

## 7.013 Practice Quiz 2 2004

**Question 1**

A. The primer shown below is used to sequence the following template DNA.

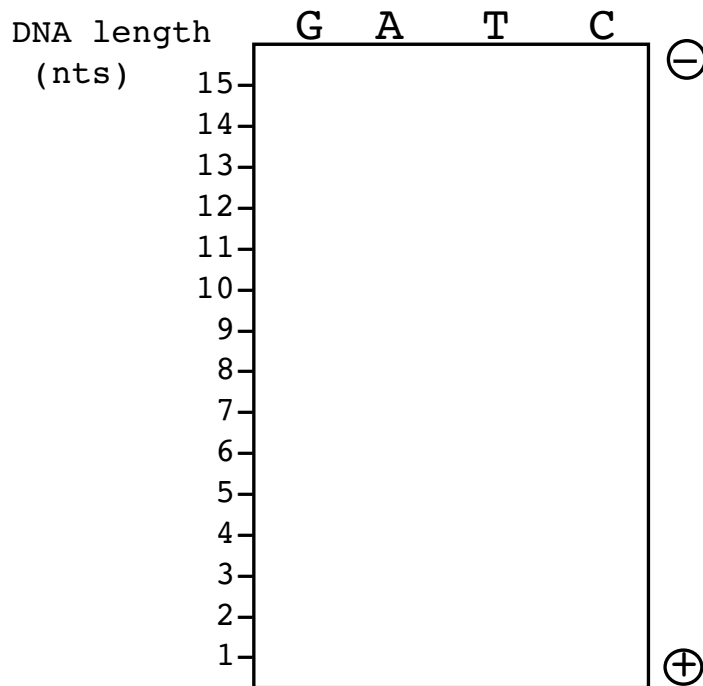
primer:

template DNA:

5' -ACTGAC-3'

5' -ACCACTAACGTCAGT-3'

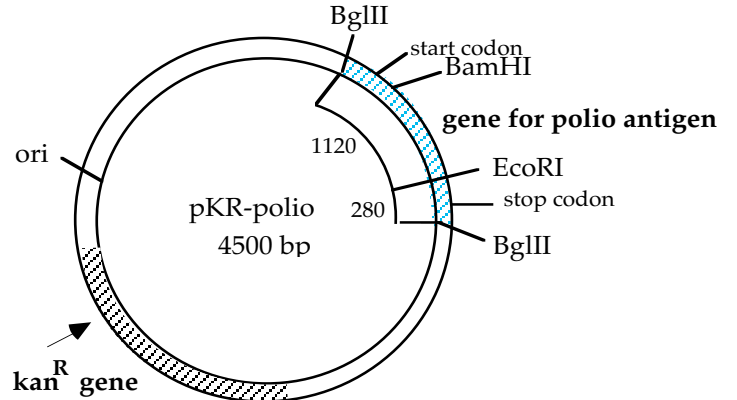
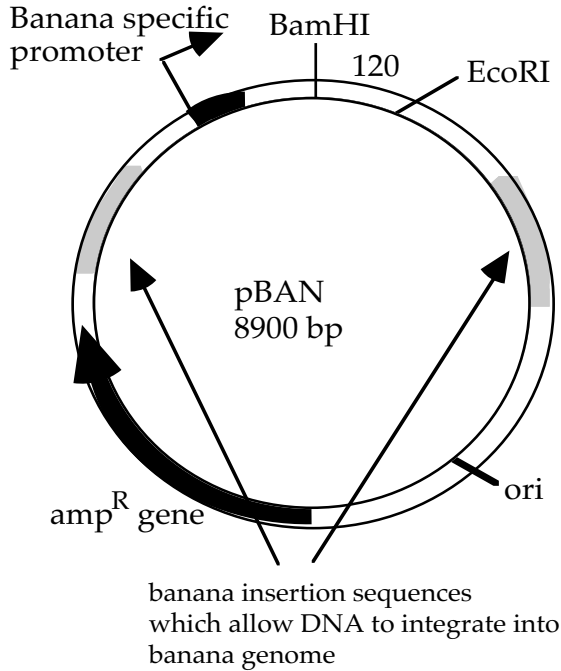
Draw the resulting DNA fragments that would be produced from each of the 4 sequencing reactions at the correct position (length in nucleotides) as they would appear on the diagram of the sequencing gel below.



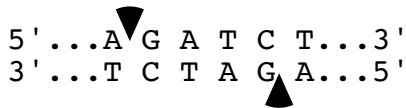
B.

Polio has been practically eliminated from the American population, however, in countries where people have little or no access to vaccinations, it is still prevalent. As a biologist with a global vision, you seek to create a transgenic banana that produces the protein used in the vaccine against polio. By consuming these bananas, individuals will develop immunity against the disease. The gene for this protein has already been cloned into a plasmid with a kanamycin-resistance gene (pKR-polio). You need to attach to the gene a banana-specific promoter and DNA sequences that will allow the gene to be incorporated into banana DNA. These sequences are contained in the pBAN plasmid, which carries a gene for ampicillin resistance. Maps of these two plasmids are shown on the next page, including important restriction sites and distances (in base pairs) between the sites.

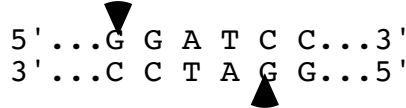
Question 1 continued



**BglIII:**



**BamHI:**



**EcoRI:**



a) An end generated by digestion with *Bam*HI can be ligated to an end generated by digestion with *Bgl*II. Why is this possible?

b) You want to insert the gene encoding the polio antigen into *pBAN*. Devise a strategy to accomplish this. Identify the enzyme(s) you would use to cut *pBAN*, the enzyme(s) you would use to cut *pKR-polio*, and the steps necessary to generate the intact plasmid.

c) You next transform *E. coli* with the plasmids you have made. You grow the transformed cells on media containing (circle one):

ampicillin

kanamycin

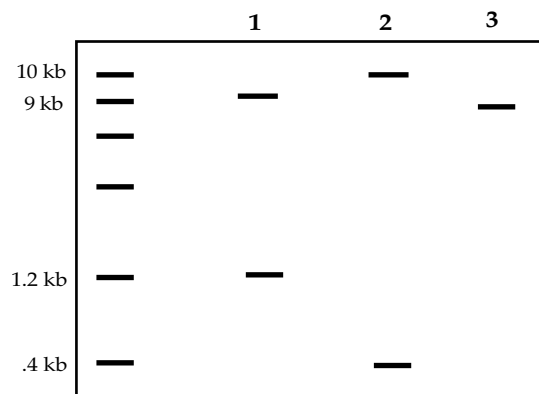
both ampicillin and kanamycin

neither ampicillin nor kanamycin

Why?

### Question 1 continued

You isolate plasmid DNA from three colonies that pass your antibiotic resistance test. You digest the DNA with the restriction enzyme *EcoRI*. You size separate the resulting fragments from each plasmid on an agarose gel. You find the following results. DNA fragment sizes are indicated to the left.

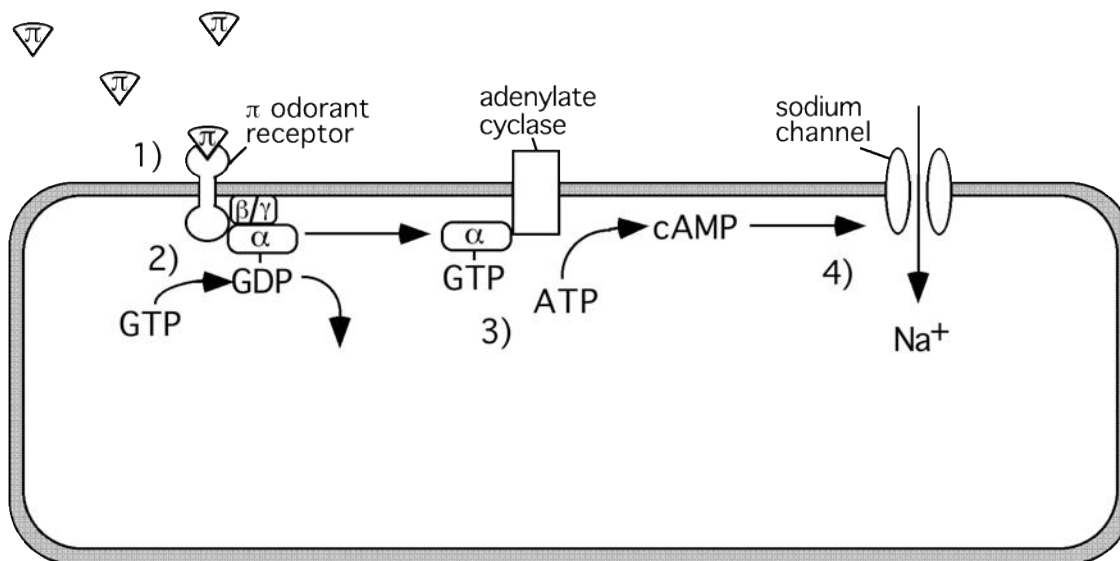


d) i) Draw plasmids associated with the colonies 1, 2, and 3. Indicate all relevant features such as the promoter, the origin of replication, the genes for ampicillin resistance and the polio antigen,

ii) Which of the three plasmids would allow synthesis of the protein in a banana? Explain your reasoning.

## Question 2

Consider the following signal transduction pathway involved in smelling pumpkin pie.



- 1) A pie odorant molecule ( $\pi$ ), one of the many aromatic compounds present in pumpkin pie, binds to a specific G protein-coupled olfactory receptor in an olfactory cell in your nose.
- 2) The receptor-odorant complex activates a G protein, which displaces GDP and then binds to a molecule of GTP.
- 3) The  $\alpha$  subunit of the G protein dissociates and activates adenylyate cyclase, which catalyzes the production of cAMP from ATP.
- 4) cAMP binds to a sodium channel, opening it and allowing  $\text{Na}^+$  to enter the cell. This creates a nerve stimulus, which travels to the brain. Your brain integrates the  $\pi$  odorant signal with signals from other olfactory receptors in your nose. The brain interprets the signal as the odor of pumpkin pie. You think, "Mmm...pie."

a) How does binding of the  $\pi$  odorant molecule stimulate the receptor protein and how does this activate the G protein?

b) Although it might be nice to have the sensation of smelling pie continuously, once the  $\pi$  odorants are gone, signaling stops. How is the signaling pathway turned off? Discuss why once the G protein is activated, it does not continuously activate adenylyate cyclase. What chemical reaction is involved in this process?

c) List two advantages to having multiple steps in a signal transduction pathway.

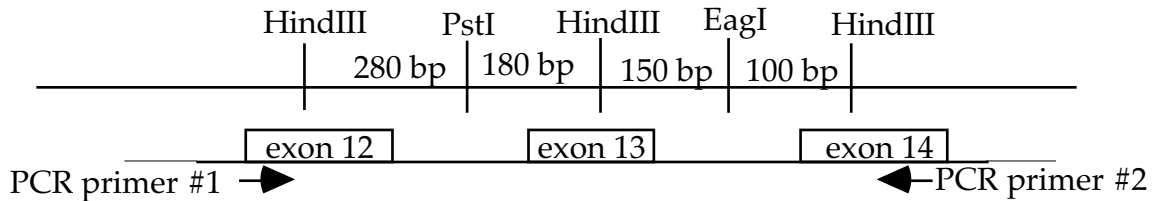
d) A turkey odorant molecule comes in contact with the  $\pi$  odorant receptor. Would this have an effect on the nerve stimulus being sent from this olfactory cell? Explain your answer.

**Question 3**

You are studying a family with hemophilia, a sex-linked recessive disease, caused by mutations in the Factor VIII gene. The Factor VIII gene contains 35 exons. The complete sequence and exon/intron structure of this gene are known. The start codon is in exon 3; the stop codon is in exon 34.

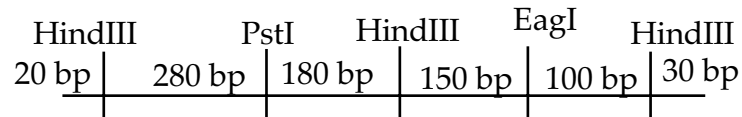
A partial restriction map and a diagram showing the location of exons 12, 13 and 14 is shown below. You synthesize two PCR primers, which anneal to sequences located within exons 12 and 14, as shown.

Wild-type(normal) Allele:

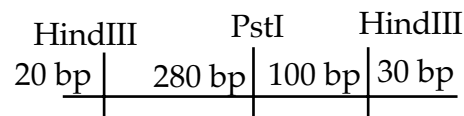


Using these PCR primers, you amplify DNA from a normal male and his hemophiliac brother. You determine the restriction map for the PCR product from these two individuals, shown below:

PCR-amplified DNA fragment from normal male:



PCR-amplified DNA fragment from hemophiliac brother:

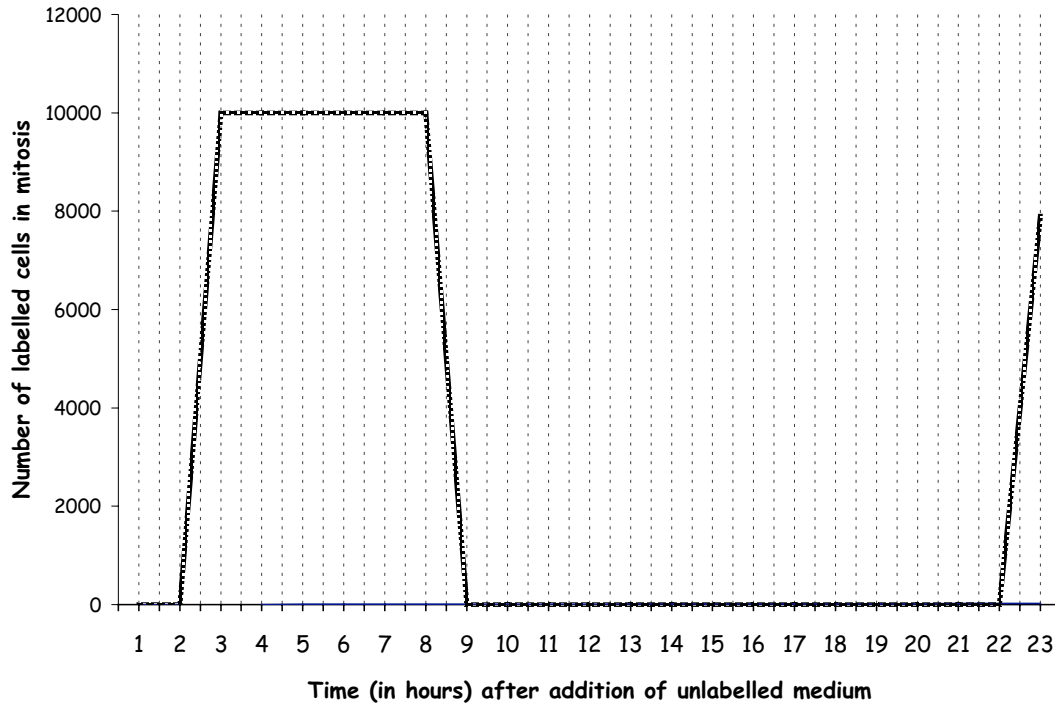


Briefly describe the likely DNA alteration in the hemophiliac.

**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  $G_2$  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  $G_1$  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

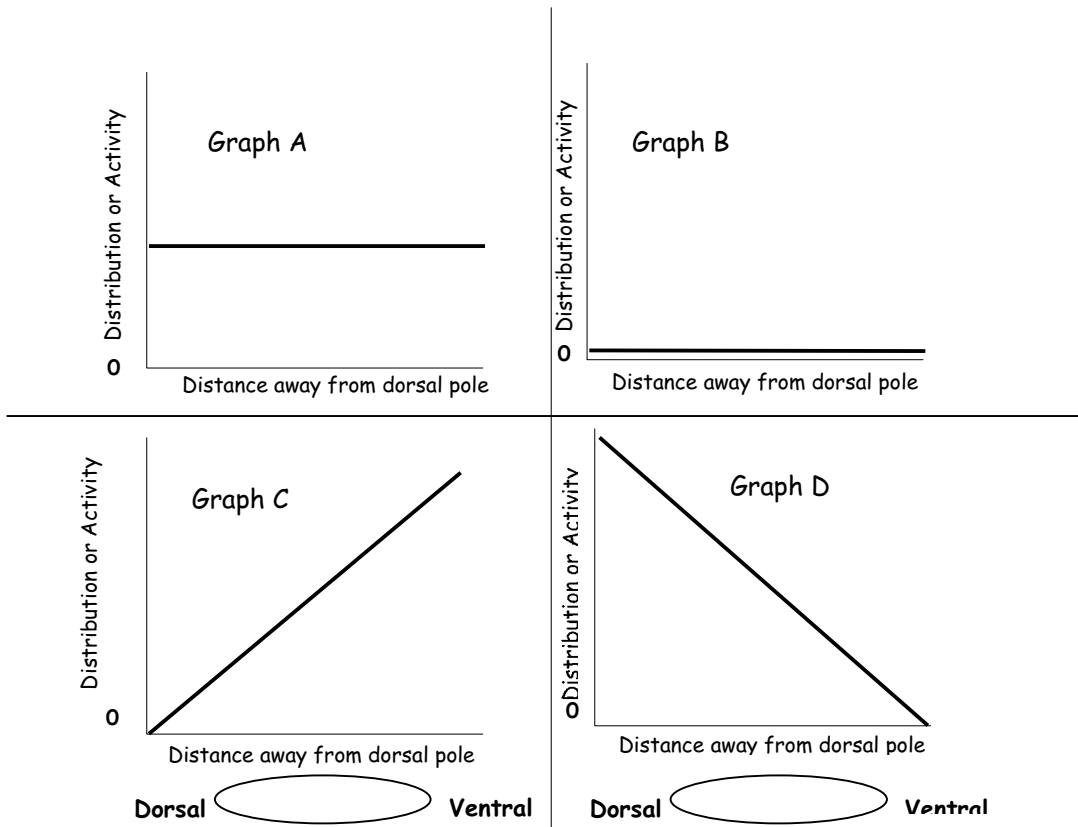
**Question 5**

A. As discussed in class, the activity of the transcription factor  $\beta$ -catenin is important for forming dorsal structures in the frog. Prior to fertilization,  $\beta$ -catenin is synthesized throughout the egg cytoplasm but is degraded by GSK-3. GSK-3 can itself be inhibited by the "Dishevelled" protein:



Upon fertilization, release of Dishevelled ultimately leads to  $\beta$ -catenin activity in specific regions of the frog zygote. From the graphs below, select the one that most closely approximates the distribution or activity of the following proteins in the following conditions.

Graph	Protein and Conditions
B	The distribution of $\beta$ -catenin protein prior to fertilization
	The distribution of GSK-3 protein prior to fertilization
	The activity of $\beta$ -catenin prior to fertilization
	The distribution of $\beta$ -catenin after fertilization
	The distribution of GSK-3 after fertilization
	The distribution of Dishevelled after fertilization
	The activity of $\beta$ -catenin after fertilization
	The activity of GSK-3 after fertilization





B. When a zygote has reached the 8-cell stage, the egg yolk is concentrated in the vegetal pole. A region called the gray crescent is also found near the vegetal pole and is required for gastrulation and development:

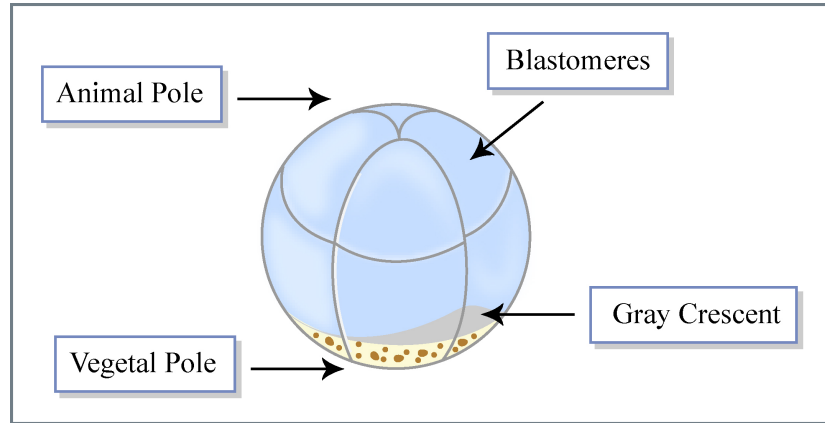
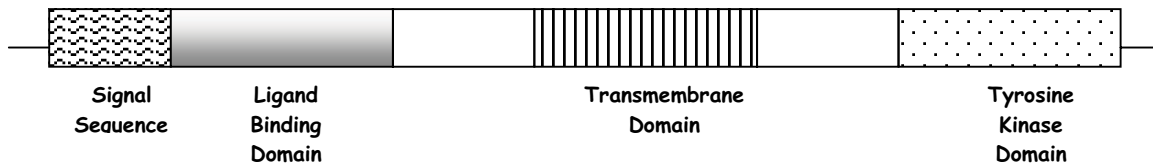


Figure by MIT OCW.

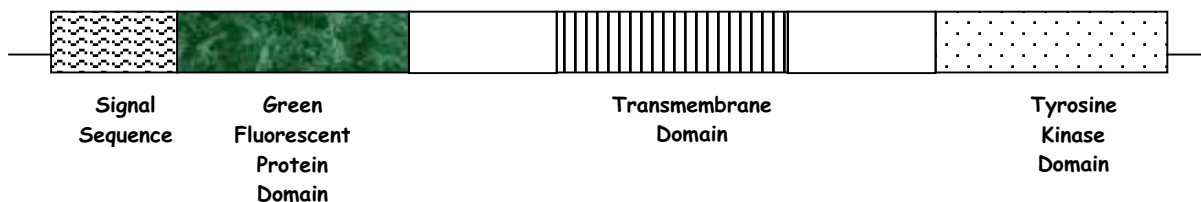
- a) In the above diagram, depict how you would divide the blastomere so that two fully functional embryos could result.
- b) If something like this were to occur naturally in a human blastula, would the result be identical or fraternal twins? Circle one.
- c) What is the difference between identical and fraternal twins?

**Question 6**

a) You have isolated a gene encoding a protein homologous to the yeast tyrosine kinase W. You suspect it will be fantastically interesting and worthy of a PhD at the very least. The putative linear protein structure is depicted below. **Label the N and C termini.**



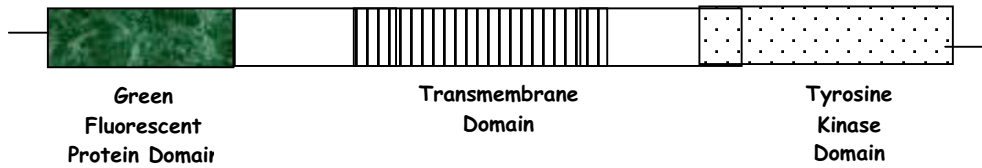
To assess the protein's localization, you generate a yeast strain in which Green Fluorescence Protein (GFP) is inserted in frame to replace the binding domain. GFP fluoresces green under appropriate light, and can thus be seen in living cells. This protein is diagramed below.



- b)
  - i) In wildtype cells, where would you expect the GFP domain to ultimately localize? (Circle one.)

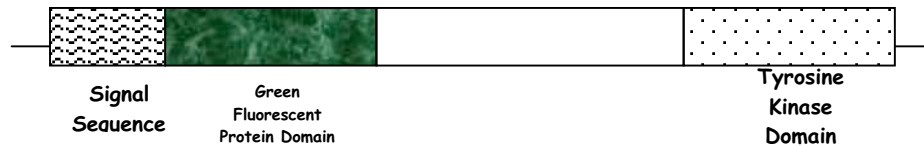
cytoplasm	inner side of vesicle membrane	outer side of vesicle membrane	inner side of plasma membrane	outer side of plasma membrane	completely secreted outside of cell (supernatant)
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ii) In mutant cells lacking the secretion signal sequence, where would you expect the GFP domain to ultimately localize? (Circle one.)



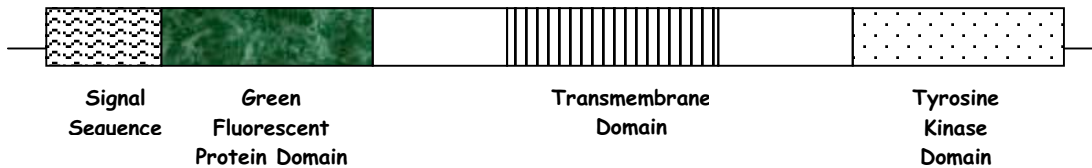
cytoplasm	inner side of vesicle membrane	outer side of vesicle membrane	inner side of plasma membrane	outer side of plasma membrane	completely secreted outside of cell (supernatant)
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iii) In mutant cells lacking the transmembrane domain, where would you expect the GFP domain to ultimately localize? (Circle one.)



cytoplasm	inner side of vesicle membrane	outer side of vesicle membrane	inner side of plasma membrane	outer side of plasma membrane	completely secreted outside of cell (supernatant)
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iv) In mutant cells that are unable to fuse transport vesicles with the golgi membrane, where would you expect the GFP to ultimately localize? (Circle one.)



cytoplasm	inner side of vesicle membrane	outer side of vesicle membrane	inner side of plasma membrane	outer side of plasma membrane	completely secreted outside of cell (supernatant)
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ANSWERS

Question 1

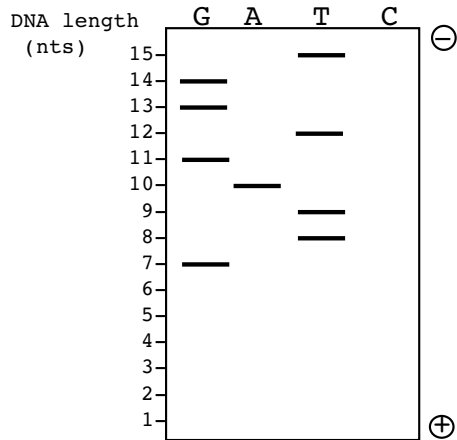
A.

primer:

template DNA:

5' -ACTGAC-3'

5' -ACCACTAACGTCAGT-3'



a) An end generated by digestion with BamHI can be ligated to an end generated by digestion with BglII. Why is this possible?

*Digestion with BglII or BamHI produce the same overhangs, or sticky ends. Thus the ends produced by cutting with BglII are complementary to the ends produced with BamHI, base pairing and ligation can occur.*

b) You want to insert the gene encoding the polio antigen into pBAN. Devise a strategy to accomplish this. Identify the enzyme(s) you would use to cut pBAN, the enzyme(s) you would use to cut pKR-polio, and the steps necessary to generate the intact plasmid.

*Cut pBAN with BamHI to linearize.*

*Cut pKR-polio with BglII, this gives a 1400 bp fragment containing the gene for the polio antigen and a 3100 bp fragment.*

*Size select the DNA from the pKR-polio plasmid to obtain the 1400 bp fragment.*

*Ligate the 1400 bp fragment together with the cut pBAN plasmid.*

c) You next transform *E. coli* with the plasmids you have made. You grow the transformed cells on media containing (circle one):

ampicillin

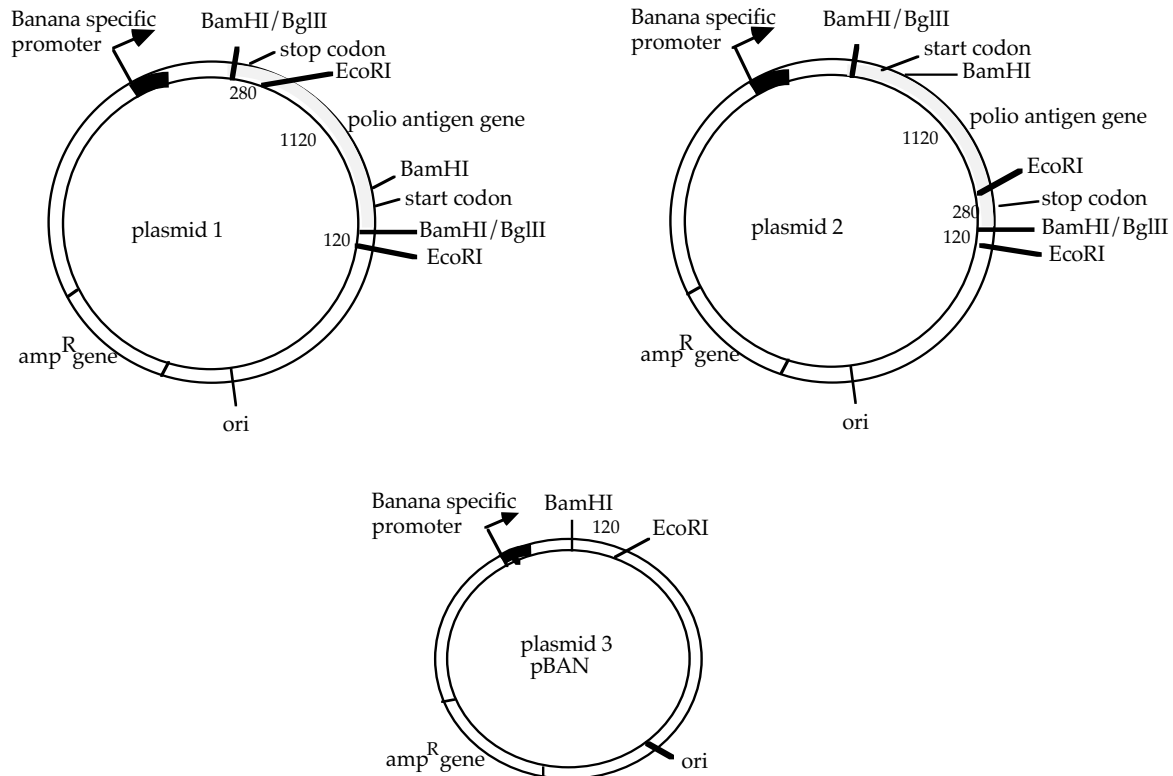
kanamycin

both ampicillin and kanamycin

neither ampicillin nor kanamycin

*Why? The media should contain ampicillin only, because the plasmid with the promoter and banana insertion sequences has a gene for ampicillin resistance.*

d) i) Draw plasmids 1, 2, and 3, indicating all relevant features such as the promoter, the origin of replication, the genes for ampicillin resistance and the polio antigen, restriction sites and distances (in base pairs) between the sites.



B.

ii) Which of the three plasmids would allow synthesis of the protein in a banana? Explain your reasoning.

*Only plasmid two would allow synthesis of functional protein.*

*The polio antigen gene must be correctly oriented with respect to the promoter.*

### Question 2

a) How does binding of the  $\pi$  odorant molecule stimulate the receptor protein and how does this activate the G protein?

*The binding of a  $\pi$  odorant molecule to the extracellular domain of the receptor changes the conformation of the protein. This allows the intracellular domain of the receptor to bind the G protein heterotrimer. This binding induces the  $\alpha$  subunit to exchange GDP for GTP, activating the  $\alpha$  subunit and releasing it from the  $\beta\gamma$  subunit.*

b) Although it might be nice to have the sensation of smelling pie continuously, once the  $\pi$  odorants are gone, signaling stops. How is the signaling pathway turned off? Discuss why once the G protein is activated, it does not continuously activate adenylate cyclase. What chemical reaction is involved in this process?

*The  $\alpha$  subunit of the G protein has an intrinsic GTPase activity. This activity converts GTP bound to the  $\alpha$  subunit to GDP. Since the GTP is required for the  $\alpha$  subunit to be active, the conversion of GTP to GDP deactivates the  $\alpha$  subunit of the G protein. The inactive  $\alpha$  subunit then can rejoin the  $\beta\gamma$  subunit to await another signal from the receptor.*

c) List two advantages to having multiple steps in a signal transduction pathway.

*Multiple steps in the pathway can provide a large amplification of the signal. It also allows multiple points of regulation. In addition, if several different receptors work through the same transduction pathway, integration of different inputs is possible.*

d) A turkey odorant molecule comes in contact with the  $\pi$  odorant receptor. Would this have an effect on the nerve stimulus being sent from this olfactory cell? Explain your answer.

No, a turkey odorant molecule would have no effect on the stimulus from this cell. The  $\pi$  odorant receptor specifically recognizes the  $\pi$  odorant. However, the turkey odorant molecule would affect olfactory cells specialized for turkey.

**Question 3**

There is a deletion of 330 bp in the DNA, including exon 13 and possibly part of exon 14, which also removes a Hind III site and an EagI site

**Question 4**

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

G<sub>0</sub> phase      G<sub>1</sub> phase      G<sub>2</sub> phase      M phase       S phase      Lunar phase

b) Estimate the length of the G<sub>2</sub> 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 G<sub>1</sub> 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

A

Graph	Protein and Conditions
<b>B</b>	<b>The distribution of <math>\beta</math>-catenin protein prior to fertilization</b>
<b>A</b>	<b>The distribution of GSK-3 protein prior to fertilization</b>
<b>B</b>	<b>The activity of <math>\beta</math>-catenin prior to fertilization</b>
<b>D</b>	<b>The distribution of <math>\beta</math>-catenin after fertilization</b>
<b>A</b>	<b>The distribution of GSK-3 after fertilization</b>
<b>D</b>	<b>The distribution of Dishevelled after fertilization</b>
<b>D</b>	<b>The activity of <math>\beta</math>-catenin after fertilization</b>
<b>C</b>	<b>The activity of GSK-3 after fertilization</b>

B. When a frog embryo has reached the 8-cell stage, the egg yolk is concentrated in the vegetal pole. A region called the gray crescent is also found near the vegetal pole and is required for gastrulation and development:

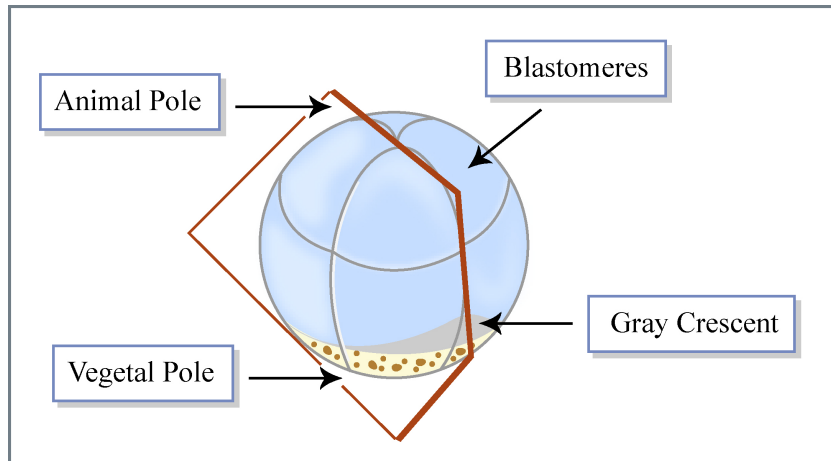


Figure by MIT OCW.

a) In the above diagram, depict how you would divide the blastomere so that two fully functional embryos could result. 3 points

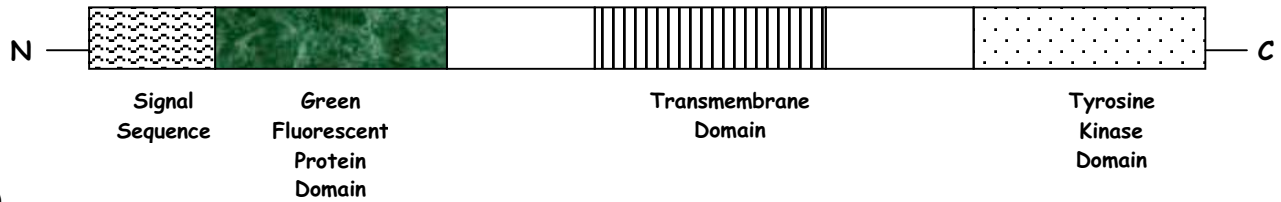
b) If something like this were to occur naturally in a human blastula, would the result be identical or fraternal twins? Circle one.

c) What is the difference between identical and fraternal twins?

*Identical twins have the same DNA, while fraternal twins are from separate eggs/sperm and should have different DNA (like siblings).*

Question 6

a) To assess the protein's localization, you generate a yeast strain in which Green Fluorescence Protein (GFP) is inserted in frame to replace the binding domain. GFP fluoresces green under appropriate light, and can thus be seen in living cells. This protein is diagramed below. Label the N and C termini

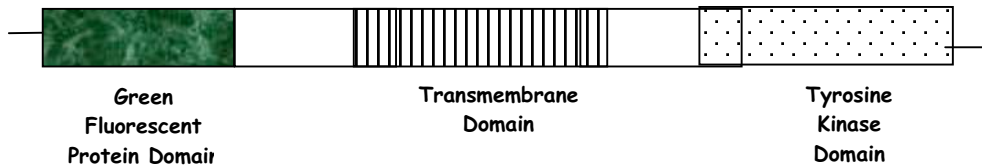


b)

i) In wildtype cells, where would you expect the GFP domain to ultimately localize? (Circle one.)

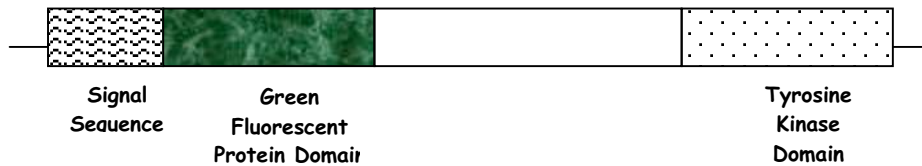
cytoplasm	inner side of vesicle membrane	outer side of vesicle membrane	inner side of plasma membrane	<b>outer side of plasma membrane</b>	completely secreted outside of cell (supernatant)
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ii) In mutant cells lacking the secretion signal sequence, where would you expect the GFP domain to ultimately localize? (Circle one.)



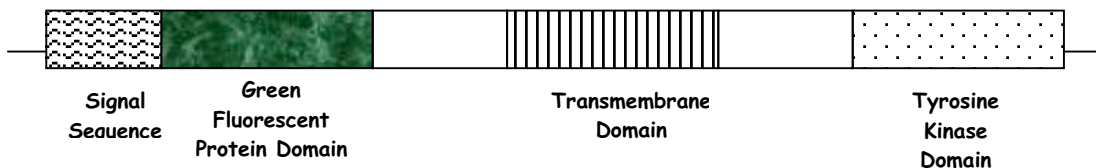
<b>cytoplasm</b>	inner side of vesicle membrane	outer side of vesicle membrane	inner side of plasma membrane	outer side of plasma membrane	completely secreted outside of cell (supernatant)
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iii) In mutant cells lacking the transmembrane domain, where would you expect the GFP domain to ultimately localize? (Circle one.)



cytoplasm	inner side of vesicle membrane	outer side of vesicle membrane	inner side of plasma membrane	outer side of plasma membrane	<b>completely secreted outside of cell (supernatant)</b>
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iv) In mutant cells that are unable to fuse transport vesicles with the golgi membrane, where would you expect the GFP to ultimately localize? (Circle one.)



cytoplasm	<b>inner side of vesicle membrane</b>	outer side of vesicle membrane	inner side of plasma membrane	outer side of plasma membrane	completely secreted outside of cell (supernatant)
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