Birla Institute of Technology & Science (BITS), Pilani
Intro to Mol Bio and Immuno (PHA F215); **Second Semester 2021-22**
Comprehensive Exam (**Closed Book**), 13th May 2022; **Maximum Marks: 40**, **Time: 90 Min**

Q1. You have isolated two mutant mammalian cell lines defective in apoptosis – one has a mutation in Apaf1, and the other has a mutated effector Bcl2. Since both these proteins are involved in the intrinsic pathway of apoptosis, you assume that both cell lines will undergo apoptosis if injected with cytochrome c in the cytoplasm. To your surprise, the Apaf1 cell line does not undergo apoptosis, but the effector Bcl2 cell line does. Explain these results. **[3]**

Q2. You are interested in the mechanism by which olfactory receptors regulate the sense of smell. Upon binding of odorants, these GPCRs activate olfactory-specific G proteins known as G_{olf}. Activated G_{olf} triggers the activation of adenylyl cyclase, which ultimately results in the opening of cAMP-gated Na+ channels; the influx of Na+ depolarizes the olfactory neurons. Humans have ~350 different olfactory receptors, each recognizing a different set of odorants. Each olfactory neuron displays only one type of olfactory receptor, and responds to only one set of odorants. You have identified one of these neurons to study.
What do you predict would happen to odor detection (i.e. increased odor detection, decreased odor detection, no change in odor detection) if cells were treated in the following fashion? Explain your answer.

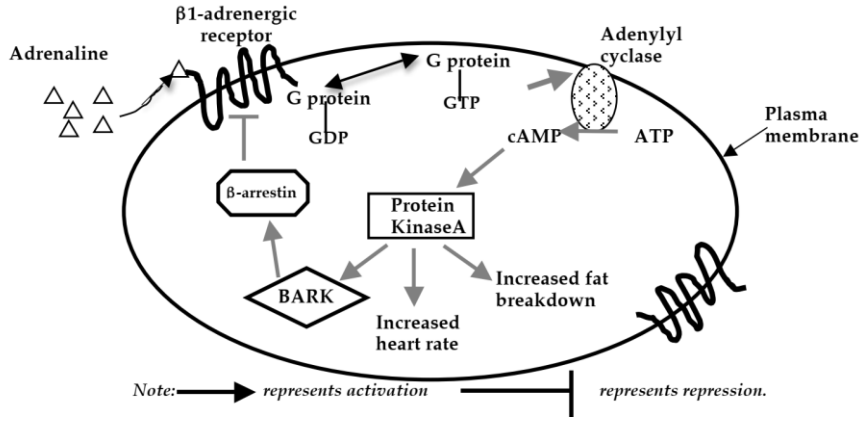
- **a)** You inhibit the RGS protein [Regulators of G protein signaling (RGS) are proteins that function to activate the GTPase activity of G-protein α-subunits] that normally works on the α subunit of the G_{olf} protein involved in this pathway. **[2]**
- **b)** You add a drug that increases the activity of cyclic AMP phosphodiesterase. **[2]**
- **c)** You add a drug that inhibits adenylyl cyclase. **[2]**

Q3. Explain the roles of protein kinase and protein phosphatase in the regulation of signaling pathways. **[3]**

Q4. Make a diagram of an APC that is interacting with and activating a Th cell. Note: Include **ALL** the relevant molecules on your schematic that is required for proper activation. **[3]**

Q5. a) You purify a novel viral protein (Protein R) and intend to further characterize it. Accordingly, you develop antibodies against Protein R. You inject Protein R into a rabbit, draw out some blood from the rabbit after a month and confirm the presence of Protein R specific antibody in the rabbit's blood. You wait for two months and then re-inject Protein R into the same rabbit. Draw the primary and secondary antibody responses specific to Protein R and explain the dynamics. **[3]**
b) How the innate immune system influencing the antibody titer in this experiment? **[3]**
c) Your friend decides to inject an attenuated form of the virus into a rabbit instead of injecting only Protein R derived from this virus. How this is going to change the overall immune response? **[4]**

Q6. During a summer hike you suddenly spot a huge grizzly bear. This emergency situation triggers a fight or flight response through a signaling pathway as shown below.



a) Name the 1st and the 2nd messenger in this pathway and give one reason that explains why the 2nd messenger may be important in this pathway. **[2]**

b) In order to further understand this cellular pathway, you examine this pathway in a cell after the following perturbations.

A. Perturbation 1: Application of cholera toxin, which prevents G proteins from hydrolyzing GTP to GDP.

B. Perturbation 2: Treatment with nebivolol, a molecule that competes with adrenaline to bind to the β1-adrenergic membrane receptor but does not stimulate the pathway.

i. In which of the two treatments will the adrenaline cause a constitutive (continual) activation of adenylyl cyclase? Explain. **[2]**

ii. In which of the two treatments will you see very low or no activation of adenylyl cyclase in the presence of adrenaline? Explain. **[2]**

Q7. A pathogenic, extra-cellular bacterium has 3 major antigens on its surface, A1, A2, and A3. A1 antigen has only B-cell epitope, A2 has only T-cell epitope, whereas A3 antigen has both B and T-cell epitopes. Explain which of the antigen would mount the most significant antibody response and why. **[3]**

Q8. A group of mice in which the B7 protein has been "knocked out" are being immunized with Mycobacterium tuberculosis. One batch is treated with a polysaccharide extract of the bacteria and a second batch is treated with a protein derived from the bacteria. What would be the likely outcome in both the cases? Explain. **[3+3=6]**

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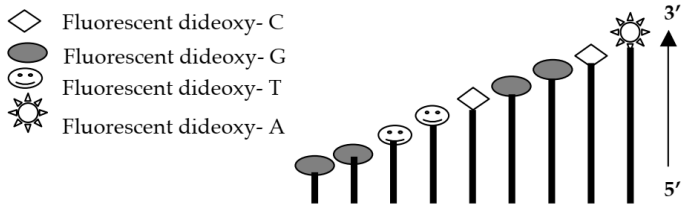
You have identified a yet unknown protein, named as Protein D, which is highly correlated with colon cancer. You found out a single amino acid mutation in this protein made it tumorigenic. You purified the wild- type and disease-associated forms of Protein D and determined their amino acid sequence. The only difference you found was at the 6th position shown in bold below.

Wild- type variant: N-gly^5-**trp**^{6}-ala^7-C
Mutant variant: N-gly^5-**ser**^{6}-ala^7-C

Shown below is a portion of the fluorescence dideoxy- sequencing gel, which gives the sequence of the coding strand of the DNA that corresponds to amino acids 5-7 of the mutant form of Protein D.



Q1. Write the sequence of the double stranded DNA that corresponds to amino acids 5-7 of the wild-type form of Protein D and label its 5' and 3' ends. **[3]**

The following is the DNA sequence of the wild type allele of Gene D that you want to amplify using the polymerase chain reaction (PCR).

```
5′CTCGAGGTGAATATGAAAG----  Gene D  ----CATTTGGCGCGTAATCGATA3′
3′GAGCTCCACTTATACTTTC----  Gene D  ----GTAAACCGCGCATTAGCTAT5′
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Q2. If you amplify a DNA sequence through PCR, what are the reaction components that you would absolutely need? Briefly state the function of each of these components. **[5]**

Q3. Give the sequence (10 bases long) of a set of primers, which you would use for the PCR reaction. **[2]**

Q4. In the PCR reaction, you need a three-step reaction cycle, which results in a chain reaction that produces an exponentially growing population of identical DNA molecules. Each step of a reaction cycle is performed at a specific temperature i.e. 94°C for Step 1, 60°C for step 2 and 72°C for Step 3. Briefly explain why the three steps are performed under different temperatures. **[3]**

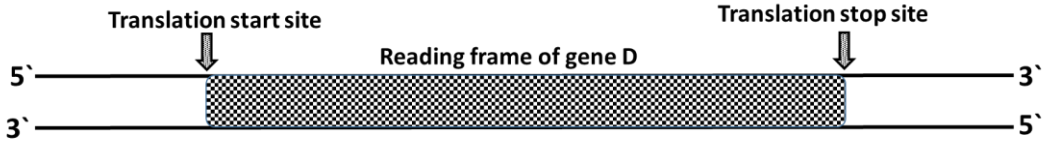
Q5. You need a large amount of Protein D encoded by Gene D. Therefore, you decide to engineer a eukaryotic cell line that will secrete a large amount of Protein D, so that you can purify Protein D from the medium.

a) List any four components, of the host eukaryotic cell translation machinery, which are absolutely required for synthesis of proteins, and briefly (few words) indicate what each does. **[4]**

b) List two components of the host eukaryotic cellular machinery, which are absolutely required for export of Protein D from the cell, and briefly (few words) indicate what each does. **[2]**

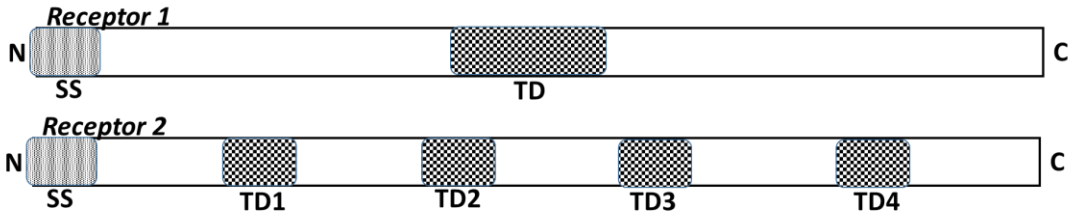
Q6. You isolate a cDNA for Gene D. However, when you transfect the normal cell line with a vector containing Gene D, you find that Protein D is produced in the cytoplasm but not secreted. You conclude that something is therefore wrong with the Gene D. What modifications would you make to the cDNA for Gene D that would plausibly allow Protein D to be secreted? **[3]**

Q7. The following is a schematic of the cDNA for Gene D. Identify the region in Gene D, where you would make the above modification. **[3]**



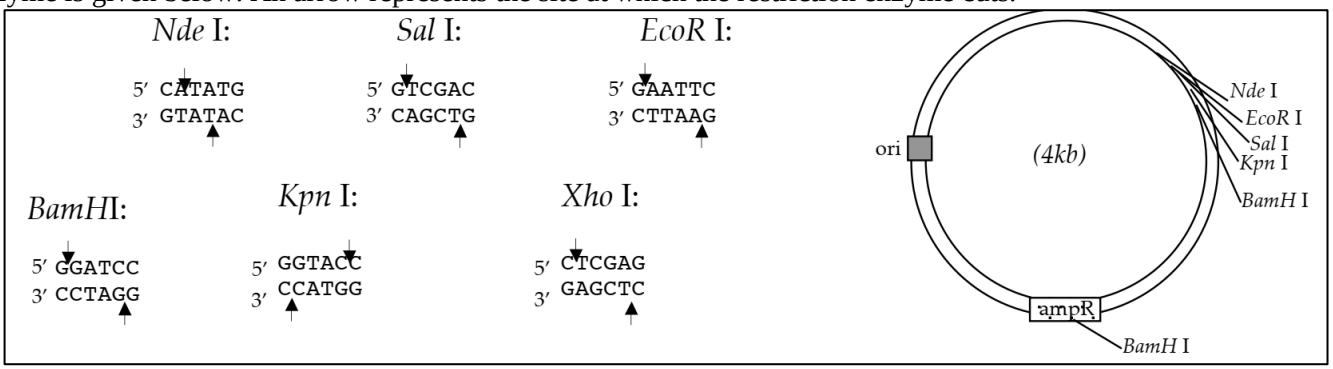
Q8. Assuming your manipulation in **Q7** is successful, would the modified/secreted version of Protein D be of the same size, larger than, smaller than its unmodified/cytoplasmic version? Explain your answer. **[3]**

Q9. Interestingly, you find that this protein, when secreted, can bind to either of following cell membrane receptors (Receptor 1 and Receptor 2). The transmembrane domains (TD) and the signal sequence (SS) are shown in the schematic.

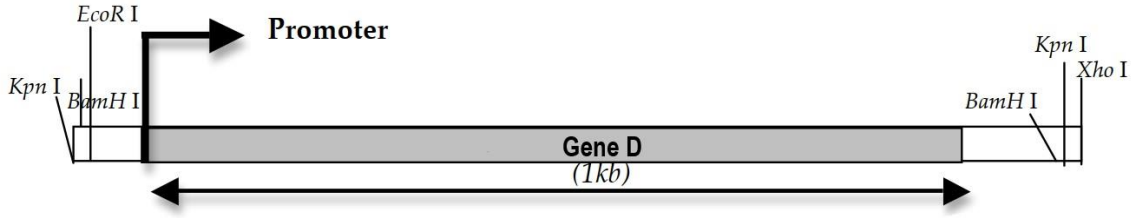


Draw the two receptors as they would be inserted into the Endoplasmic reticulum (ER) and plasma membrane, label the N and the C termini and include all the TD domains that are shown in the schematic above. **[2+2]**

Q10. You decide to use the following plasmid vector to clone Gene D. The recognition sequence for each restriction enzyme is given below. An arrow represents the site at which the restriction enzyme cuts.



A schematic of Gene A is given below. You want to clone Gene D into the plasmid vector. Give three different strategies that you could use to clone Gene A into the vector, and obtain colonies that contain a recombinant plasmid.



Strategy	Restriction enzyme(s) used to cut….	
	Gene A	**Plasmid vector**
1		
2		
3		

[2×3=6]

Q11. Which strategies (Choose from Strategy 1, 2, 3) would allow a directional cloning? **[2]**

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