Solution key-7.016 Problem Set 6- 2018

Question 1 (5pts)

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
CTGGAGTGTATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA3′
```

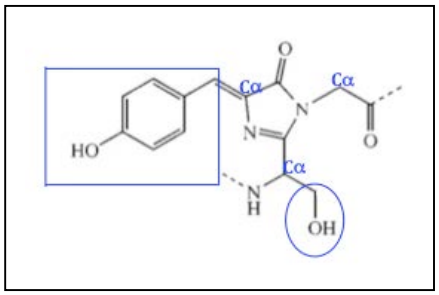
a) Write the sequence of the first 6 bases of the GFP mRNA: **5'-UACACA-3'** *(0.5pts)*

b) Box the 5'untranslated region **(5'UTR).** *(0.5pts)*

c) Write the first 3 amino acids of the GFP protein. **N- Met-Ser-Lys-C** *(0.5pts)*

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?** *(1pt with 0.5 for explanation)*
Yes, this is a silent mutation with no change in amino acid sequence in the primary structure of the protein. The protein will have the same amino acid sequence and will be functional.

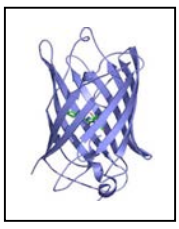
e) The following is the line angle drawing of the fluorophore in GFP. This fluorophore originates from the **cyclization and oxidation of the Ser^{65}-Tyr^{66}-Gly^{67} tripeptide**.



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}. *(0.5pts)*

ii. Label the alpha carbon (α-C) contributed by each of the three amino acids. *(0.5pts)*

f) The following is the structure of monomeric GFP.



i. What is the highest level of its protein structure: **primary/secondary/tertiary/ quaternary?** ***Tertiary*** *(0.25pts)*

ii. What secondary structure is most prominent in this protein: **α- helices or β-sheets?** ***β- sheets*** *(0.25pts)*

Image courtesy of Clipart Library.

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**.

Both approaches can be used to study the localization of this intracellular protein. But antibody can detect and bind to an intracellular protein of cells that are permealized/ dead. In comparsion, the fusion protein will be expressed and functional in living cells. (0.5pts)

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. *(0.5pts)*

A polyclonal antibody is a collection of different antibodies, each directed against a specific epitope (antibody binding site on a antigen) of the SAME antigen. They may also exhibit varying affinities for the different epitopes of the same antigen. In comparison, a monoclonal antibody can recognize a single epitope on the same antigen and has a constant affinity for the antigen.

A polyclonal antibody is prepared by injecting the antigen (along with an adjuvant) into an organism such as a rabbit. You repeat the injections until you can detect an antibody titer in the rabbit's blood. In order to generate a monoclonal antibody, you inject the antigen (along with an adjuvant) intravenously in an organism such as mouse. You isolate the B cells from the spleen of this mouse after about 2 weeks and mix them with myeloma cells in a special growth medium that promotes cell fusion and formation of hybridoma cells. The hybrid cells will grow continuously (a feature of myeloma cells) and make antibodies specific to the antigen (a feature of B cells).

Question 2 (3pts)

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?** *(1pt with 0.5pts for explanation)*

No, the λ_{max} emission wavelength of EthBr would be longer than its λ_{max} excitation wavelength. The λ_{max} excitation wavelength represents light energy to promote EthBr to the excited state. When EthBr returns to the ground state, energy is emitted and therefore you observe fluorescence of EthBr. So the excitation wavelength will be shorter (higher energy) than the emission wavelength (lower energy).

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

These are dyes that can be applied to living cells without causing cell death. Hoechst and DAPI are examples of supravital dyes. These dyes are cell permeable and bind to AT rich sequences in the minor groove of double helical DNA in live or fixed (dead) cells. (0.5pts)

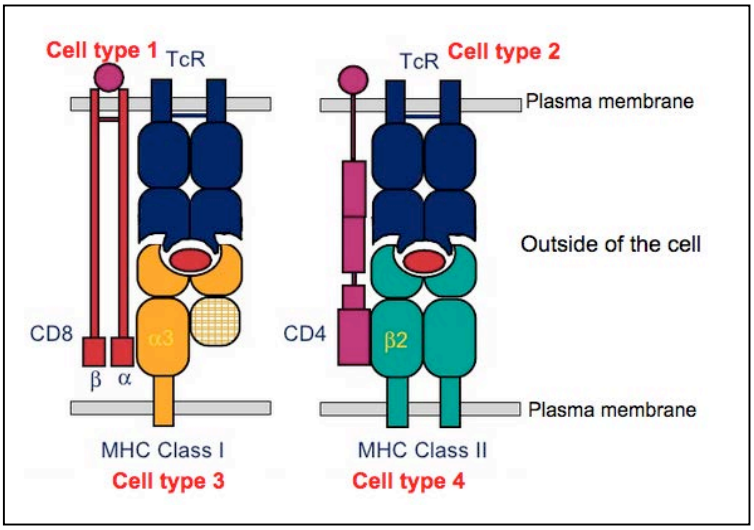
c) Microarrays are the 2D- arrays built on functional silicon chips that display a collection of DNA or cDNA samples. You have the following microarrays: *(1.5pt with 0.5pts for each)*

- **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: *Yes since this microarray has the entire genomic information including the coding regions of Her-2 gene.* **Microarray B:** *Yes since this microarray has the cDNA corresponding the expressed region of the gene that has the mutation.* **Microarray C:** *No, since this microarray does not include the coding regions of the expressed genes.*

Question 3 (4pts)
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**? *(MHC-I if included is OK) (1pt with 0.5pts for each)*

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**? *(MHC-I if included is OK) (1pt with 0.5pts for each)*

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**?
(1pt with 0.5pts for each)

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

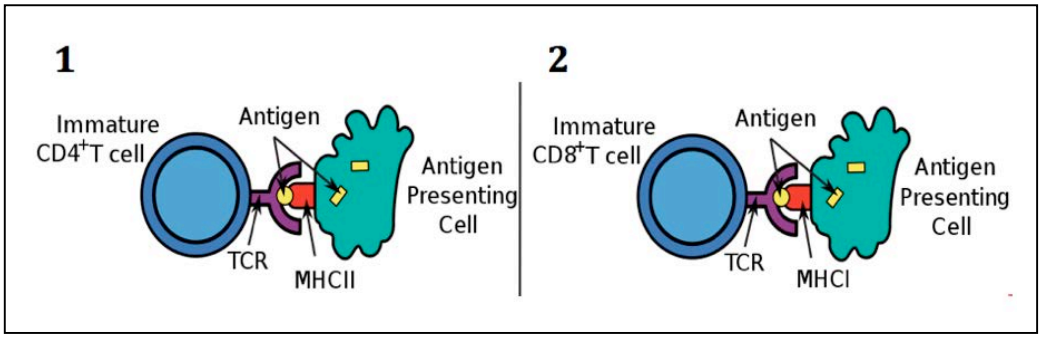
i. B cell activation and humoral (antibody-mediated) immune response: **Cell type 1/ 2/ 3/ 4**? *(0.25pts)*

ii. Cytotoxic T cell mediated killing of an infected cell: **Cell type 1/2/ 3/ 4**? *(0.25pts)*

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.
This is due to allelic exclusion, which is process that avoids dual specificity of T and B cells for antigens. A successful VDJ recombination sends a signal back to the nucleus to suppress recombination on other chromosomes/ homolog. If however, the first allele recombination fails, no suppression occurs and there is a VDJ recombination in the 2nd allele of the Immunoglobulin and/ or TcR gene. (0.5pts)

Question 4 (5pts)
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.

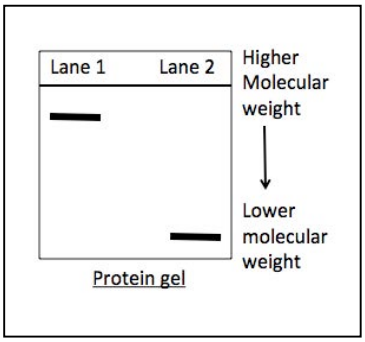
a) Which of the following schematics (**1 or 2**) represents the immune response that will be triggered following vaccination with Gardasil.



Schematic 1: Here you are using viral proteins, which can be engulfed by APC to trigger humoral immune response. (1pt with 0.5pts for explanation)

b) Following vaccination with Gardasil, why is the secondary immune response against HPV **faster and more effective** than the primary immune response?
During the primary immune response, the memory B and T_H cells, against the specific antigen, are generated. During the secondary immune response, these can proliferate to produce plasma B cells that produce and secrete antibody, which has a higher affinity for the antigen than surface antibody. The secreted antibody molecules can bind to the antigen to neutralize its infecting ability. Furthermore, they also promote the phagocytosis of antibody bound antigen. (1pt with 0.5pts for explanation)

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**?
Secreted IgG (0.25pts)

ii. From the gel, which B cell-type produces the antibody type shown in **Lane 2**?
Plasma B cells likely, they produce secreted antibody (IgG), which is monomeric as opposed to IgM of the memory B cells, which maybe pentameric. (0.25pts)

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?**
SAME with potential for slight variation, since they both recognize the SAME antigen. But the antigen-binding site of secreted antibody has a higher affinity for the antigen. (0.25pts)

ii. The **same or different Constant** regions? **Why?** *(0.25pts)*
DIFFERENT, since the secreted antibody lacks the transmembrane domain.

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. *(1pt with 0.5 for each)*

*-VDJ recombination: The immunoglobulin gene has approximately 100 variable (V) segments, 30 diversity (D) segments and 6 joining (J) segments, which can undergo **DNA recombination** in order to produce different combinations of V, D and J segments for heavy chains and V and J segments for light chains to produce millions of antibody molecules each of which is unique to a specific antigen.*

In addition, more variations at the DNA level can be generated through…

-Somatic hypermutations

- Class switching

-Affinity maturation

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?
The self reactive T cell are usually eliminated in the thymus during the process of their development by negative selection or clonal deletion. If this goes wrong then autoimmune disorders such as MS arise. (0.25pts)

ii. Immunosuppressive drugs are often prescribed to patients with autoimmune diseases. What would be the most common side effect of these drugs?
The patient will be prone to opportunistic infections since the patient's immune system will be compromised. (0.25pts)

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. *(0.5pts with 0.25pts for each)*

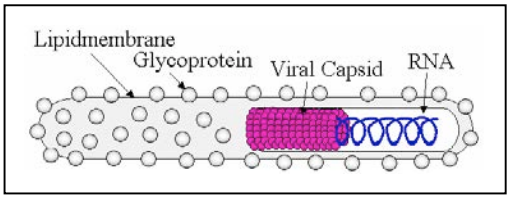
***Similarity:** They both have memory and represent specificity*

***Difference:** CRISPR-Cas9 defense mechanism is inherited where as adaptive immune response is acquired over time*

Question 5 (3pts)

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?** *No, you can't since the virus has a single stranded RNA genome with no regular complementary base pairing. (0.5pts)*

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

LESS STABLE, since the RNA is composed of ribonucleotides (NTPs) monomers, which have an extra hydroxyl group (-OH group) at the 2'C position of the ribose base. This makes RNA more reactive and less stable compared to DNA. So the genome of Ebola virus would be less stable compared to the genome of a DNA virus. (0.5pts)

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.**
Type C, since it uses the host DNA polymerase to replicate its genome and this enzyme has proofreading ability. (0.5pts)

c) Which of these viruses (**A/ B/ C**) brings protein(s) along with its genome, into the host cell, at the time of infection?
Type A, since this is minus stranded RNA virus that has a genome with polarity opposite to the mRNA of the host cell. Therefore, it needs to bring in its own RNA dependent RNA polymerase at the time of infection for its genome to replicate and eventually be translated. (0.5pts)

d) Do all viruses use host translation machinery to make viral proteins? Why or why not?
Yes, depending on their genome some viruses may bring into the host cells their proteins and genome, which may be critical for their replication. But ALL viruses always use the host translation machinery to make the viral proteins, which are packaged with the genome to make new viral particles. (0.5pts)

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?
It is segmented and this allows mixing of genome of different viral strains that are co-infecting a cell resulting in the mergence of new strains. In addition, the viral RNA dependent RNA polymerase lacks the proofreading ability and this enhances the mutation rate resulting in the rapid emergence of the new viral strains. (0.5pts)

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? *The gp120 (gp41) protein on the surface of HIV envelop interact with the CD4 receptor (and CCR) on the surface of T helper cells to inject the viral genome and viral proteins such as reverse transcriptase and integrase.*

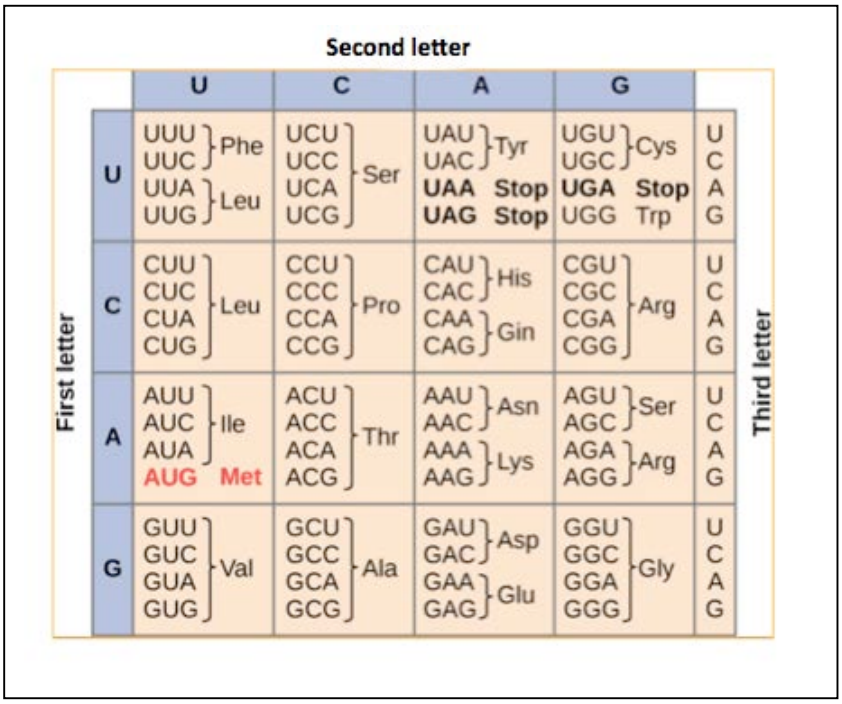
b) In recent years, therapies have been developed to fight AIDS using nucleoside analogs. One drug used to combat AIDS is Azidothymine (AZT). The structure of AZT is very similar to thymidine except that in AZT, the 3'-OH group on the deoxyribose sugar is replaced by an azido (N_3) group. Which process of the life cycle of HIV do you think is inhibited by AZT and how does this work?
AZT is a thymidine analogue (a nucleotide used in the synthesis of DNA). Therefore AZT interferes with the synthesis of DNA from RNA by reverse transcriptase. This enzyme incorporates AZT more effectively into the growing DNA chain and this blocks the further elongation of the chain because the growing end has no 3'-OH group on the deoxyribose sugar. So the viral concentration decreases over time with response to the treatment.

Question 6 continued

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?

Multiple separate mutations in different viral components are far less likely to occur than the single mutations, so combination therapy reduces the chances of generating drug resistant strains of HIV.

CODON CHART



7.016 Introductory Biology
Fall 2018