7.012 Quiz 3 practice

Quiz 3 on Friday, November 12th 10 – 11 AM

Review Session Wednesday 11/10 from 7-9 pm

Tutoring Session Thurs. 11/11 from 4-6 pm

Final Exam is on Monday, December 13th 9 - noon

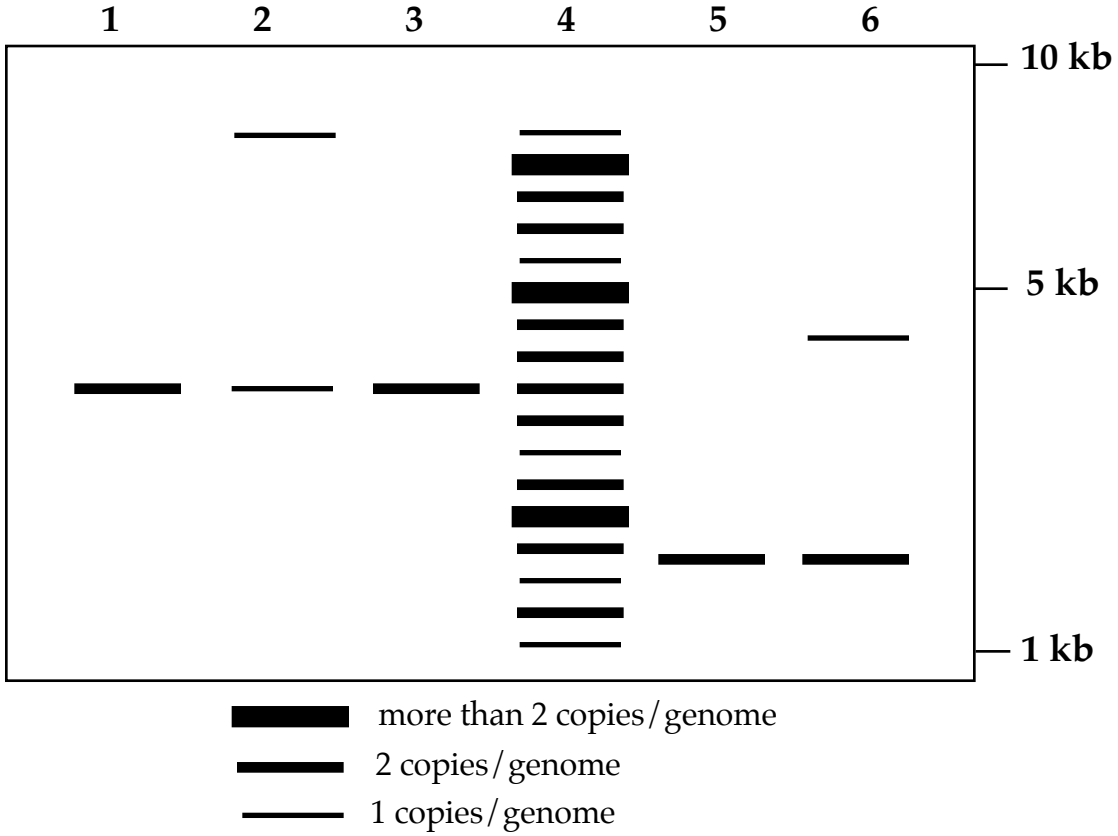
Question 1

A) You infected mice with mouse mammary tumor virus (a retrovirus). After a period of time, most infected mice had developed breast tumors, whereas uninfected mice did not. You isolated cell lines from over 50 independent tumors. You demonstrated that all of these lines had virus integrations in the same chromosomal location. Can one conclude that the virus integrates into cellular DNA at only one site? Explain.

B) The *ras* oncogene is involved in a variety of human and animal cancers. DNA was isolated from a number of normal and cancerous tissues.

-Cellular DNA was digested with *Eco*RI.
-Digested DNA was separated by gel electrophoresis and transfered to a nitrocellulose membrane.
-The membrane was probed with the radioactive labelled cloned ras DNA and then the membrane was exposed to x-ray film.
-The resulting autoradiograph is shown below.

1) white blood cells from a healthy human
2) human lymphoma cells (cancerous)
3) human bladder carcinoma cells (cancerous)
4) human sarcoma cells (cancerous)
5) blood from a healthy mouse
6) mouse myeloma cells (cancerous)



Question 1 continued

a) How do you explain the presence of sequences complementary to the oncogene in the DNA from healthy human and mouse samples? Why don't they have cancer?

b) Why is the hybridizing band from sample 1 a different size than that from sample 5?

c) For each cancer examined above, based on the autoradiogram, choose the most likely mechanism of transformation and explain your choice:

1) point mutation within the gene

2) chromosomal rearrangement involving the gene

3) gene amplification

4) oncogenic retroviral insertion.

Question 2

You are studying the cell cycle in haploid yeast cells. You isolate a cell that is a temperature-sensitive cell division cycle (*cdc*) mutant, *cdcX*-. *cdcX*- grows normally at 25 °C, but arrests at 36 °C at the point in the cell cycle where the expression of the normal *cdcX* gene is required.

To determine where in the cell cycle expression of *cdkX* is required, you design experiments based on the following facts:

1) The drug nocodazole arrests, but does not kill yeast in mitosis (M phase).
2) Cell density can be measured to determine if the yeast cells have completed a cell division.

For your experiments:

- You incubate *cdkX*- cells at 25 °C with nocodazole until all the cells are synchronized. You then shift the cells to 36 °C and remove the nocodazole.
  The cells divide once and then arrest.

- You incubate *cdkX*- cells at 36 °C until all arrest. You then add nocodazole and shift the cells to 25 °.
  The cells arrest without dividing.

a) Given these experiments you can assume that the protein encoded by the *cdkX* gene is not required at what phase(s) of the cell?

Another mutant *cdkY-* is a cold-sensitive mutant that arrests at 18 °C, but grows normally at 25 °C. *cdkY-* cells arrest between the S and G2 phases of the cell cycle. You make *cdkX- cdkY-* double mutants and perform the following experiments.

- You incubate *cdkX- cdkY-* double mutants at 36 °C until all the cells are synchronized and then shift the cells to 18 °.
- **The cells arrest without dividing.**

- You incubate *cdkX- cdkY-* double mutants at 18 °C until all until all the cells are synchronized and then shift the cells to 36 °C.
- **The cells divide once and then arrest.**

b) Given these experiments you can assume that the protein encoded by the *cdkX* gene is not required at what phase(s) of the cell?

c) In what stage(s) of the cell cycle do you expect the protein encoded by the *cdkX* gene to act?

d) A cell in the process of DNA replication (cell A) is fused with a cell in early G1 (cell B). How does the timing of the cell cycle for the **cell B** nucleus in the fused cell compare to that expected for the **cell B** nucleus if the cells remained independent?

e) What soluble factor(s) are found in cell A but <u>not</u> in cell B (check all that apply)?

___ pRB protein
___ TGFβ
___ Human papilloma virus
___ a cyclin dependent kinase
____ a G1 cyclin
___ a cAMP dependent kinase
___ a G2 cyclin
___ an S-phase cyclin
___ an oncogene

Question 3

Part I

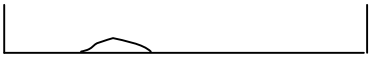
As a premier cancer biologist, you often plate cells in dishes, feeding them serum with growth factors and allowing them to grow for 2 weeks. Sometimes after incubation of strains you observe the following when looking at the side of a culture dish.

Strain A

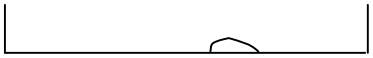
Strain B

a) Which plate shows abnormal cells? Explain.

b) Predict the behavior of these cell lines if grown without added growth factors by **drawing** what the plates will look like after incubation **without** growth factors. Simply modify the existing figure below for your answer. (Note: one cell from each strain is initially deposited in each dish.)



Strain A



Strain B

<u>Part II</u>

A fellow researcher gives you two cancerous cell lines to examine and determine possible mutations. The results are shown below.

Cell Line	**Mutation**
WT	none (wild type DNA)
1	a deletion at the same region on both copies of chromosome 4
2	a point mutation in a gene on only one copy of chromosome 7

c) Based on this data above, identify the type **of cancer gene that is mutated in each of the cell lines.**

Cell Line	**Cancer Gene (oncogene or tumor suppressor gene)**
WT	none
1	
2	

Question 3 continued

You learn that cell line 1 is a skin cancer cell line. The region you identified as deleted on chromosome 4 in these cells normally contains a gene called *p16*.

d) What is the role of the *p16* gene product in the normal cell based on the information above?

You obtain another cell line (cell line 3) that has one wild-type copy of chromosome 4 and one mutant copy of chromosome 4 (as described above in cell line 1).

e) Will cell line 3 display a cancerous phenotype when grown in the **presence** of growth factors? **Yes/No** (Circle one.)

Explain briefly.

f) Will cell line 3 display a cancerous phenotype when grown in the **absence** of growth factors? **Yes/No** (Circle one.)

Explain briefly.

Part III

g) Cell line 2 is a breast cancer cell line that expresses a mutant version of a receptor protein called KIT. Choose from the following options to explain the role of KIT in normal cells.
Circle one.

Activation of KIT causes cells to die

Activation of KIT promotes progression through the cell cycle.

Activation of KIT has no effect on the cell cycle.

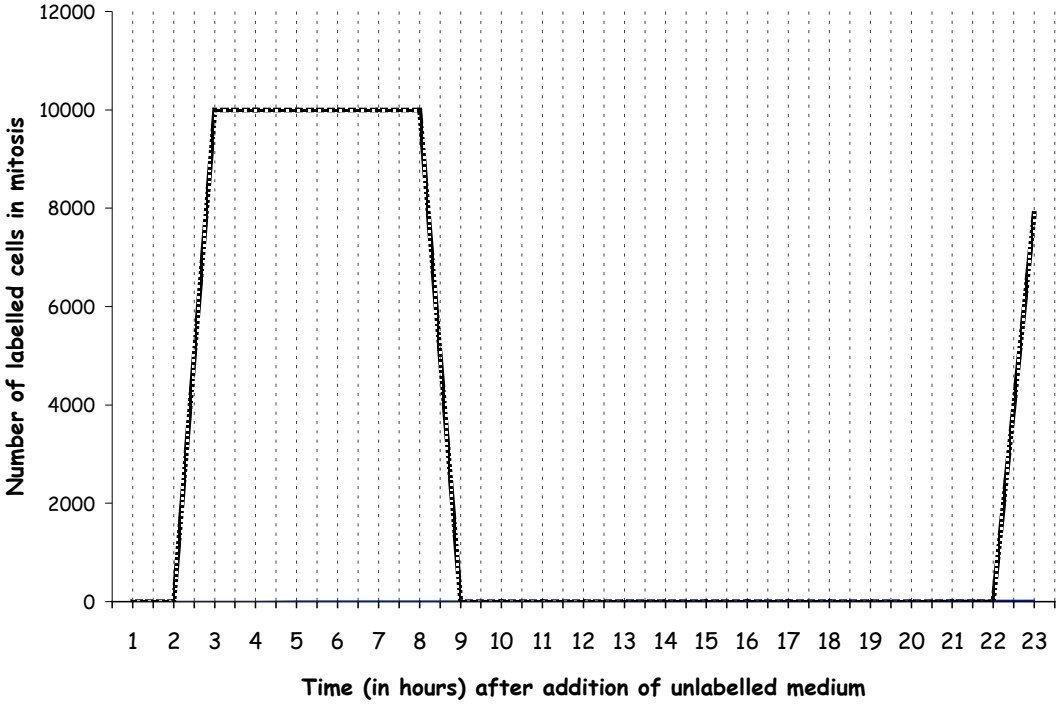
Activation of KIT causes cells to enter G0.

h) Specifically how could a point mutation in the gene encoding the KIT receptor cause the abnormal behavior depicted in Part I.

Question 4

You know in yeast that mitosis takes one hour. You decide to further study the cell cycle in yeast cells using radioactive dTTP. Cells grown in radioactive dTTP incorporate this radioactive nucleotide into their DNA.

You label a population of asynchronously growing yeast cells by adding radioactive dTTP to the medium for **one minute**. You then replace this medium with medium containing unlabelled dTTP and continue growing the cells. At one hour time points following the replacement you count the number of radioactively labeled cells in mitosis. Your data is shown below.



a) Cells are in which phase of the cell cycle when incorporating radioactive dTTP into their DNA? (Circle one.)

G_0 phase G_1 phase G_2 phase M phase S phase Lunar phase

b) Estimate the length of the G2 phase from the graph. (Circle one.)

Can't be determined 0 hrs ~2-3 hrs ~6-7 hrs ~9-10 ~11-12 hrs ~13-14 hrs ~20 hrs ~22 hrs

c) Estimate the length of the S phase from the graph. (Circle one.)

Can't be determined 0 hrs ~2-3 hrs ~6-7 hrs ~9-10 ~11-12 hrs ~13-14 hrs ~20 hrs ~22 hrs

d) Estimate the duration of the cell cycle. (Circle one)

Can't be determined 0 hrs ~2-3 hrs ~6-7 hrs ~9-10 ~11-12 hrs ~13-14 hrs ~20 hrs ~22 hrs

e) Estimate the length of the G1 phase from the graph. (Circle one.)

Can't be determined 0 hrs ~2-3 hrs ~6-7 hrs ~9-10 ~11-12 hrs ~13-14 hrs ~20 hrs ~22 hrs

Question 5

You are an immunologist who wants to make the big bucks. You decide to leave the world of science and get a job as a script-consultant on a new medical drama (ER-like) show. You test the writers with a few questions to see just how much they know.

a) Compare how macrophages and B cells **recognize** antigen.

b) Compare how macrophages and B cells **present** antigenic peptides (epitopes).
They present epitopes exactly the same way on their MHC II molecules on the surface.

c) Macrophages and B cells present antigens to _____ _____-cells. (Fill in blanks.)

d) Name 2 components of the innate or nonspecific immune system.

_____ _____

Below are short descriptions given to you by the writers of scenarios in the early episodes.

Scenario #1

One of the characters on the show is diagnosed with leukemia, a cancer of the blood system. She is very sick until her boyfriend bravely agrees to donate his bone marrow. The bone marrow transplant is successful and our character lives!

e) You tell the writers that the bone marrow transplant from the boyfriend is **unlikely** to be successful. Give the reason and the molecular basis for why.

Because of the **different** major histocompatability molecules the marrow will be rejected.

Scenario #2

The leading doctor gives birth to a baby boy. After some time, the child shows no acquired or specific immune response and is diagnosed with a rare disorder, Severe Combined Immune Deficiency (SCID), and as a result the boy must live in a germ-free environment. Several causes of SCID have been described and are listed below.

f) For each cause, indicate which of the following branches of immune system are affected. (**Cellular =Cell-Mediated**)

Cause	**Cell-Mediated, Humoral or Both**
T cells fail to develop	
DNA recombination deficiency	
Absence of MHC class I molecules	
Lack of MHC class II molecules	

Scenario #3

Patients in the hospital are coming down with multiple infections. Lab results show that the sick are infected with a bacterium, *S. aureus*, that secretes "Protein A" which binds the constant region of antibodies.

g) What cell recognizes the constant region of secreted antibodies? _____

h) Why might the effect of Protein A allow **multiple** (non *S. aureus*) infections?

i) What branch of the immune system does *S. aureus* evade using Protein A?

Humoral Cellular or Cell-Mediated Both

Scenario #4

Many patients are coming into the emergency room with a disease caused by an unknown pathogen! A doctor studies this pathogen in order to create a vaccine against it.

j) He discovers that the infectious agent is an intracellular bacterium and its cell surface is coated with human-like proteins. Considering the mechanism of the pathogen, the doctor decides to generate a live-attenuated vaccine instead of a heat-killed vaccine.

i) What are the two advantages of using a live-attenuated vaccine vs. a heat killed vaccine in this case?

ii) What is a disadvantage of using a live-attenuated vaccine?

Question 6

a) Shown below is a schematic of the production of a heavy chain polypeptide for an antibody. At the top is the chromosomal arrangement found in an immature B cell, at the bottom is shown the heavy chain polypeptide.

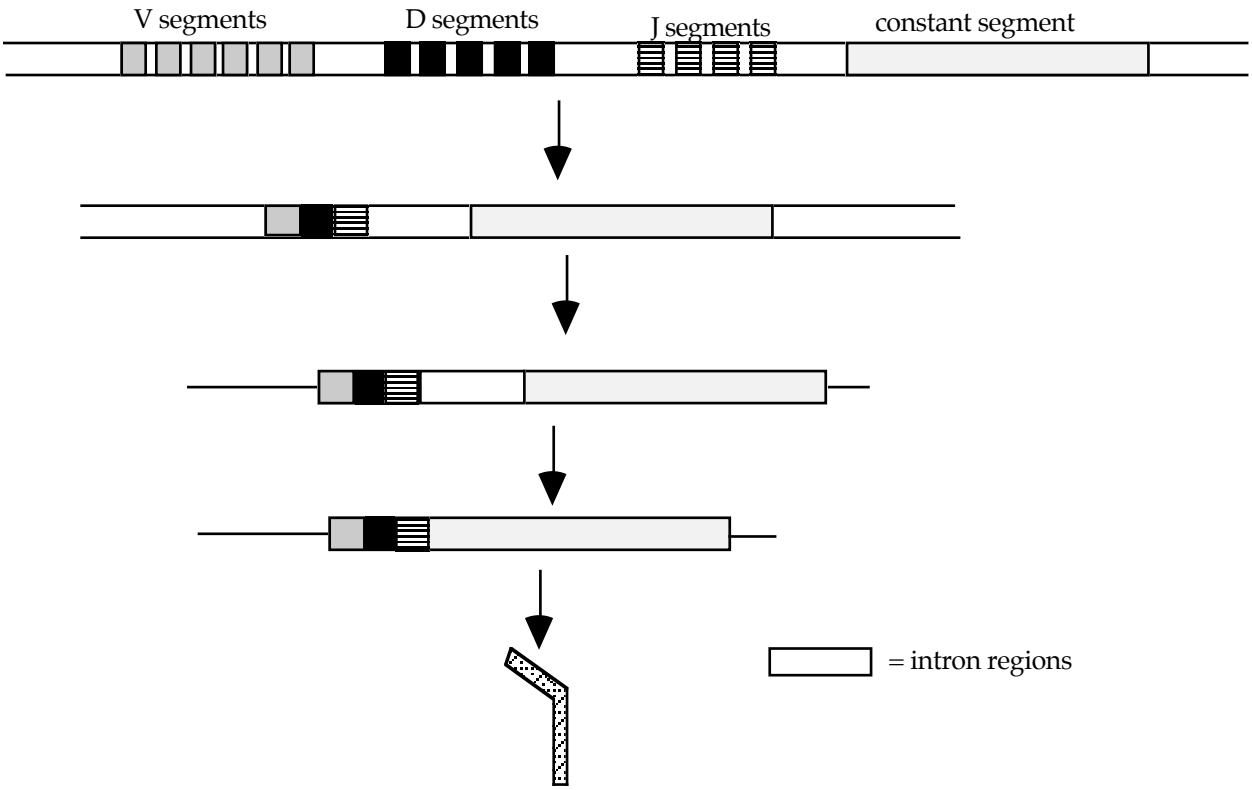
i) Label the process indicated by <u>each</u> arrow. Choose the one best option for each from:

protein processing	RNA ligation
transcription	RNA splicing
translation	DNA rearrangement
transduction	DNA ligation

ii) Indicate on the diagram below where you would expect to find each of the following components;

Promoter Transcription terminator start codon stop codon

iii) Indicate on the diagram below the variable and the constant region of the heavy chain polypeptide.



b) Indicate whether each of the following statements is true or false. If false, correct the statement or provide a brief explanation for why it is false.

i) Memory cells are the basis for a strong secondary humoral immune response.

ii) T cells produce antibodies that bind antigen.

iii) Macrophages present antigenic peptides on MHC II proteins to B cells.

iv) Clonal expansion means that any B cells present at the time of exposure to an antigen will be stimulated to proliferate.

v) The two antigen binding sites formed by the variable regions in a single antibody molecule bind to two different antigenic determinants.

c) When a rabbit protein is injected into rabbits, no antibodies against this protein are generated. If, however, the same rabbit protein is injected into guinea pigs, the guinea pigs generate antibodies against the rabbit protein. Briefly (in one or two sentences) explain this observation.

d) The genomes contained in almost all of the somatic cells in an adult human are identical. Name one (diploid) cell type that is an exception to this and specify the process by which the genetic variation occurred.

e) Will siblings have the exact same antibody repertoire? What about identical twins? Briefly explain your reasoning.

Answers

Question 1

A) One cannot conclude that the virus is able to integrate at only one site. However, one might propose that the virus can only cause cancer when it integrates into a certain chromosomal location or next to a specific gene. In fact, viruses can integrate many places throughout the genome. The reason you only observed integration events at one site is because you have examined only those events that cause tumors. Perhaps the integration of the virus next to a proto-oncogene can cause it to become oncogenic, possibly by activating expression of the oncogene in the wrong place or at the wrong time.

B)

a) The sequences that are complementary to the probe in normal cell DNA correspond to the cellular proto-oncogene. The individuals from that the material came don't have cancer because they have not acquired the mutations necessary to turn the proto-oncogene into an oncogene.

b) Random sequence variation between mouse and human DNA alters the restriction map around the gene. The two species diverged from a common ancestor during the process of evolution, and DNA sequence variation has been accumulating since. Some of these variations occur in restriction enzyme sites.

c) -lane 2: a chromosomal rearrangement or a point mutation at one of *Eco*RI restriction sites are the most probable mechanisms because one copy of the gene has changed its location with respect to at least one of the flanking *Eco*R1 sites.

-lane 3: a point mutation within the coding sequence of the gene is the probable mechanism of transformation because there is no obvious change in the Southern blot--none of the restriction sites have been altered.

-lane 4: gene amplification has created many copies of the gene which probably are present in several tandem arrays in the sarcoma DNA.

- lane 6: retroviral transduction has brought an extra copy of the oncogene into the cell. Since the smaller fragment is still present in two copies per cell, there has probably not been any change in the "resident" proto-oncogenes.

Question 2

a) Given these experiments you can assume that the protein encoded by the cdkX gene is not required at what phase(s) of the cell?

M phase

b) Given these experiments you can assume that the protein encoded by the cdkX gene is not required at what phase(s) of the cell?

G2 phase or M phase

c) In what stage(s) of the cell cycle do you expect the protein encoded by the cdkX gene to act?

G1 or S phase

d) How does the timing of the cell cycle for the **cell B** nucleus in the fusion cell compare to that expected if the cells remained independent?

In the fusion cell, the DNA of cell B immediately begins replication (S-phase). If cell B had remained independent, S-phase would have occurred later.

e) What soluble factor(s) are found in cell A but <u>not</u> in cell B (check all that apply)?

___ RB protein
___ TGFβ
___ Human papilloma virus
___ a cyclin dependent kinase
_X_ a G1 cyclin
___ a cAMP dependent kinase
___ a G2 cyclin
_X_ an S-phase cyclin
___ an oncogene

Question 3

Part I

As a premier cancer biologist, you often plate cells in dishes, feeding them serum with growth factors and allowing them to grow for 2 weeks. Sometimes after incubation of strains you observe the following when looking at the side of a culture dish.

Strain A

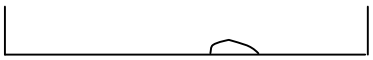
Strain B

a) Which plate shows abnormal cells? Explain.

The plate on the left. Strain A shows no contact inhibition. Normal cells stop growing when they touch each other. Abnormal cells pile up.

b) Predict the behavior of these cell lines if grown without added growth factors by **drawing** what the plates will look like after incubation **without** growth factors. Simply modify the existing figure below for your answer. (Note: one cell from each strain is initially deposited in each dish.)

Strain A



Strain B

Part II

A fellow researcher gives you two cancerous cell lines to examine and determine possible mutations. The results are shown below.

Cell Line	Mutation
WT	none (wild type DNA)
1	a deletion at the same region on both copies of chromosome 4
2	a point mutation in a gene on only one copy of chromosome 7

c) Based on this data above, identify the type of cancer gene that is mutated in each of the cell lines.

Cell Line	Cancer Gene (oncogene or tumor suppressor gene)
WT	none
1	**TSG**
2	**ONCOGENE**

You learn that cell line 1 is a skin cancer cell line. The region you identified as deleted on chromosome 4 in these cells normally contains a gene called *p16*.

d) What is the role of the *p16* gene product in the normal cell based on the information above?

It is a gatekeeper of the cell cycle (the brake linings) preventing progression through cell cycle unless all checks out. It inhibits cell proliferation.

You obtain another cell line (cell line 3) that has one wild-type copy of chromosome 4 and one mutant copy of chromosome 4 (as described above in cell line 1).

e) Will cell line 3 display a cancerous phenotype when grown in the **presence** of growth factors? **Yes/No** (Circle one.)

Explain briefly.

No, it the p16 mutation is recessive to wild-type. Basically the mutant chromosome 4 gives a recessive cancerous phenotype. Cell line 3 has a WT copy of chromosome 4 which is sufficient to give the WT (NORMAL) phenotype

f) Will cell line 3 display a cancerous phenotype when grown in the **absence** of growth factors? **Yes/No** (Circle one.)

Explain briefly.

It will not grow. THERE ARE NO GROWTH FACTORS. Cell line 3's cancerous phenotype is recessive. (Not simply that the cells wouldn't grow because cell line 3 contains a WT copy of the p16 gene, and enough p16 protein is present to block proceeding through the cell cycle given the absence of growth factors.)

Part III

g) Cell line 2 is a breast cancer cell line that expresses a mutant version of a receptor protein called KIT. Choose from the following options to explain the role of KIT in normal cells. Circle one.

Activation of KIT causes cells to die.

Activation of KIT promotes progression through the cell cycle.

Activation of KIT has no effect on the cell cycle.

Activation of KIT causes cells to enter G0.

h) Specifically how could a point mutation in the gene encoding the KIT receptor cause the abnormal behavior depicted in Part I.

Any mutation in the receptor that would cause it to be constitutive, ligand-independent activation, dimerization, always active, etc. would be enough to cause the cancer phenotype.

Question 4

a) Cells are in which phase of the cell cycle when incorporating radioactive dTTP into their DNA? (Circle one.) **3 points**

G_0 phase G_1 phase G_2 phase M phase **S phase** Lunar phase

b) Estimate the length of the G2 phase from the graph. (Circle one.) **3 points**

Can't be determined 0 hrs **~2-3 hrs** ~6-7 hrs ~9-10 ~11-12 hrs ~13-14 hrs ~20 hrs ~22 hrs

c) Estimate the length of the S phase from the graph. (Circle one.) **3 points**

Can't be determined 0 hrs ~2-3 hrs **~6-7 hrs** ~9-10 ~11-12 hrs ~13-14 hrs ~20 hrs ~22 hrs

d) Estimate the duration of the cell cycle. (Circle one.) **3 points**

Can't be determined 0 hrs ~2-3 hrs ~6-7 hrs ~9-10 ~11-12 hrs ~13-14 hrs **~20 hrs** ~22 hrs

e) Estimate the length of the G1 phase from the graph. (Circle one.) **3 points**

Can't be determined 0 hrs ~2-3 hrs ~6-7 hrs ~9-10 **~11-12hrs** ~13-14 hrs ~20 hrs ~22 hrs

Question 5

You are an immunologist who wants to make the big bucks. You decide to leave the world of science and get a job as a script-consultant on a new medical drama (ER-like) show. You test the writers with a few questions to see just how much they know.

a) Compare how macrophages and B cells **recognize** antigens.

MØs nonspecifically engulf antigen.
B cells take up antigens that their surface antibodies specifically bind.

b) Compare how macrophages and B cells **present** antigenic peptides (epitopes).

They present epitopes exactly the same way on their MHC II molecules on the surface.

c) Macrophages and B cells present epitopes to __*helper*__ __*T*__-cells. (Fill in blanks.)

d) Name 2 components of the innate or nonspecific immune system.

Skin, MØs, complement, mucus lining, mucocilliary ladder, lysozyme in tears, sweat, etc.

Below are short descriptions given to you by the writers of scenarios in the early episodes.

Scenario #1

One of the characters on the show is diagnosed with leukemia, a cancer of the blood system. She is very sick until her boyfriend bravely agrees to donate his bone marrow. The bone marrow transplant is successful and our character lives!

e) You tell the writers that the bone marrow transplant from the boyfriend is **unlikely** to be successful. Give the reason and the molecular basis for why.

*Because of the **different** major histocompatability molecules the marrow will be rejected.*

Scenario #2

The leading doctor gives birth to a baby boy. After some time, the child shows no acquired or specific immune response and is diagnosed with a rare disorder, Severe Combined Immune Deficiency (SCID), and as a result the boy must live in a germ-free environment.

Several causes of SCID have been described and are listed below.

f) For each cause, indicate which of the following branches of immune system are affected.

Cause	Cellular / Cell-Mediated, Humoral or Both
T cells fail to develop	***BOTH***
DNA recombination deficiency	***BOTH***
Absence of MHC class I molecules	***Cellular or CELL-Mediated***
Lack of MHC class II molecules	***HUMORAL***

Scenario #3

Patients in the hospital are coming down with multiple infections. Lab results show that the sick are infected with a bacterium, *S. aureus*, that secretes "Protein A" which binds the constant region of antibodies.

g) What cell recognizes the constant region of secreted antibodies? _____MØ_____

h) Why might the effect of Protein A allow **multiple** (non *S. aureus*) infections?

Protein A sequesters all antibodies to all antigens by binding to the antibody. This will prevent Macrophages from ridding the pathogen and will in fact precipitate out antibodies from the blood.

i) What branch of the immune system does *S. aureus* evade using Protein A?

Humoral Cellular or Cell-Mediated Both

Scenario #4

j) He discovers that the infectious agent is an intracellular bacterium and its cell surface is coated with human-like proteins. Considering the mechanism of the pathogen, the doctor decides to generate a live-attenuated vaccine instead of a heat-killed vaccine.

i) What are the two advantages of using a live-attenuated vaccine vs. a heat killed vaccine in this case?

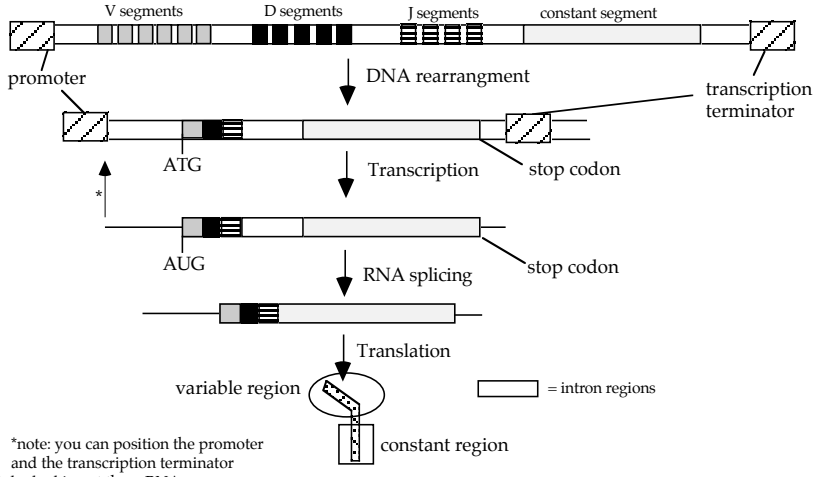
It'll mimic the disease by invading cells, thus it will illicit both a humoral and cellular response.

Surface proteins will not be denatured by heat.

ii) What is a disadvantage of using a live-attenuated vaccine?

Could acquire virulence factors, Need a "cold chain" (expensive refrigeration), it may make people sick.

Question 6



a)

b)

i) True.

ii) False. B cells produce antibodies that bind antigens.

iii) False. Macrophages present antigen to T helper cells. Only T cells can recognize epitopes in MHC II moleculeses on macrophages and B cells.

iv) False. Clonal expansion means that only the B cells that express antibodies that recognize a particular foreign antigen will proliferate when exposed to that particular antigen.

v) False. The two antigen binding sites of an antibody molecule bind to identical antigenic determinants.

c) The rabbit protein is recognized as foreign (non-self) by the guinea pig.

d) B cells, by gene rearrangement of Ab genes (VDJ rearrangement). Also, T cells (by rearrangement of T cell receptor genes).