# Question 1

Familial adenomatous polyposis (FAP) affects nearly 1/8000 people in the USA. Patients having FAP are genetically **predisposed** to colon cancer. Mutations in the APC gene have been identified as the probable cause of FAP.

(a) The following diagram represents the gel electrophoretic profiles of both the PCR amplified APC DNA (top panel) and APC protein (bottom panel) isolated from white blood cells (WBCs) and colon cancer cells of two individual patients. *(A profile of the APC DNA and APC protein in a normal individual is provided as a reference. Please note the intensity of the bands while answering this question).*
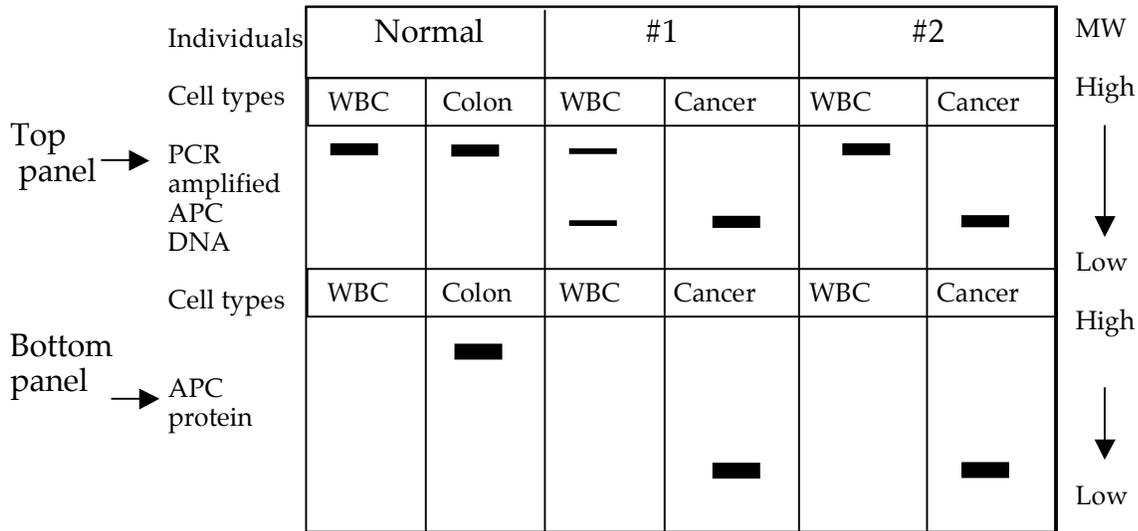


i. Explain why in the normal individual, the APC protein is detected only in colon cells even though the APC DNA is present in both colon cells and WBCs.

ii. One of these two individuals **does not** have FAP but still develops colon cancer. Given the data above, which individual would this be? Explain how this individual got colon cancer.

iii. Complete the following table based on the information provided in the gel profile above. *(Use the symbols '+' to represent the wild-type allele of the APC gene, '-' to represent the loss of function mutation and 'M' to represent the gain of function mutation. The genotype of the APC gene in a normal individual is provided as a reference).*

| Individuals | Genotype of APC gene | | Is the genotype of WBC different from colon cancer cells? If yes, explain why. |
|---|---|---|---|
| | WBC | Colon cells or Colon cancer cells | |
| Normal | +/+ | +/+ | |
| #1 | | | |
| #2 | | | |

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## Question 1 continued

(b) Vincristine is an inhibitor of microtubule assembly and is used as an important chemotherapeutic drug. Explain how the disruption of microtubule assembly may prevent cancer cell growth.

(c) During drug screening you identify two compounds A and B that have the potential to kill colon cancer cells and normal cells as shown by the following graph.



#1: Cancer cells + Compound A
#2: Cancer cells + Compound B
#3: Normal cells +Compound B
#4: Normal cells + Compound A
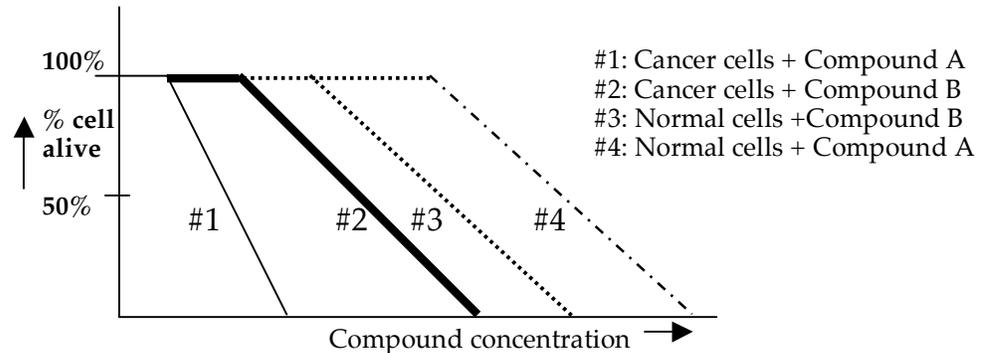
Which of these two compounds is a better candidate for colon cancer treatment? Explain why.

(d) Many patients show signs of severe anemia as a side effect of chemotherapy and are prescribed erythropoietin (EPO). EPO is a secreted protein, produced by the kidney, which binds to its receptor on erythroid precursor cells and stimulates the formation of red blood cells (RBCs). Four different mutations are described below. For each mutation, list whether the RBC production would **increase, decrease or not change** in an individual that was homozygous for this mutation as compared to the wild-type situation. Briefly explain your reasoning for each mutation.

     i.    A mutation in the EPO gene, which results in deletion of the signal sequence of the EPO protein.

    ii.    A mutation in the EPO receptor, which results in the deletion of its transmembrane domain.

    iii.    A mutation in the EPO receptor that results in the deletion of its cytoplasmic domain.

    iv.    A mutation that results in a constitutively active promoter for the EPO gene.

# Question 2

You are studying two characteristics in a plant; growth (slow or fast) and seed size (small or large). You cross a true-breeding plant with large seeds and slow growth to a true-breeding plant with small seeds and fast growth. All of the resulting F1 plants have small seeds and grow slowly.

(a) What are the genotypes of the true breeding parental plants? Use the nomenclature outlined below.
- *In each case, use the uppercase letter for the allele associated with the dominant phenotype and the lowercase letter for the allele associated with the recessive phenotype.*
- *For the seed size (i.e. large or small) use D or d to designate the alleles.*
- *For the growth (i.e. slow or fast) use G or g to designate the alleles.*

| Parents | Genotypes |
|---|---|
| Large seeds and slow growth | |
| Small seeds and fast growth | |

(b) You then cross two of the F1 plants that have small seeds and grow slowly. If these two genes are unlinked, about how many total offspring will you need to obtain 100 plants that have large seeds and are fast growing?

(c) You find that the two genes are linked, and plan to determine the map distance between the gene that regulates seed size and the gene that regulates plant growth rate. You test cross an F1 plant to a plant with large seeds that is fast growing. What are the genotypes and phenotypes associated with the non-recombinant and recombinant progeny?

| Progeny | Phenotypes | Genotypes |
|---|---|---|
| Recombinant | | |
| | | |
| Non-recombinant | | |
| | | |

# Question 3

(a) For the pedigree below, identify the most likely mode of inheritance and state the genotypes of the numbered individuals in the following tables. *(Note: The filled squares or circles represent the abnormal phenotype. Assume that the unaffected people marrying into the family are homozygous for the wild type allele of the gene. Use the letter "A" for the allele associated with the dominant phenotype, "a" for the allele associated with the recessive phenotype. Assume complete penetrance).*
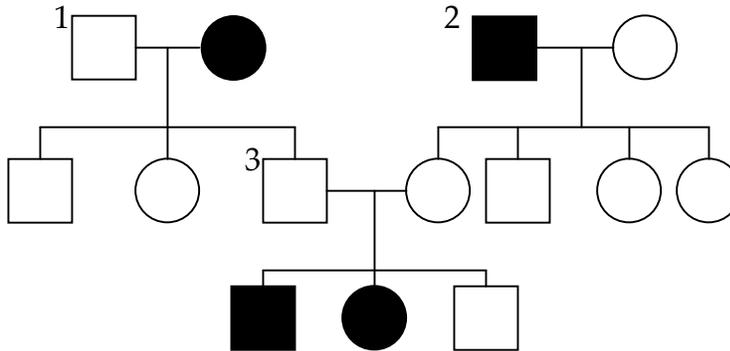


| Mode of inheritance: | |
|---|---|
| Individuals | Genotypes |
| #1 | |
| #2 | |
| #3 | |

(b) Individual #4 in the pedigree below has an intestinal disease that shows an autosomal recessive mode of inheritance. He marries a woman (#5) who develops Huntington's disease that has an autosomal dominant mode of inheritance. Their son (#6) had Huntington's disease but not the intestinal disorder. Furthermore, the gene that regulates the intestinal disorder is linked to the gene involved in Huntington's disease. *(Note: Individuals with Huntington's disease are shaded grey in the pedigree below; stripes are used to represent the individuals affected by the intestinal disorder. Assume complete penetrance).*
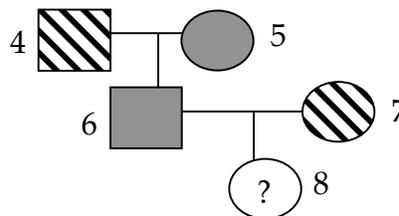


    i.    Write the genotypes of the following individuals for **both** the disorders *(Note: Use the letters H or h to designate the alleles for the Huntington's disease gene and the letters B or b to designate the alleles of the gene associated with the intestinal disorder. In each case use the uppercase letter for the dominant phenotype and lowercase letter for the recessive phenotype).*

| Individuals | Genotypes |
|---|---|
| #6 | |
| #7 | |

    ii.    What is the probability that Individual #8 is affected by both disorders, if the genetic distance between the two genes is 10cM?

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# Question 4

Her-2 is a gene that encodes a **glycoprotein** that is a member of the family of Epidermal growth factor (EGF) **receptor tyrosine kinases**. Her-2 gene amplification is observed in 30% of breast cancers.

(a) In **a breast cancer patient** that over-expresses Her-2 gene, would you characterize the gene encoding Her-2 protein as an oncogene, tumor suppressor gene or a proto-oncogene? Explain.

(b) From the choices given below, circle the molecule(s) that may be present in Her-2 receptor glycoprotein.
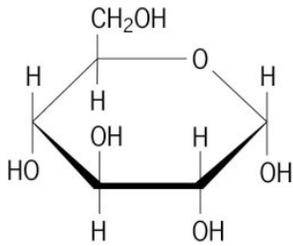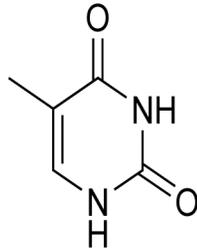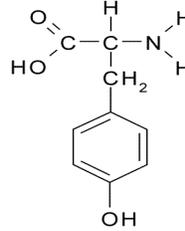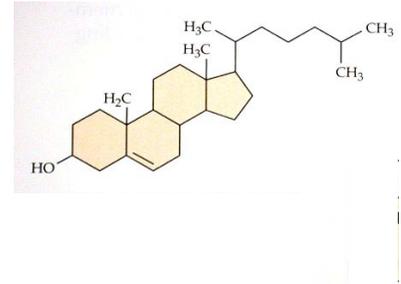


(i)                 (ii)                 (iii)                 (iv)

(c) Breast cancer patients that show the amplification of the Her-2 gene can be effectively treated with Herceptin, a monoclonal antibody that specifically binds to and prevents the dimerization of Her-2 receptors.

   i.    Based on your knowledge of immunology, each Herceptin antibody molecule can potentially bind to how many Her-2 receptor molecules?

   ii.    The heavy and the light chains of the Herceptin antibody should join together to form an intact functional antibody. Name the **strongest** type of interaction/bond that holds the chains together to form an antibody.

(d) The following schematic represents the binding of Herceptin to the extracellular domain of the Her-2 receptor. *For simplicity only the side- chains of the important amino acids in the binding site are shown. The C$^\alpha$ of each amino acids is indicated with an* *.
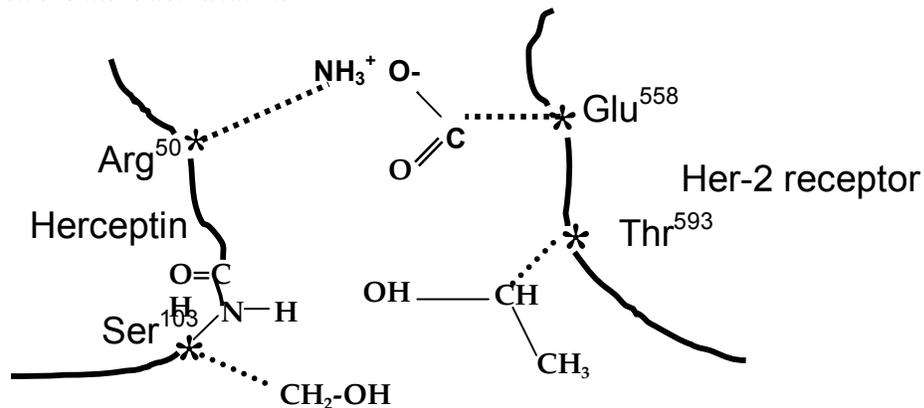
# Question 4 continued

Circle the **strongest interaction** that exists between……

    i.    Side-chains of $Arg^{50}$ of Herceptin and $Glu^{558}$ of Her-2 receptor.

        **Hydrogen**        **Ionic**        **Hydrophobic Interaction**        **Covalent**

    ii.    Backbone of $Ser^{103}$ of Herceptin and side-chain of $Thr^{593}$ of Her-2 receptor.

        **Hydrogen**        **Ionic**        **Hydrophobic Interaction**        **Covalent**

(e) The $Glu^{558}$ of Her-2 protein is encoded by 5'GAA3' codon. Write down the t-RNA anti-codon for $Glu^{558}$ and label its 5' and 3' ends.

(f) You come across the following mutations of $Glu^{558}$ in the Her-2 gene.  For each mutation, explain whether the binding of Herceptin with Her-2 protein will be disrupted. *(Note: A table of 20 essential amino acids and a codon chart is provided on the last two pages of this exam).*

| Mutations | Herceptin – Her-2 binding (Yes/No?) | Explain |
|---|---|---|
| 5'GAA3' to 5'UAA3' | | |
| 5'GAA3' to 5'GAG3' | | |
| 5'GAA3' to 5'GAC3' | | |

(g) Below are three different options for a hypothetical linear stretch of amino acids present in the Her-2 receptor.  For each option, the amino acids in Region 1 could represent a part of the transmembrane domain that spans the lipid bilayer, and the amino acids in Region 2 represent a part of the cytoplasmic kinase domain.

                        **Region 1**        |        **Region 2**

    Option 1: ……… lys-cys-gly-ala-val-trp-glu-lys-arg………...

    Option 2: ……… leu-ala-gly-cys-ala-val-lys-tyr-glu………...

    Option 3: ……… .gly-thr-tyr-ser-ala-glu-ala-his-met……….

    i.    Which option(s) most likely includes a stretch of Her-2 receptor that spans the lipid bilayer? Explain **in one sentence** why you selected this option.

    ii.    Which option most likely includes a stretch of Her-2 receptor that can be phosphorylated? Explain **in one sentence** why you selected this option.

# Question 5

Consider the following signal transduction pathway that is activated by the binding of EGF ligand to its specific membrane receptor.

- *EGF ligand binds to the EGF receptor.*
- *Ligand bound EGF receptors become active through phosphorylation and dimerization.*
- *Active EGF receptor causes Ras to exchange its bound GDP for GTP and become active.*
- *Active Ras activates the kinase cascade (RAF, MEK and MAPK) through phosphorylation.*
- *This increases the expression of c-myc gene which **promotes** cell proliferation.*
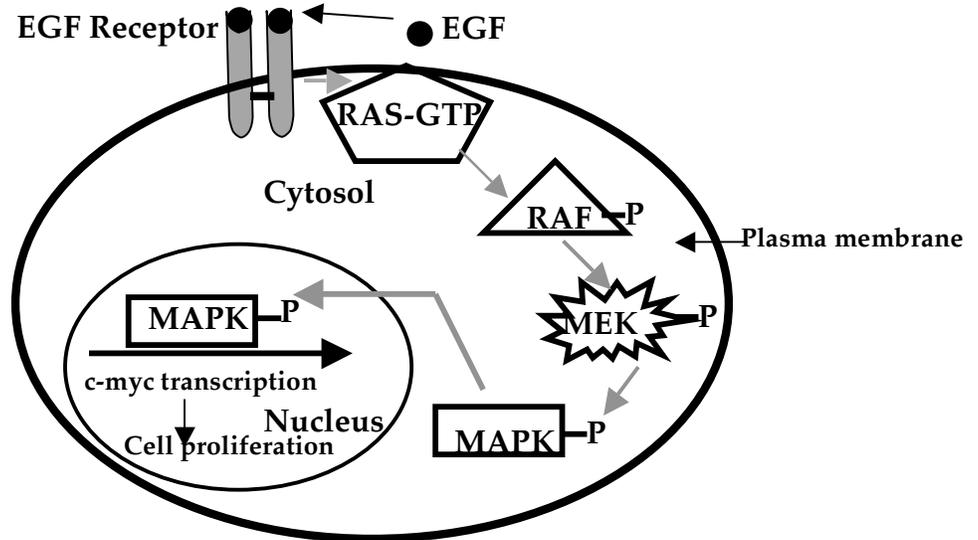


(a) Consider the following cells that have mutations in different components of the EGF signal transduction pathway.

- *Mutant 1 (m1): Ras protein that continues to stay in its GDP bound form.*
- *Mutant 2 (m2): RAF protein that lacks its kinase domain.*
- *Mutant 3 (m3): EGF receptor that lacks its extracellular domain.*
- *Mutant 4 (m4): MAPK that is constitutively phosphorylated at its active site.*
- *Mutant 5 (m5): c-myc gene that has a constitutively active promoter.*

Complete the table for each of the following cells. Indicate whether c-myc is expressed and  state the change in cell proliferation relative to wild type cells **in the presence of EGF**.

| Mutations in the cell | c-myc expressed (Yes/No?) | Cell proliferation increased//unchanged/ no proliferation? Explain. |
|---|---|---|
| Wild type | Yes | |
| Homozygous for m1 and m2 | | |
| Homozygous for m4 and m5 | | |
| Homozygous for m3 and m5 | | |

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(b) Shown below is the diagram of the cell cycle and the different phosphorylation states of the rentinoblastoma (Rb) protein. This protein is encoded by a tumor suppressor gene and acts at G1/S phase.

G0

M

G2

G1

Phospho-Rb

Rb

S
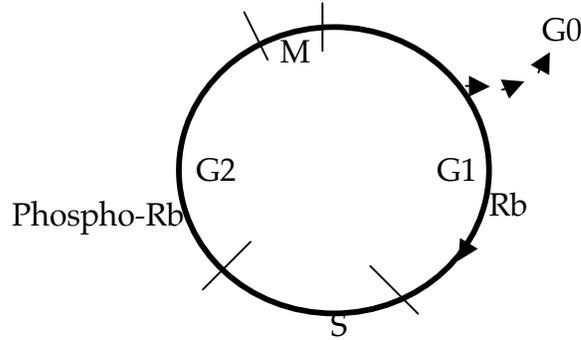
What two **classes** of enzymes can control the phosphorylation of the Rb protein?

(c) You construct a conditional mutant allele of Gene R ($R^{ts}$), which is active at a low growth temperature but is inactivated by higher (non-permissive) temperature. Using the cells with the $R^{ts}$ mutation, you grow a synchronized cell culture (all cells are growing at same stage of cell cycle and at exactly the same pace) and monitor the DNA content **per cell** over time, with the following results:

Shift to high temperature

DNA content per cell (n)

4

2

0

Time
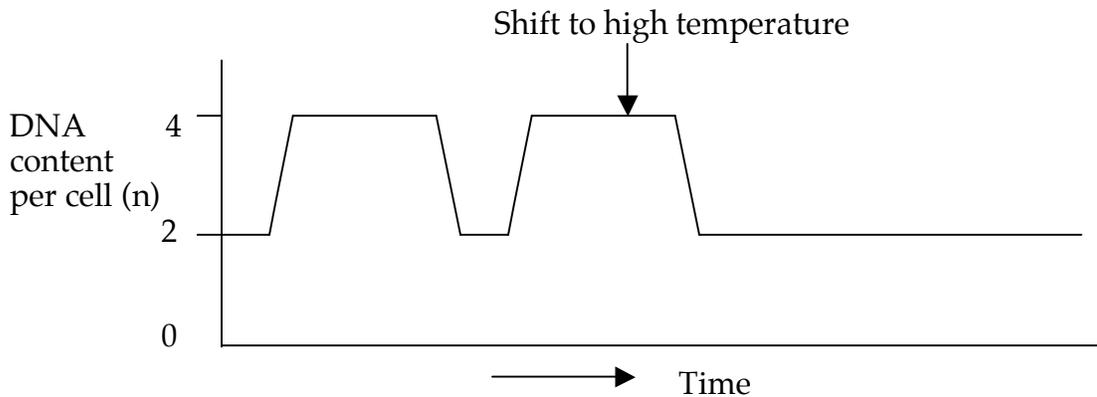
(i)     On the diagram above, draw a **box** around one complete cell division cycle at the permissive temperature.

(ii)    Within the box you have drawn, draw vertical lines to separate the phases of the cell cycle, and label each phase using the abbreviations: M, S, G1 and G2.

(iii)   Draw a **star** within the appropriate box marking the phase of the cell cycle in which DNA replication occurs.

(iv)    The vertical arrow on the diagram above indicates the time at which you shifted the cells to the non-permissive temperature. In what phase of the cell cycle is the activity of Gene R required?

# Question 6

The functional state of G proteins such as Ras depends on their binding to GTP or GDP. The schematic below represents a GTP molecule. GTP is also used to build nucleic acids and as an energy source equivalent to ATP.
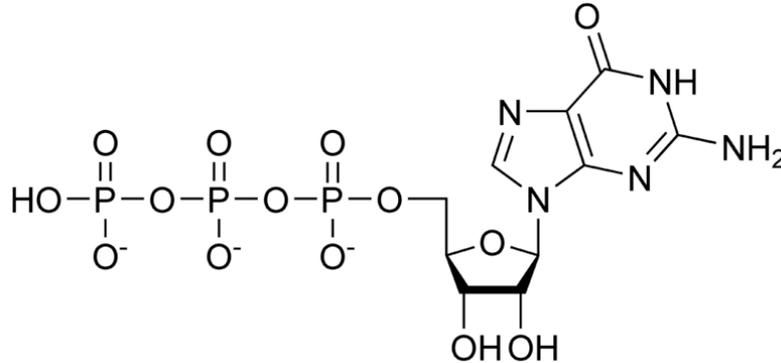


(a) Circle the part(s) of this molecule that associates with and activates G proteins.

(b) Draw a box around all the parts of this molecule that are added to the **growing chain** of nucleic acid.

(c) Make a triangle around the **reactive group** that forms a covalent bond with the **incoming base** of a growing nucleic acid polymer.

(d) Name the molecule(s) that are produced from GTP if it is used as an energy source.

(e) Star the atom(s) that can form a hydrogen bond with the complementary nucleotide.

(f) Put an arrow(s) next to the part of this molecule that you would alter if it were to be used as a chain terminator during DNA sequencing. For each arrow, indicate the change you would make.

(g) The molecule represented in the schematic above can be a direct precursor of…………
*Circle all that apply.*

Carbohydrates       Amino acids          Proteins        Cholesterol              DNA
          mRNA              tRNA          rRNA    Phospholipids       micro-RNA

(h) The following diagram represents a complex three-dimensional conformation of a micro RNA (miRNA) molecule.
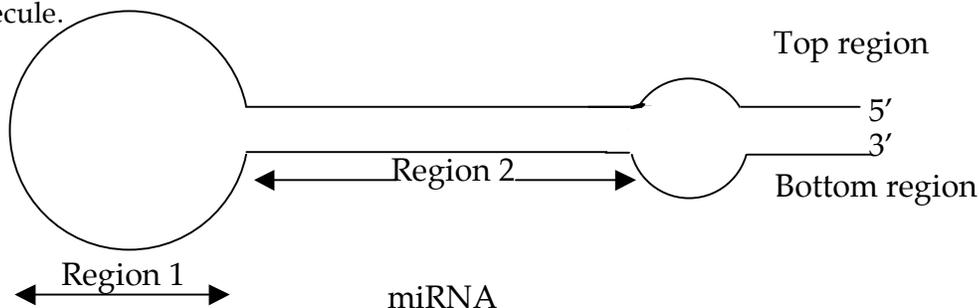
## Question 6 continued

    i.    Within Region 2, what bonds/interactions are **primarily involved** in stabilizing the RNA structure?

    ii.    In Region 1, if the sequence of the top region is AUGGCUAA, can you predict the % of bases i.e. %A, %U %C and %G of the bottom region? **Explain**. *(Note: Your choices are Yes/No).*

    iii.    In the Region 2, if the sequence of the top region is AUGGCUAA, can you predict the % of bases i.e. %A, %U %C and %G of the bottom region? **Explain**. *(Note: Your choices are Yes/No).*

## Question 7

Oncogenic mutations can result from DNA rearrangements or duplications. Below is a partial sequence of two different genes. Gene 1 encodes a protein that is always expressed at high levels. Gene 2 encodes a growth stimulating protein that is only expressed when the cell has received growth promoting signals. On occasion, Gene 2 gets positioned near the promoter for Gene 1. Some of these DNA rearrangements allow an increase in expression of the growth-promoting Gene 2. This can result in uncontrolled cell division. The direction of transcription is shown by an arrow.

Gene 1: expressed at high levels. A Partial sequence of gene 1 is shown below. The italicized and underlined sequence represents the promoter.

```
     1        10         20         30         40         50         60         70
     I--------I----------I----------I----------I----------I----------I----------I
5' ATCGGTCTCGGCTACTACGTAAACGCGCGCATATATCGATATCTAGCTAGCTATCGGTCTCGGCTACTAC
3' TAGCCAGAGCCGATGATGCATTTGCGCGCGTATATAGCTATAGATCGATCGATAGCCAGAGCCGATGATG

              80         90        100        110        120        130        140
     ---------I----------I----------I----------I----------I----------I----------I
5' GCATGTATCGATATAATCTAGCTAGCTTCTCTTCTCTCTCTCCCCCGCGGGGGCTAGTACTATGTATGGT
3' CGTACATAGCTATATTAGATCGATCGAAGAGAAGAGAGAGAGGGGGCGCCCCCGATCATGATACATACCA

              150        160        170        180        190        200        210
     ---------I----------I----------I----------I----------I----------I----------I
5' CGTCTCGGCTACTACGTAAACGCGCGCATATATCGATATCTAGCTAGCTATCGGTCTCGGCTACTACGTA
3' GCAGAGCCGATGATGCATTTGCGCGCGTATATAGCTATAGATCGATCGATAGCCAGAGCCGATGATGCAT
```

Gene 2: growth stimulating protein. The following is the portion of gene 2 that is inserted near Gene 1; the bases of one codon are underlined to indicate the reading frame.

```
5' TCTCGGCTACTACGTAAACGCGCGCATATATCGATATCTAGCTACTATCGGTCTCGGCTACTACGTAAAC
3' AGAGCCGATGATGCATTTGCGCGCGTATATAGCTATAGATCGATCATAGCCAGAGCCGATGATGCATTTG
```

You have found four different rearrangements such that:

    1) Gene 2 sequence inserts immediately after base pair 83 (shown in bold)
    2) Gene 2 sequence inserts immediately after base pair 136 (shown in bold)
    3) Gene 2 sequence inserts immediately after base pair 155 (shown in bold)
    4) Gene 2 sequence inserts immediately after base pair 183 (shown in bold)

## Question 7 continued

(a) Would rearrangement (1) result in an oncogenic mutation?  Explain.

(b) Would rearrangement (2) result in an oncogenic mutation?  Explain.

(c) Would rearrangement (3) result in an oncogenic mutation?  Explain.

(d) Would rearrangement (4) result in an oncogenic mutation?  Explain.

## Question 8

In nature, the influenza A virus has persisted in wild aquatic birds for millions of years and does not typically produce harmful effects. This is a single (-) stranded, segmented, RNA virus that does not replicate via a DNA intermediate.  Its genome is comprised of 8 distinct RNA strands that together encode 10 different proteins. The following is a schematic of the influenza virus.
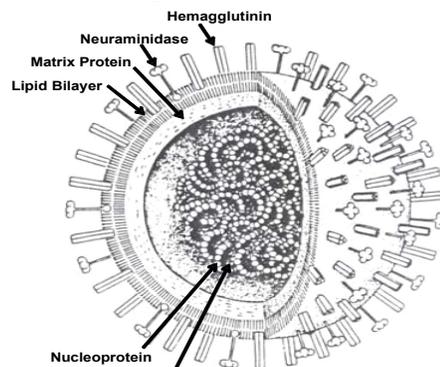


(a) Influenza virus is unable to make more viral RNA genome within the host cells using exclusively the host cell proteins.

      i.     Explain why that is so.

      ii.     Explain how the virus overcomes this issue and replicates its genome in the host.

(b) Explain why the influenza A virus can rapidly mutate to produce new variants.

## Question 8 continued

(c) You decide to generate vaccine against Influenza A virus using either a live-attenuated form of the virus or heat–killed viral particles.

   i. Which of these two vaccine strategies produces both antibody and Tc mediated immune responses? Explain why.


   ii. If the vaccine generated against the virus leads to a cell mediated immune response, name the proteins/enzymes (secreted by the cytotoxic T cells) that cause the killing of virus infected cells and propose a mechanism through which they do so.


   iii. If the vaccine generated results only in a humoral response and production of antibodies....
   • Identify the most likely components of the virus, from the diagram provided at the beginning of this question, to which the raised antibodies will bind.


   • Propose a mechanism through which the **secreted antibodies** can counteract the viral infection.


(d) If an individual is exposed a second time to same strain of Influenza A virus, the secondary immune response observed is much faster and stronger than the primary response. Propose **an explanation** for an increase in the rate and strength of the secondary immune response.


(e) Pigs can be infected both by the avian and human forms of the Influenza virus. This allows them to act as virus mixing bowls in which the two viral strains can readily exchange RNA strands. Thus the virus that emerges from such double-infected species can represent a unique assortment of genes, generating new strains of virus. The HIN1 virus that causes Swine flu is one such example. Why is a newly emerged virus considered a threat that is significant enough to cause a global pandemic, compared to a seasonal viral strain?


(f) You come across a patient who is suffering from a mysterious illness that kills the brain cells. You grind the damaged brain cells and treat them separately **in vitro** with DNAse, RNAse, proteases and UV. You use the treated samples to infect normal mice. You observe that all these samples, except the one treated with proteases, can cause the same illness in infected mice. Based on the data provided, what is the most likely cause of this illness?

# Question 9

You have started up a company to produce a vaccine against a particular DNA virus that causes disease in humans. To begin with, you want to screen the viral genes to identify those which encode proteins that can be used as potential antigens to create this vaccine.

You intend to adopt the following strategy to identify such genes:
- *Isolate the viral genome.*
- *Digest the genome using a specific restriction enzyme.*
- *Clone the digested viral DNA fragments into a specific cloning vector to make a genomic library.*
- *In bacterial cells, select a clone producing viral antigen from viral gene(s).*

(a) Why would you make a viral genomic library, rather than a cDNA library, to screen for viral genes?

(b) Shown below are the restriction sites for four restriction enzymes. *Cutting sites are indicated by a slash (/).*

| Enzyme X | Enzyme Y | Enzyme Z | Enzyme R |
|---|---|---|---|
| 5'–CC/CG GG–3' | 5'–TC/CG GA–3' | 5'–T/TCGA A–3' | 5'–TCC/GGG–3' |
| 3'–GG GC/CC–5' | 3'–AG GC/CT–5' | 3'–A AGCT/T–5' | 3'–AGG/CCC–5' |

Usually, fragments cut with different restriction enzymes cannot be ligated together. However, DNA fragments cut with enzyme X and enzyme Y can be ligated together.

    i.    Based on the sequences given above, write the 6-base pair sequence of the double-stranded DNA molecule at the site of ligation of an X-cut DNA fragment to a Y-cut DNA fragment. Be sure to indicate the 5' and 3' ends of each DNA strand.

    ii.    Will the resulting sequence be cut by……………… *circle 'Yes' or 'No' for each:*

- Restriction enzyme X        (Yes/No)

- Restriction enzyme Y        (Yes/No)

- Restriction enzyme Z        (Yes/No)

- Restriction enzyme R        (Yes/No)

## Question 9 continued

(c) You will use one of the three plasmids shown in the schematic below as the cloning vector to make your library. Assume that you would clone fragments of the viral DNA into the restriction site X. You plan to select the transformed bacteria on Tetracycline media. **Circle** the best choice of vector for your experiment. **Explain** why you selected this option.



Plasmid #1      Plasmid #2      Plasmid #3
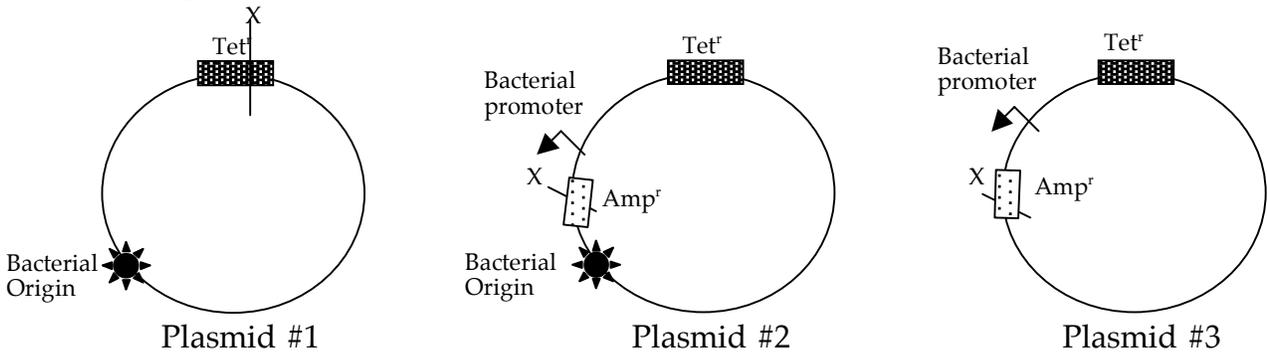
(d) After you have transformed *E. coli* cells with your library, you grow them on media containing Tetracycline to select for the bacterial cells that have been transformed. What additional selective media would you replica-plate these colonies onto to identify the clones that contain viral DNA?

(e) After various DNA based assays, you identify one colony that contains a gene which encodes a viral protein to use as the basis of your vaccine. You decide to express it in the milk of transgenic cows in order to produce it in large quantities. You need to fuse the viral gene to a cow-specific promoter so it will be expressed in the cow cells. For the promoter you choose, under what condition(s) and in what cell type(s) should the promoter be active? **Explain.**

(f) You transfer the viral gene into a new plasmid vector to introduce it into cow cells. From the choices below…



Plasmid #4      Plasmid #5      Plasmid #6
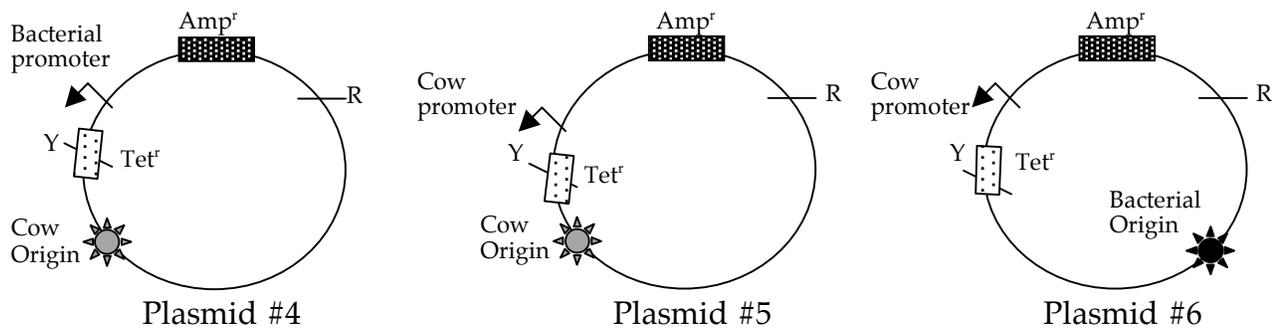
   i.   Which of the above vectors could you use to maintain and express the viral gene in cow cells?

   ii.  One way to make your transgenic cow is to have the viral gene insert into the cow genome. If you are employing this approach and do **not** want the original plasmid vector to be maintained in the cow, which vector could you use?

## Question 9 continued

(g) You introduce the recombinant vector into embryonic cow cells in culture.  Before you start growing up transgenic cows, you decide to check the sequence of the transgene in the cells to make sure it is correct.  You will first PCR amplify your gene of interest, then **sequence the PCR product**.  You have three primers which anneal to your DNA construct as diagrammed below:

**Promoter**

5'————————————————————3'
3'————————————————————5'
        1                    2
                                    3
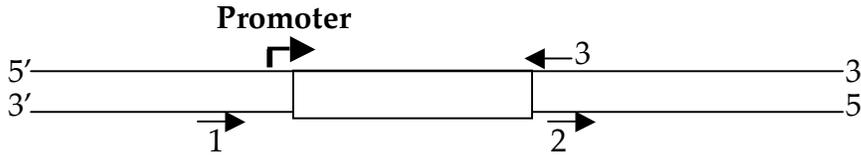
From the list below, check each item to indicate which reaction(s), if any, it should be added to.

| Reagents | PCR amplification | DNA sequencing |
|---|---|---|
| Primer #1 | | |
| Primer #2 | | |
| Primer #3 | | |
| DNA polymerase | | |
| dNTPs mix | | |
| ddNTP | | |
| Genomic DNA from transgenic cow cell | | |
| Reverse transcriptase | | |

(h) Finally, you use specific antibodies to purify the viral protein from the cow's milk. **Circle** the part(s) of the antibody which will bind to the viral protein.

Heavy chain          Light chain          Constant region          Variable region


## Question 10

An important set of neural connections is between motor neurons of the spinal cord and their target muscles in the limbs. Lateral neurons express the transcription factor **LIM-1** and the receptor tyrosine kinase **EPH-4**, and innervate (grow towards and create synapses with) dorsal limb muscles. In contrast, medial neurons express the transcription factor **ISL-1**, do not express EPH-4, and innervate the ventral limb muscles.  Both dorsal and ventral limb muscles express and secrete **ephrin-4**, the ligand for the EPH-4 receptor.



● =ephrin-4
☐ =LIM-1 + EPH-4
■ =ISL-1

Spinal Cord
Lateral neurons
Medial neurons
Dorsal muscle
Ventral muscle
Limb
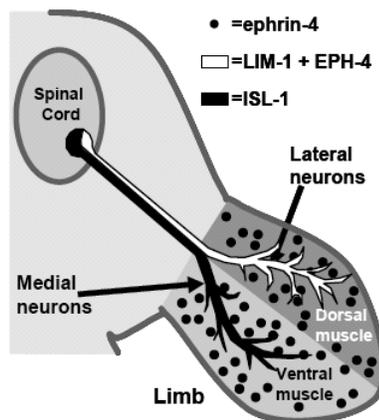
# Question 10 continued

(a) In order to ask whether LIM-1 and ISL-1 play a role in axonal pathfinding, researchers introduced a LIM-1 expressing construct into developing medial neurons. The resulting neurons expressed EPH-4 and innervated dorsal limb muscle rather than ventral. Conversely, expressing ISL-1 in lateral neurons prevented LIM-1 and EPH-4 expression, and the resulting neurons innervated ventral muscle, rather than dorsal muscle. Diagram the regulatory relationships between **EPH-4**, **LIM-1**, **ISL-1**, and **dorsal muscle innervation** that you can derive from these results. In your diagram, use an $\rightarrow$ to indicate that it activates and a $\perp$ to indicate inhibition.

(b) Axon pathfinding takes place in immature neurons. Once axons find their targets on the muscle, chemical synapses form to create the neuromuscular junctions. In the term **chemical synapse**, to what class of molecules does "chemical" refer?

In neuromuscular synapses, the axon is presynaptic and the muscle is postsynaptic. Neuromuscular synapses are often excitatory, and the output is muscle contraction. The neurotransmitter is acetylcholine (Ach), and binds to nicotinic acetylcholine receptors (AchR), which are **ligand-gated Na+ channels**. Ach is destroyed by acetylcholinesterase (Ach-esterase) after it is released into the synaptic cleft.
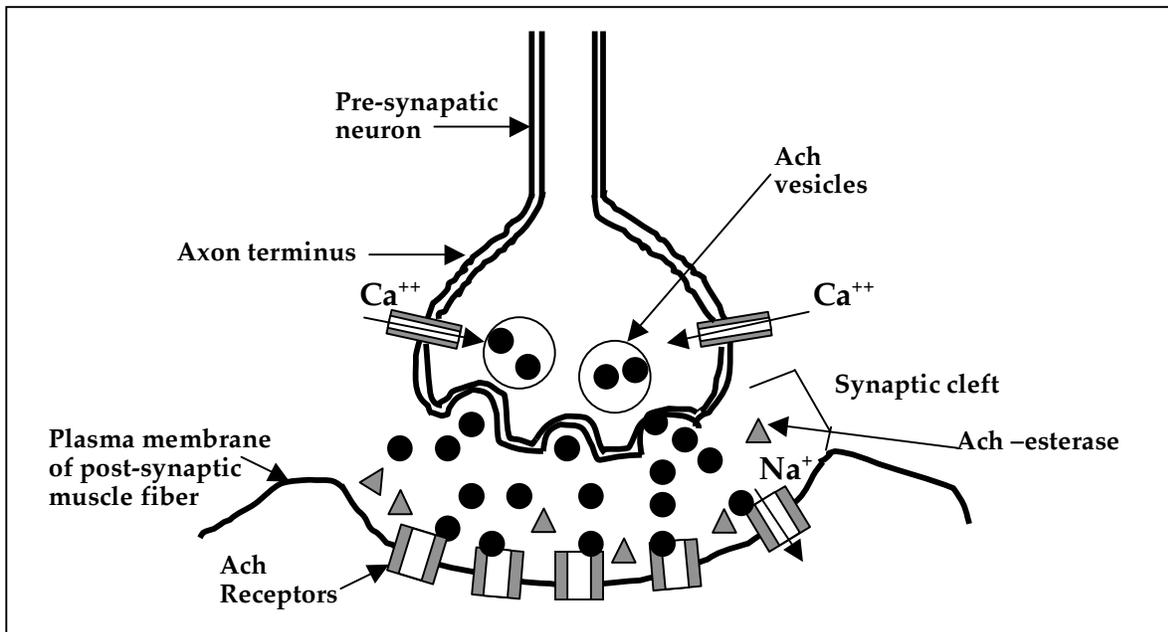


(c) In Myasthenia gravis, that targets the Ach receptor, muscles fail to contract, leading to profound weakness. Explain **one type of change** in Ach receptor that could cause this disorder, and why.

## Question 10 continued

(d) Lambert-Eaton syndrome is associated with the production of antibodies to pre-synaptic voltage-gated calcium channels. Explain why such antibodies cause a decrease in muscle contraction in affected individuals relative to normal individuals.

(e) Botulism is an illness caused by Botulinum toxin, produced by the bacterium Clostridium botulinum. The toxin inactivates synaptobrevin, a membrane protein of synaptic vesicles, required for exocytosis. Would the frequency of muscle contraction increase or decrease in patients with Botulism relative to unaffected individuals? Explain.

(f) Dementia with Lewy Bodies (DLB) is a major cause of senile dementia, similar to Alzheimers. The disorder can be treated with Acetylcholinesterase, and Ach receptors are normal in these patients. What is the most likely cause of DLB?

## Question 11

The liver is an organ which consists of lobules, each containing three major cell types in characteristic organization. The most prevalent type of cells are **hepatocytes**, which filter the blood, **endothelial cells** which line the sinusoids, vessels that carry blood between groups of hepatocytes, and a few **Kupfer cells** which also line the sinusoids.
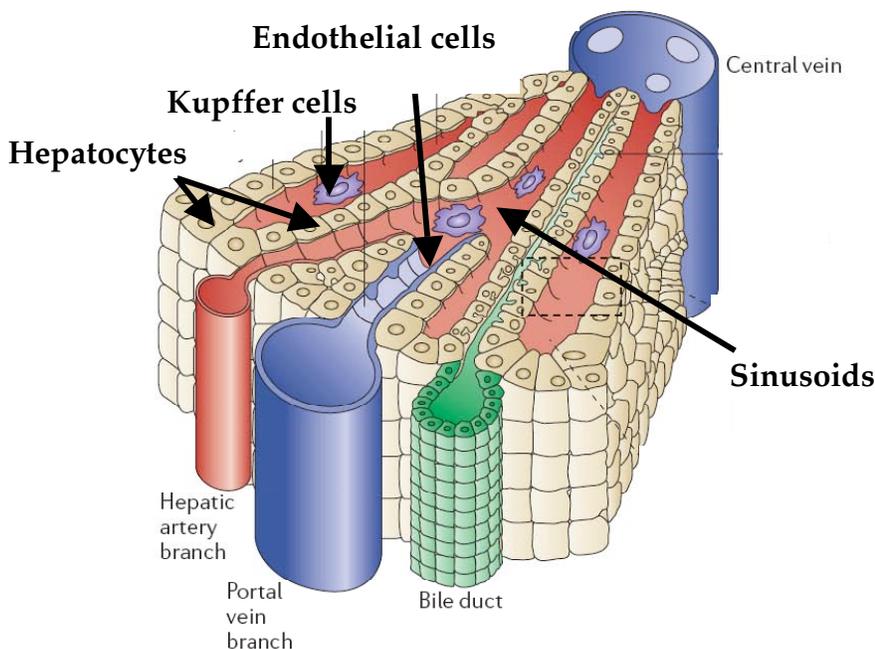
# Question 11 continued

HepaHope is a bioengineering company that is testing a "hybrid" liver. This consists of a suspension of single hepatocytes on a synthetic support. It works like a kidney dialysis machine, filtering the patient's blood to remove ammonia and detoxify wastes. One issue with this device is that the hepatocytes constantly need replenishing, as they stop functioning about 24 hours after being added to the device.

(a) In general what structural aspect of the normal liver is missing from the HepaHope device?

(b) You consult with the company and suggest that hepatocyte function may be prolonged by addition of signaling molecules. You decide to test ligands from four major signaling pathways to see if they prolong liver cell function. These ligands are Fgf3, Delta, Wnt8 and BMP4. Factors are tested in combination, with the encouraging results below.

| Factor | Hepatocyte Function (hours) |
| --- | --- |
| No factors | 24 |
| All four factors | 72 |
| BMP+Wnt8+Fgf | 72 |
| BMP+Fgf+Delta | 24 |
| Wnt8+Fgf+Delta | 72 |

i. Which ligand(s) is most important in prolonging the liver function? What next factor combination(s) would you assay to test the ligands that you identified?

ii. If any of these ligands normally regulate liver function (that is, in the intact liver), where would you expect to observe expression of …. *(Your choices are hepatocytes, endothelial cells, kupfer cells and sinusoids).*
   - These ligands?

   - The receptors for these ligands?

(c) Hepatocytes have a half-life (time of survival) of about 10 days. BrdU is a thymine analog that is incorporated normally into DNA, but is used in a pulse/chase assay to distinguish DNA synthesized during the pulse. Describe a pulse/chase experiment with BrdU that would measure hepatocyte half-life.

(d) Damaged liver can regenerate well, suggesting the involvement of liver stem cells. Some evidence suggests that that Kupfer cells are liver stem cells. How would you test whether Kupfer cells are stem cells, using a transplant approach? *Note: Carbon tetrachloride treatment can be used to specifically destroy the liver.*

## Question 11 continued

(e) The sinusoids are tubes that arise from single cells that associate to form a sheet which eventually forms a tube. What is the term for conversion of single cells to cell sheets?

(f) What changes will the following perturbations have on single cells versus cell sheets? *Your options are: death; adhesion; movement; shape; no change.* **For each perturbation, select the most likely option(s) and explain your choice.**

| Perturbation | Effect on | | Explanation |
| --- | --- | --- | --- |
| | Single cell | Cell sheet | |
| Removal of extracellular matrix | | | |
| Tight junction disruption | | | |
| Actin depolymerization | | | |
| Prevention of homotypic adhesion | | | |
| Stabilization of microtubules | | | |

## Question 12

(a) The liver arises from ventral embryonic endoderm. Using some or all of the frog embryos below, how would you use the technique of fate mapping to address from where in the embryo the liver arises?



ventral        dorsal

*Early embryo (4 hours old)*

*liver*

ventral        dorsal
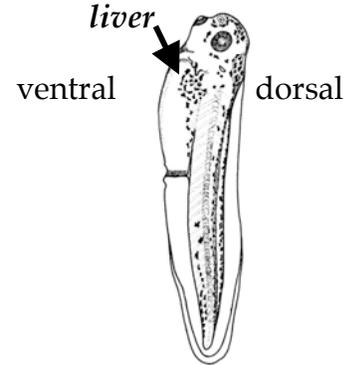
*Older embryo (40 hours)*

(b) Hepatocytes are differentiated liver cells. **HNF4a is a hepatocyte-specific transcription factor necessary for activation of all liver differentiation genes, including albumin.** In order to understand the mechanism by which HNF4a acts, you express HNF4a in the developing pancreas, which is also derived from endoderm. In this case, expression of albumin and other liver-specific genes is not activated. Explain this observation.

(c) DNMT is a DNA methylase, expressed throughout development, which adds methyl groups to cytosine. You decide to regenerate animals by SCNT using the nuclei either from adult hepatocytes, 40 hour embryonic hepatocytes, or 40 hour hepatocytes treated with an inhibitor of the DNA methlyase DNMT. Which of the three nuclei would be the best choice? Explain why you would prefer one over the other.

|          | **2nd base in codon** | | | | |
| **1st base in codon** | | **U** | **C** | **A** | **G** | **3rd base in codon** |
| **U** | Phe | Ser | Tyr | Cys | U |
|       | Phe | Ser | Tyr | Cys | C |
|       | Leu | Ser | STOP | STOP | A |
|       | Leu | Ser | STOP | Trp | G |
| **C** | Leu | Pro | His | Arg | U |
|       | Leu | Pro | His | Arg | C |
|       | Leu | Pro | Gln | Arg | A |
|       | Leu | Pro | Gln | Arg | G |
| **A** | Ile | Thr | Asn | Ser | U |
|       | Ile | Thr | Asn | Ser | C |
|       | Ile | Thr | Lys | Arg | A |
|       | Met | Thr | Lys | Arg | G |
| **G** | Val | Ala | Asp | Gly | U |
|       | Val | Ala | Asp | Gly | C |
|       | Val | Ala | Glu | Gly | A |
|       | Val | Ala | Glu | Gly | G |

# STRUCTURES OF AMINO ACIDS at pH 7.0
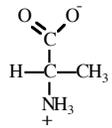
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(ala)
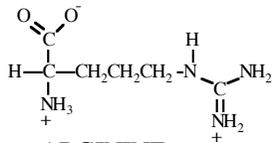
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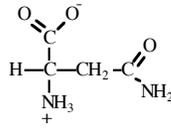
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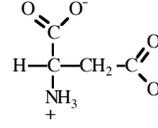
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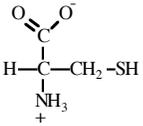
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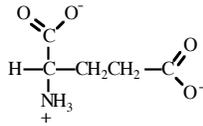
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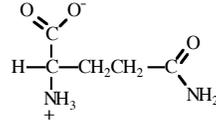
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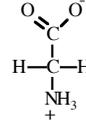
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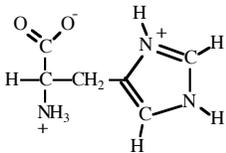
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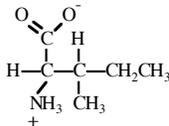
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(ile)
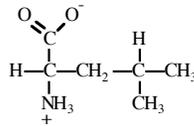
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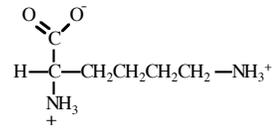
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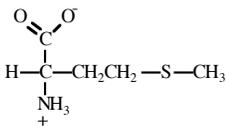
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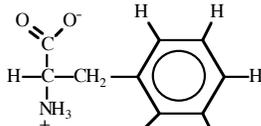
**PHENYLALANINE**
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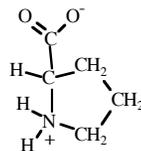
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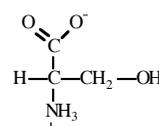
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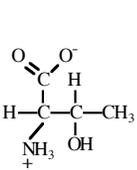
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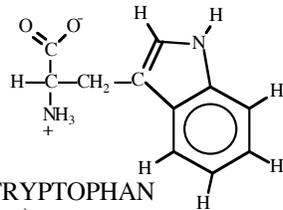
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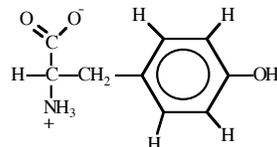
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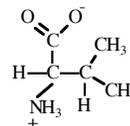
**VALINE**
(val)

7.013 Introductory Biology
Spring 2013