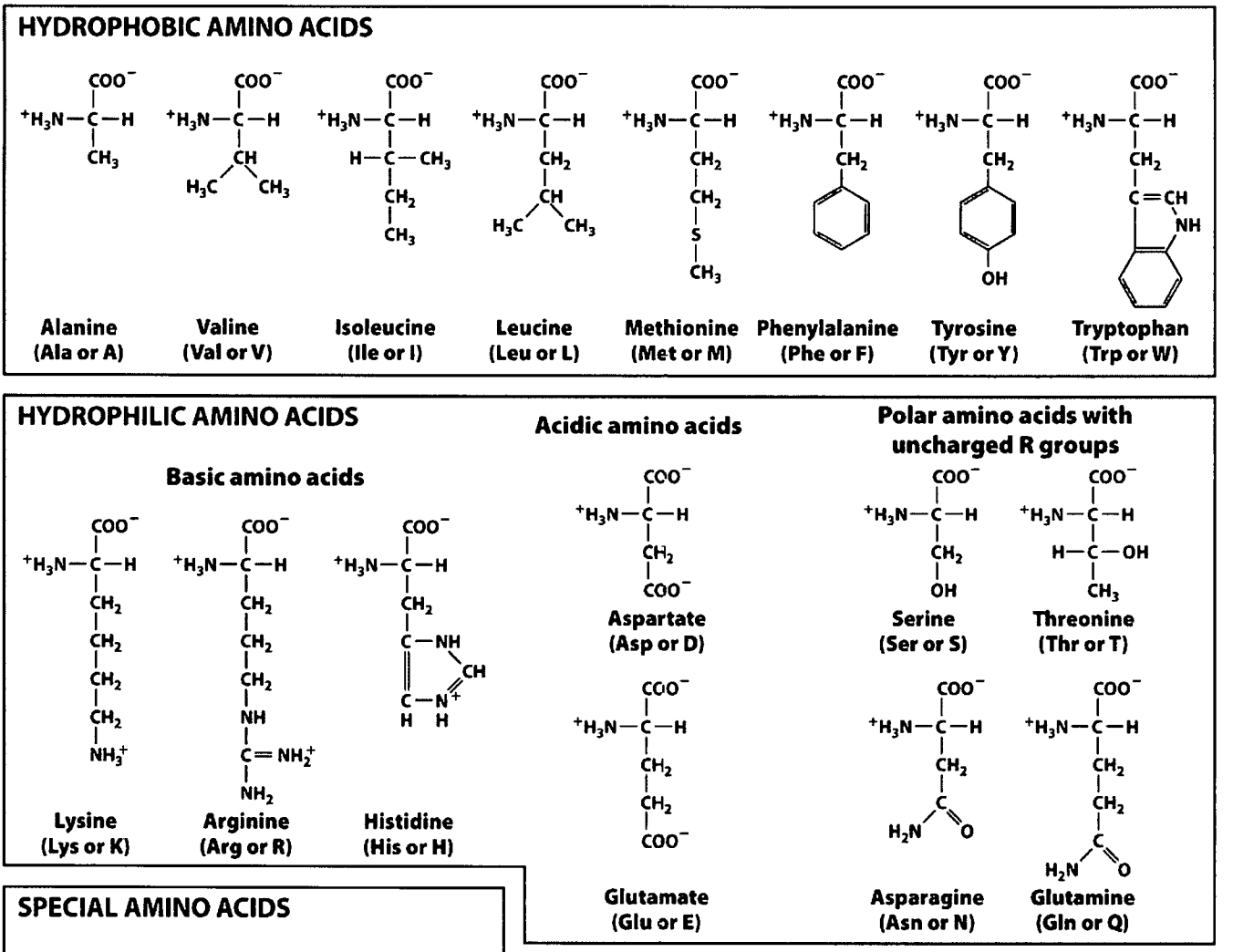

Figure 2-14
Molecular Cell Biology, Eighth Edition

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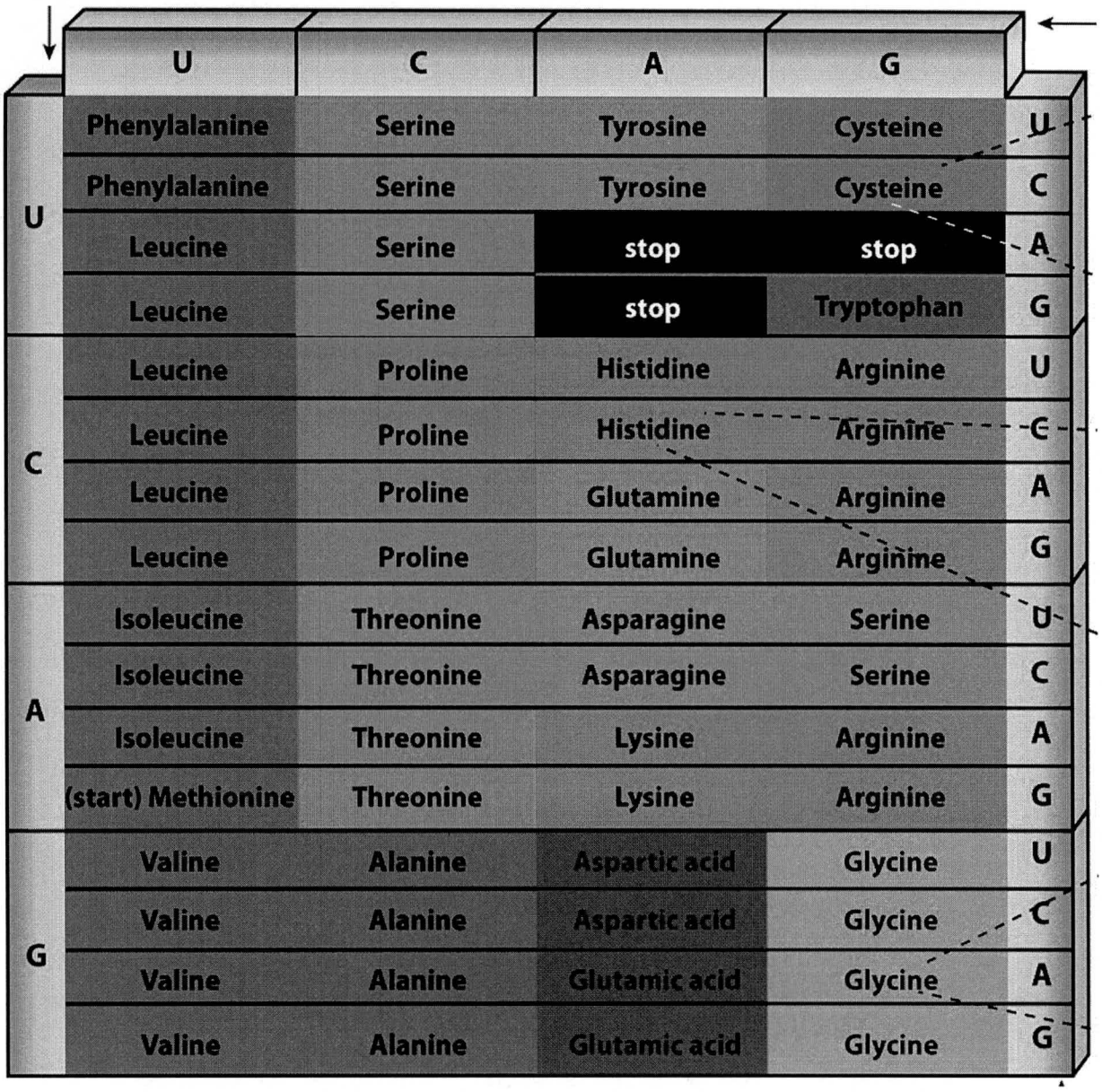
Resource #2

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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BME 205 2014 Exam Answer Sheet

First Name Last Name

9	9	9	9	9	9	9	9	9	9
8	8	8	8	8	8	8	8	8	8
7	7	7	7	7	7	7	7	7	7
6	6	6	6	6	6	6	6	6	6
5	5	5	5	5	5	5	5	5	5
4	4	4	4	4	4	4	4	4	4
3	3	3	3	3	3	3	3	3	3
2	2	2	2	2	2	2	2	2	2
1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0

Bubble in your student number with each column representing one number.

Bubble in Answer Boxes Completely:

1	A	B	C	D	E	26	A	B	C	D	E	51	A	B	C	D	E
2	A	B	C	D	E	27	A	B	C	D	E	52	A	B	C	D	E
3	A	B	C	D	E	28	A	B	C	D	E	53	A	B	C	D	E
4	A	B	C	D	E	29	A	B	C	D	E	54	A	B	C	D	E
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11	A	B	C	D	E	36	A	B	C	D	E	61	A	B	C	D	E
12	A	B	C	D	E	37	A	B	C	D	E	62	A	B	C	D	E
13	A	B	C	D	E	38	A	B	C	D	E	63	A	B	C	D	E
14	A	B	C	D	E	39	A	B	C	D	E	64	A	B	C	D	E
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16	A	B	C	D	E	41	A	B	C	D	E	66	A	B	C	D	E
17	A	B	C	D	E	42	A	B	C	D	E	67	A	B	C	D	E
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22	A	B	C	D	E	47	A	B	C	D	E	72	A	B	C	D	E
23	A	B	C	D	E	48	A	B	C	D	E	73	A	B	C	D	E
24	A	B	C	D	E	49	A	B	C	D	E	74	A	B	C	D	E
25	A	B	C	D	E	50	A	B	C	D	E	75	A	B	C	D	E