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7.016 Problem Set 6- 2018

Question 1

Green fluorescent protein (GFP) was first isolated from the pacific jellyfish *Aequoria victoria*. The gene for GFP has been isolated and is widely used as a fluorescent marker through expression of various fusion proteins. Below is the sequence of the **coding strand (mRNA like strand)** of GFP-cDNA. ***Note:*** *A codon chart is on the last page.*

```
5'TACACACGAATAAAAGATAACAAAGATGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGA
TGGCGATGTTAATGGGCAAAAATTCTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTA
TTTGCACTACTGGGAAGCTACCTGTTCCATGGCCAACACTTGTCACTACTTTCTCTTATGGTGTTCAATGCTTTTCAAGATAC
CCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTACAAAGA
TGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATT
TTAAAGAAGATGGAAACATTCTTGGACACAAAATGGAATACAACTATAACTCACATAATGTATACATCATGGCAGACAAACCA
AAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTAAAGATGGAAGCGTTCAATTAGCAGACCATTATCAACAAAA
TACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCCAAAGATCCCAACG
AAAAGAGAGATCACATGATCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAAATG
TCCAGACTTCCAATTGACACTAAAGTGTCCGAACAATTACTAAATTCTCAGGGTTCCTGGTTAAATTCAGGCTGAGACTTTAT
TTATATATTTATAGATTCATTAAAATTTTATGAATAATTTATTGATGTTATTAATAGGGGCTATTTTCTTATTAAATAGGCTA
CTGGAGTGTATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA3'
```

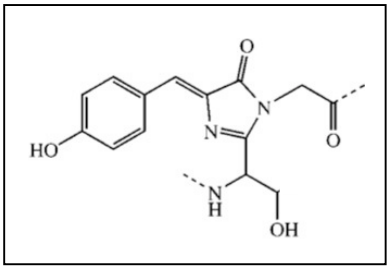
a) Write the sequence of the first 6 bases of the GFP mRNA: **5'-_____3'**

b) Box the 5'untranslated region **(5'UTR).**

c) Write the first 3 amino acids of the GFP protein. **N- _____-C**

d) You find a mutant GFP mRNA where the A (shown in red) →G. Could this mRNA be translated to make a functional GFP? **Why or why not?**

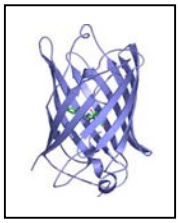
e) The following is the line angle drawing of the fluorophore in GFP. This fluorophore originates from the **cyclization and oxidation of the Ser^{65}-Tyr^{66}-Gly^{67} tripeptide**.



i. **Circle** the part that corresponds to the side-chain of Ser^{65} and **Box** the region corresponding to the side-chain of Tyr^{66}.

ii. Label the alpha carbon (α-C) contributed by each of the three amino acids.

f) The following is the structure of monomeric GFP.



i. What is the highest level of its protein structure: **primary/secondary/tertiary/ quaternary?**

ii. What secondary structure is most prominent in this protein: **α- helices or β-sheets**?

Image courtesy of Clipart Library.

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Question 1 continued

g) You want to study the localization and dynamics of an **intracellular protein** encoded by Gene A in living cells. You create a GFP-Gene A fusion gene. In comparison, your labmate creates an antibody specific to this protein and plans to use this to study its localization and function. Why is your approach better than your labmate's approach? Give **one reason**.

h) Antibodies are very useful reagents in molecular biology. They can either be polyclonal or monoclonal. List **one difference** between polyclonal and monoclonal antibodies that are targeted to the SAME protein antigen.

Question 2

a) Ethidium bromide **(EthBr)** is a DNA intercalator that inserts between the stacked base pairs and fluoresces when exposed to UV light. Would the λ_{max} emission wavelength of EthBr be shorter than its λ_{max} excitation wavelength? **Why or why not?**

b) What are supravital dyes and what are they used for?

c) Microarrays are the 2D- arrays built on functional silicon chips that display a collection of DNA or cDNA samples. You have the following microarrays:

- **Microarray A** contains a collection of the entire genomic sequences from human.
- **Microarray B** contains a collection of ALL coding sequences in humans
- **Microarray C** contains a collection of ALL regulatory sequences in human genome.

Approximately 50% of breast cancer patients have a missense mutation 5'GCG3' → 5'ACG3' in codon^{593} of the Her-2 gene. This results in Ala^{593}->Thr^{593} mutation in the Her-2 receptor protein. **Explain** whether or not you can identify this subtype of patients using…

Microarray A:

Microarray B:

Microarray C:

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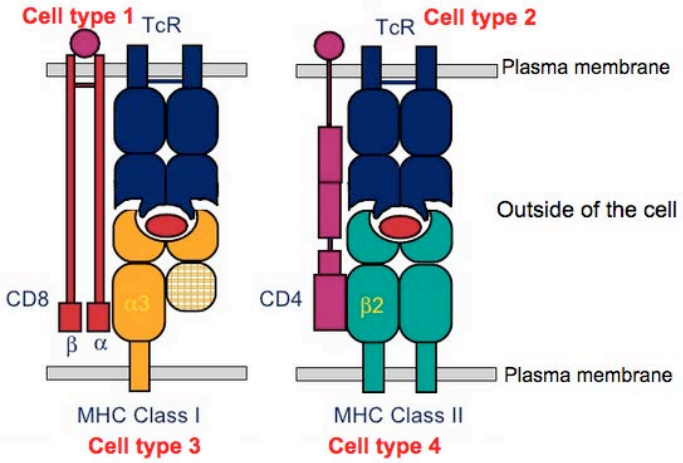
Question 3

The schematic below shows the interaction between the T cell receptors **(TcR)**, Major Histocompatibility Complexes Class I and II **(MHC Class I** and **MHC Class II**) and the accessory cell surface glycoproteins **(CD4 and CD8)** located on the surface of **cell types 1-4.**



a) Which cell type is likely a helper T cell **(T_H)**: **Cell type 1/ 2/ 3/ 4**? Which cell surface marker(s) would this cell type have: **MHCI/ MHCII/ CD4/ CD8**?

b) Which cell type is likely a cytotoxic T cell **(T_C)**: **Cell type 1/ 2/ 3/ 4**? Which cell surface marker(s) would this cell type have: **MHCI/ MHCII/ CD4/ CD8**?

c) Which cell type is most likely an antigen presenting cell **(APC)**: **Cell type 1/ 2/ 3/ 4**? Which cell surface marker(s) would this cell type have: **MHCI/ MHCII/ CD4/ CD8**?

d) Identify the cell types that interact with each other to trigger …

i. B cell activation and humoral (antibody-mediated) immune response: **Cell type 1/ 2/ 3/ 4**?

ii. Cytotoxic T cell mediated killing of an infected cell: **Cell type 1/2/ 3/ 4**?

e) An individual who is heterozygous for two alleles of the Antibody (Ab)/ Immunoglobulin (Ig) gene expresses only ONE of the two alleles for this gene. Briefly **explain** why this is critical.

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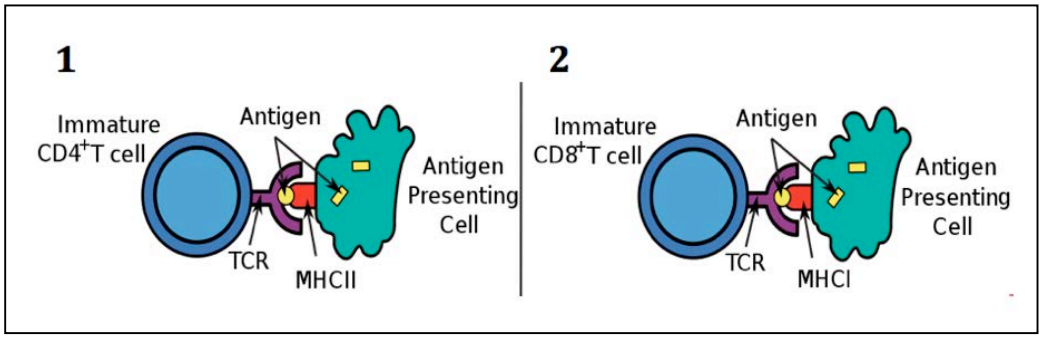
Question 4

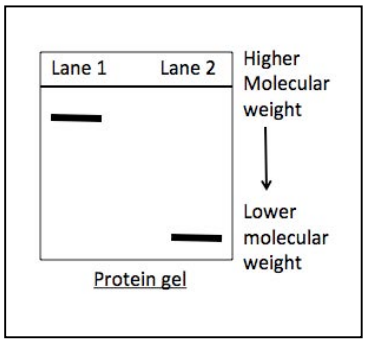
Gardasil is a preventive vaccine that was designed against the **surface proteins** of Human Papillomavirus (HPV), a **DNA virus** that causes cervical, head and neck cancer.

a) Which of the following schematics (**1 or 2**) represents the immune response that will be triggered following vaccination with Gardasil and **why?**



b) Following vaccination with Gardasil, why is the secondary immune response against HPV **faster and more effective** than the primary immune response?

c) During a primary and secondary humoral immune response to an antigen, the mature and memory B cells **produce surface antibodies.** Furthermore, the memory B cells, during secondary immune response, can give rise to **plasma B cells that produce secreted antibodies**. You isolate the antibodies that are specific to the HPV surface proteins and separate them by gel electrophoreses. The gel is shown below.



i. Which class of antibodies is present in **Lane 2** of the gel: **secreted IgG OR IgM**?

ii. From the gel, which B cell-type produces the antibody type shown in **Lane 2**?

d) If you compare the structure of secreted and surface antibodies that are specific to the **same epitope** (antigenic determinant) of an antigen, would you expect these antibodies to have …

i. The **same or different Variable** regions? **Why?**

ii. The **same or different Constant** regions? **Why?**

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Question 4 continued

e) The immune system is capable of producing antibodies (IAb) and T- cell receptors (TcR), each of which have antigen-binding sites that are specific to a particular antigen. If our genome only has approximately 20,000 genes, **list two processes** by which our immune system can produce millions of Abs and TcRs.

f) Multiple sclerosis (MS) is an autoimmune disorder in which the immune system attacks and destroys the myelin sheath of a neuron.

i. What process is disrupted in MS patients, resulting in the production of self-reactive antibodies?

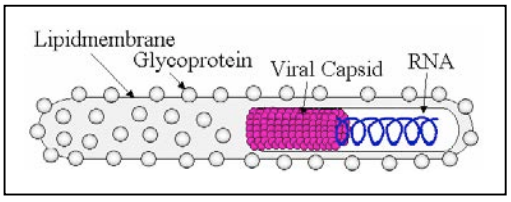
ii. Immunosuppressive drugs are often prescribed to patients with autoimmune diseases. What would be the most common side effect of these drugs?

g) Bacteria have the CRISPR-Cas9 defense mechanism that protects them from the invading viruses. Give **one similarity and one difference** between this CRISPR–Cas9 defense mechanism in bacteria and the adaptive immune response in vertebrates.

Question 5

Viruses are obligate intracellular parasites. They can have DNA or RNA genomes, which are either single- or double-stranded. The single stranded RNA viruses can be plus stranded RNA viruses, minus stranded RNA viruses or retroviruses.

a) Ebola is a **single stranded RNA virus** as shown in the schematic below.



i. If the viral genome contains 25% adenine nucleotide, can you predict the % of the remaining three nucleotides that make up its genome? **Why or why not?**

ii. Would the genome of Ebola virus be **more OR less** chemically stable than the genome of a DNA virus? **Why**?

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Question 5 continued
You are studying the following three viruses.

- **Virus A** is an enveloped, minus (-) stranded RNA virus.
- **Virus B** is an enveloped, plus (+) stranded RNA virus.
- **Virus C** is a non-enveloped, double-stranded DNA virus.

b) Which of these viruses (**A / B / C**) is likely to have the **lowest mutation rate**? **Explain.**

c) Which of these viruses (**A/ B/ C**) brings protein(s) along with its genome, into the host cell, at the time of infection?

d) Do all viruses use host translation machinery to make viral proteins? Why or why not?

e) Influenza virus is a **minus-stranded, segmented, enveloped RNA virus**. Each year, the world health organization (WHO) recommends development of a new flu vaccine based on the analysis of the newly emerged strains. Explain why this virus mutates so rapidly?

Question 6 (This question is optional and will NOT be graded)
Human immunodeficiency virus (HIV) is a retrovirus. Its genome is a single (+) stranded RNA and is packaged with the reverse transcriptase enzyme within a protein capsid. This is further packaged into an envelope that is derived from the plasma membrane of the host cell in which the virus had replicated. The surface of the envelope is covered with the envelope glycoproteins gp120 and gp41.

a) HIV specifically infects the T- helper (T_H) cells of the human immune system. If the HIV enters the host cell by means of host receptor recognizing a viral protein, what would be the most likely **ligand(s)** and its **corresponding receptor(s)** during HIV infection?

b) In recent years, therapies have been developed to fight AIDS using nucleoside analogs. One drug used to combat AIDS is Azidothymine (AZT). The structure of AZT is very similar to thymidine except that in AZT, the 3'-OH group on the deoxyribose sugar is replaced by an azido (N_3) group. Which process of the life cycle of HIV do you think is inhibited by AZT and how does this work?

c) Combination therapy is often the strategy for treating HIV patients. Why is combination therapy more successful in preventing the emergence of disease resistant clones?

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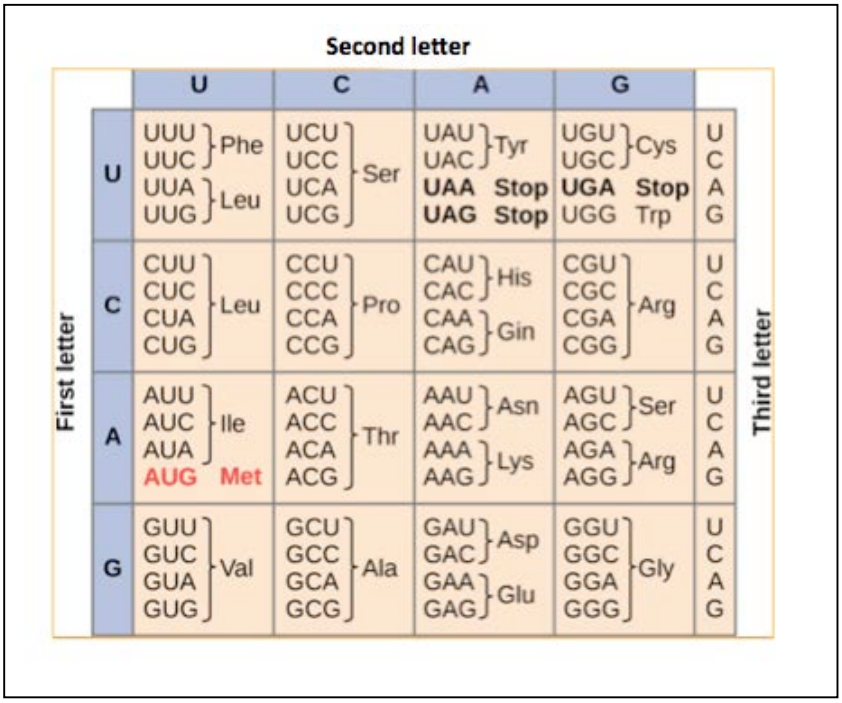
CODON CHART



First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU, UUC: Phe; UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr; **UAA Stop**; **UAG Stop**	UGU, UGC: Cys; **UGA Stop**; UGG: Trp	U C A G
C	CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His; CAA, CAG: Gln	CGU, CGC, CGA, CGG: Arg	U C A G
A	AUU, AUC, AUA: Ile; AUG: Met	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn; AAA, AAG: Lys	AGU, AGC: Ser; AGA, AGG: Arg	U C A G
G	GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp; GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly	U C A G

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