7.012 Practice Quiz 2 2004

Actual Quiz 2 (closed book) will be given Monday 10/25 at 10:00 am

No Sections on MONDAY or TUESDAY 10/25-10/26 (No Kidding.)

NOTE THE ROOM MAY BE DIFFERENT THAN THE ROOM YOUR WERE ASSIGNED FOR QUIZ 1

Quiz Review Session

Thursday, 10/21 7:00 - 9:00 pm

Tutoring Session

Friday, 10/22 4:00 - 6:00 pm

Question 1

a) 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) DNA replication is initiated at promoter sequences in the DNA.

ii) RNA polymerase requires primers to initiate RNA synthesis.

iii) Okazaki fragments are the short fragments of DNA that are produced on the leading strand at the DNA replication fork.

iv) The 5' to 3' direction of DNA synthesis implies that deoxyribonucleotides are added to the 5' OH group on the growing strand.

v) Transcription is terminated at stop codons in the mRNA.

b) Shown below is the DNA sequence of a gene from a virus that encodes a short viral peptide. Also shown is the sequence of the mRNA synthesized from this gene.

genomic DNA sequence:

5'-AGCTCATGTGCGAGTCCTGACGCTGACTAGG-3'
3'-TCGAGTACACGCTCAGGACTGCGACTGATCC-5'

mature mRNA sequence (G^* = *G* cap):

$5'-G^*$UCAUGUGCGAACGCUGACUAGGAAAAAAAA....-3'

i) In the genomic DNA sequence shown above, draw a box around each of the two exons in the gene.

ii) In the mRNA above, some nucleotides are present that are not coded for in the genomic DNA sequence. Name the two processes that have occurred to add these nucleotides to the mRNA.

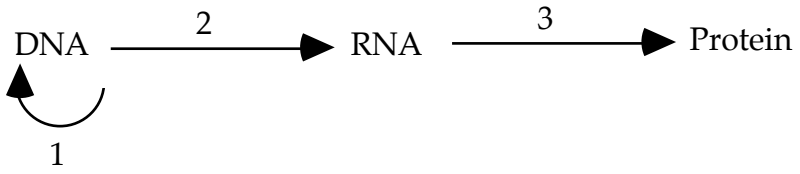
iii) How many amino acids are in the viral peptide encoded by this gene? _____

iv) Is this virus more likely to replicate in prokaryotic or eukaryotic cells? Briefly explain your reasoning.

	U	C	A	G	
U	UUU phe (F)	UCU ser (S)	UAU tyr (Y)	UGU cys (C)	UC
	UUC phe (F)	UCC ser (S)	UAC tyr (Y)	UGC cys (C)	A
	UUA leu (L)	UCA ser (S)	UAA STOP	UGA STOP	G
	UUG leu (L)	UCG ser (S)	UAG STOP	UGG trp (W)	
C	CUU leu (L)	CCU pro (P)	CAU his (H)	CGU arg (R)	UC
	CUC leu (L)	CCC pro (P)	CAC his (H)	CGC arg (R)	A
	CUA leu (L)	CCA pro (P)	CAA gln (Q)	CGA arg (R)	G
	CUG leu (L)	CCG pro (P)	CAG gln (Q)	CGG arg (R)	
A	AUU ile (I)	ACU thr (T)	AAU asn (N)	AGU ser (S)	U
	AUC ile (I)	ACC thr (T)	AAC asn (N)	AGC ser (S)	C
	AUA ile (I)	ACA thr (T)	AAA lys (K)	AGA arg (R)	A
	AUG met (M)	ACG thr (T)	AAG lys (K)	AGG arg (R)	G
G	GUU val (V)	GCU ala (A)	GAU asp (D)	GGU gly (G)	U
	GUC val (V)	GCC ala (A)	GAC asp (D)	GGC gly (G)	C
	GUA val (V)	GCA ala (A)	GAA glu (E)	GGA gly (G)	A
	GUG val (V)	GCG ala (A)	GAG glu (E)	GGG gly (G)	G

Question 2

The term "central dogma" refers to the flow of biological information from DNA to RNA to protein.



a) i) In the spaces below, indicate the process that corresponds to each arrow.

1. _____ 2. _____ 3. _____

ii) Name the initiation site for each processes, and on which molecule this site exists.

1. _____2. _____3. _____

iii) What cellular machinery carries out each process?

1. _____ 2. _____ 3. _____

b) What is a gene? Please answer in one sentence. The first sentence written will be considered as your answer.

c) Many antibiotics are compounds that interfere with the transfer of genetic information from RNA to protein. Streptomycin is a compound that affects the small ribosomal subunit in prokaryotes. Streptomycin interferes with the binding of all Methionine-tRNAs to ribosomes. What two specific effects will streptomycin have on protein synthesis in prokaryotes?

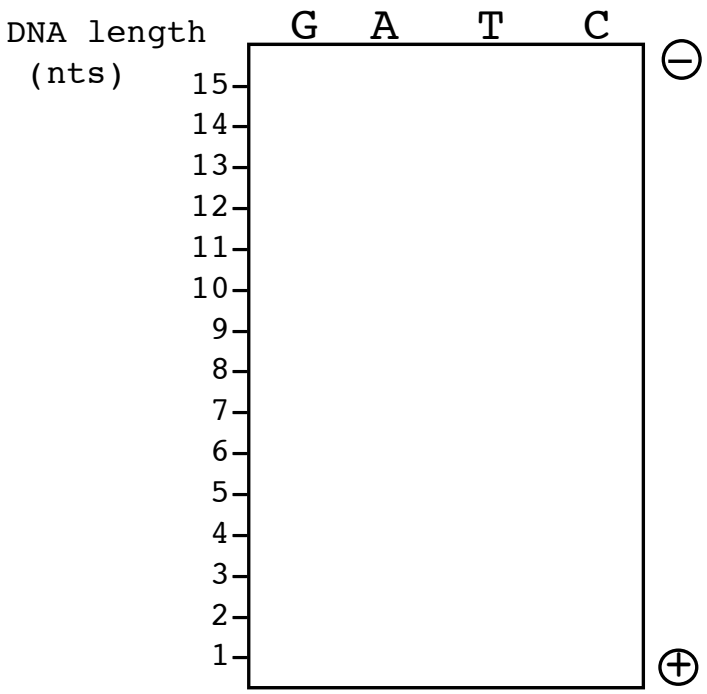
Question 3

A.

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

primer:	**template DNA:**
5'–ACTGAC–3'	**5'–ACCACTAACGTCAGT–3'**

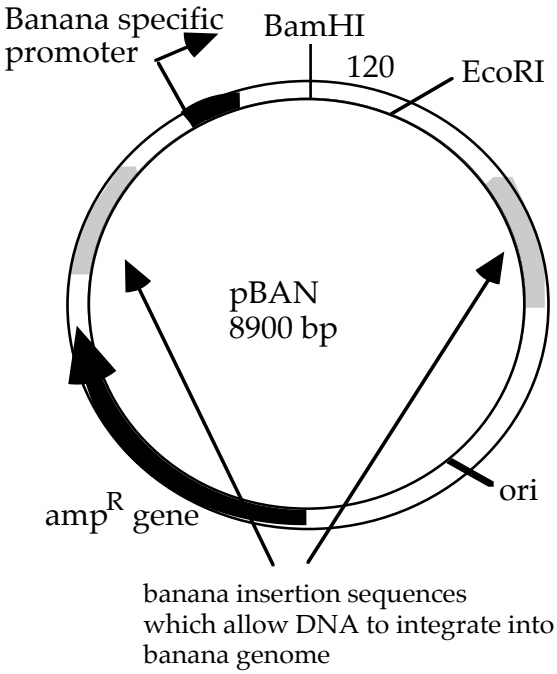
Draw the resulting DNA fragments that would be produced from each of the 4 sequencing reactions at the correct position (length in nucleotides) as they would appear on the diagram of the sequencing gel below.

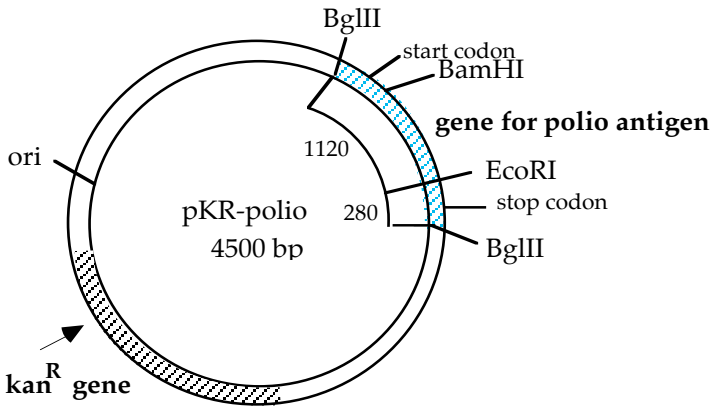


B.

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 3 continued






```
BglII:                  BamHI:                  EcoRI:

5'...A G A T C T...3'    5'...G G A T C C...3'    5'...G A A T T C...3
3'...T C T A G A...5'    3'...C C T A G G...5'    3'...C T T A A G...5
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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.

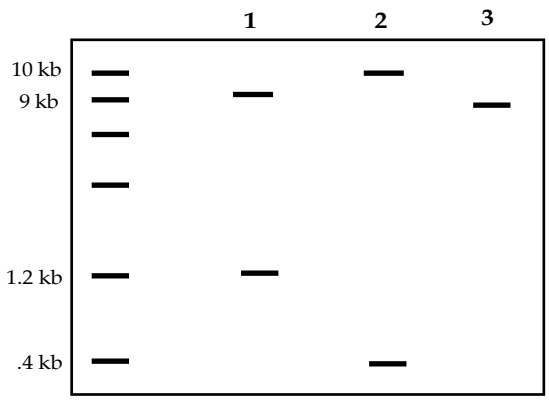
c) You next transform *E. coli* with the plasmids you have made. You grow the transformed cells on media containing (circle one):

ampicillin **kanamycin**

both ampicillin and kanamycin **neither ampicillin nor kanamycin**

Why?

You isolate plasmid DNA from three colonies that pass your antibiotic resistance test. You digest the DNA with the restriction enzyme *Eco*RI. 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.

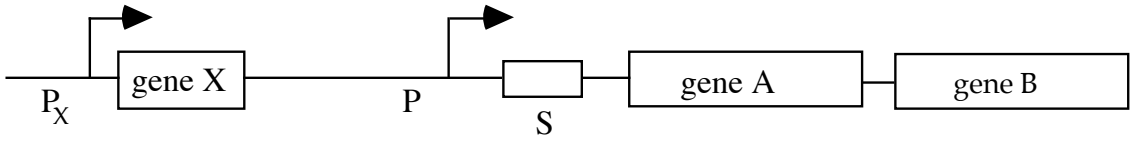


d)
i) Draw plasmids associated with the colonies 1, 2, and 3. Indicate all relevant features such as the promoter, the origin of replication, the genes for ampicillin resistance and the polio antigen,

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

Question 4

In *E. coli*, the fictitious AB operon is induced by the presence of Compound W. A diagram of the operon, its regulatory proteins and regulatory sites is shown below:



P_X promoter for the regulatory protein
X gene for the regulatory protein of the AB operon
P promoter for the AB genes
S sequence shown to be important for regulation by W
A structural gene for enzyme A
B structural gene for enzyme B

The following table shows the genotypes of different *E. coli* strains with a wild-type AB operon and various mutant AB operons, and the number of molecules of protein A per cell in the absence or presence of Compound W (–W or +W, respectively).

a) In the table below, the symbol "+" indicates that the gene or control element is functional (wt) and "–" indicates that the gene or control element is non-functional. Assume the genes not listed are wild type.

					Molecules of A		
Strain	X	P	S	A	-W	+W	Expression
Wild type	+	+	+	+	0	200	**inducible**
M1	-	+	+	+	200	200	
M2	+	-	+	+	0	0	
M3	+	+	-	+	200	200	
M4	+	+	+	-	0	0	

i) For each strain on the table above, label the expression as either inducible, uninducible or constitutive.

ii) Based on the data shown above, does the regulatory protein X act as a repressor or an activator of the AB operon? Explain your reasoning.

Question 4, continued

b) You make partial diploids of various *E. coli* mutant strains using a single-copy plasmid introduced into the *E. coli* cell. In cells of the following genotypes, predict the number of molecules of enzyme A per cell (0, 200, 400) produced in the absence or presence of Compound W (–W or +W, respectively). Put the total number of molecules of enzyme A on the lines provided.

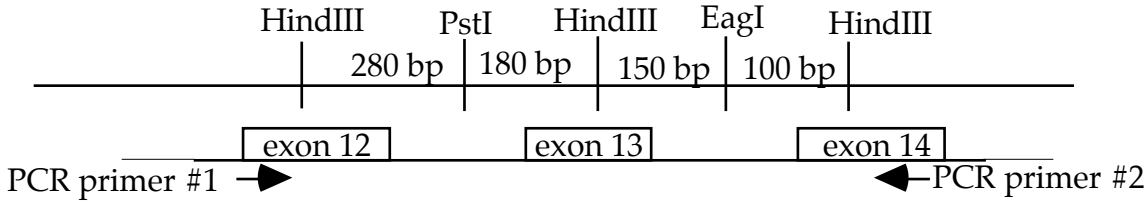
Genotype	# of molecules of enzymes A per cell –W	+W
$\frac{X^+\ P^+\ S^+\ A^+}{X^+\ P^+\ S^+\ A^+}$	0	400
$\frac{X^+\ P^+\ S^+\ A^+}{X^+\ P^+\ S^+\ A^-}$	0	200
$\frac{X^+\ P^+\ S^+\ A^+}{X^-\ P^+\ S^+\ A^+}$	_____	_____
$\frac{X^+\ P^+\ S^-\ A^+}{X^+\ P^+\ S^+\ A^+}$	_____	_____
$\frac{X^-\ P^+\ S^+\ A^+}{X^+\ P^+\ S^+\ A^-}$	_____	_____
$\frac{X^+\ P^+\ S^-\ A^+}{X^+\ P^+\ S^+\ A^-}$	_____	_____
$\frac{X^+\ P^+\ S^-\ A^-}{X^+\ P^+\ S^+\ A^+}$	_____	_____

Question 5

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.

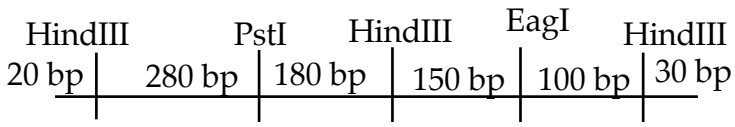
A partial restriction map and a diagram showing the location of exons 12, 13 and 14 is shown below. You synthesize two PCR primers, which anneal to sequences located within exons 12 and 14, as shown.

Wild-type(normal) Allele:

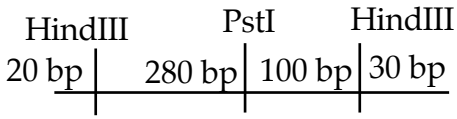


Using these PCR primers, you amplify DNA from a normal male and his hemophiliac brother. You determine the restriction map for the PCR product from these two individuals, shown below:

PCR-amplified DNA fragment from normal male:



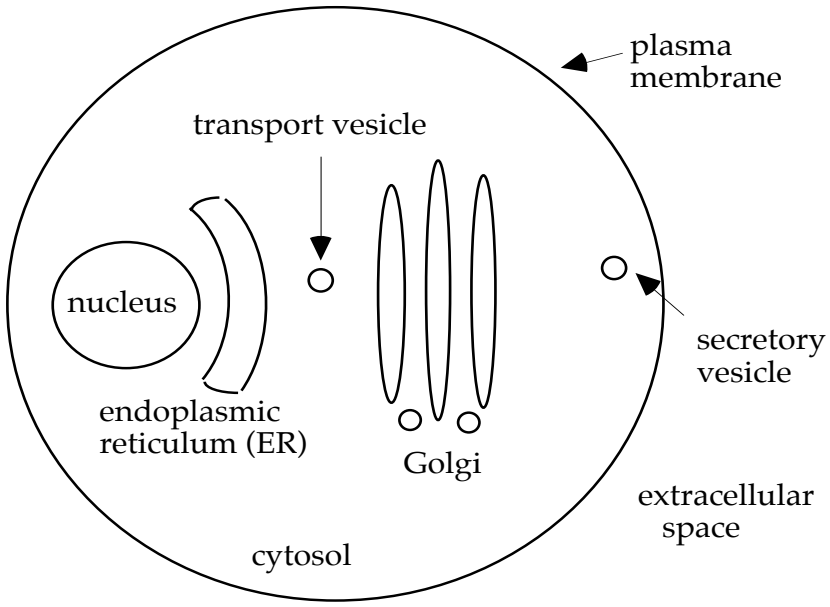
PCR-amplified DNA fragment from hemophiliac brother:



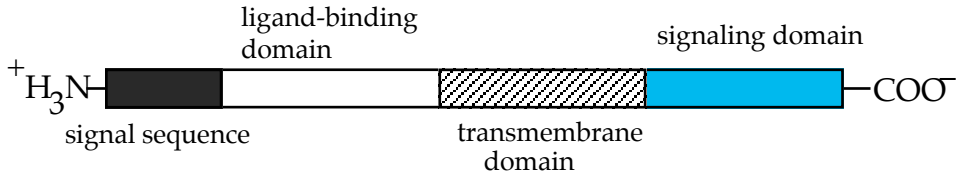
Briefly describe the likely DNA alteration in the hemophiliac.

Question 6

Use this diagram of a cell as a guide when answering the following questions.



a) Consider the following receptor protein:



i) Where would this receptor protein be localized if it's the cell was lacking SRP? Would it be functional?

ii) Where would this receptor protein be localized if its signaling domain were deleted? Would it be functional?

iii) What common characteristic do the side chains of the amino acids found in a transmembrane domain share, and why?

iv) Which domain (ligand binding, transmembrane, or signaling**) of the wild-type receptor protein is inserted into the ER lumen during translation? Why must the receptor protein be inserted in this fashion to become functional?**

b) The mature mRNA encoding a protein destined for secretion specifies a polypeptide chain of 497 amino acids; yet the protein that is actually secreted by the cell is 475 amino acids long.

i) How can you account for this size difference?

ii) Would a <u>transmembrane</u> protein also show this disparity? Briefly explain your answer.

ANSWERS
Question 1

a)

i) FALSE. DNA replication is initiated at the origin of replication. RNA polymerases bind to promoter sequences to initiate transcription.

ii) FALSE. RNA polymerase does not require primers to initiate RNA synthesis. DNA polymerase requires primers to initiate DNA replication.

iii) FALSE. Okazaki fragments are made on the lagging strand at the replication fork.

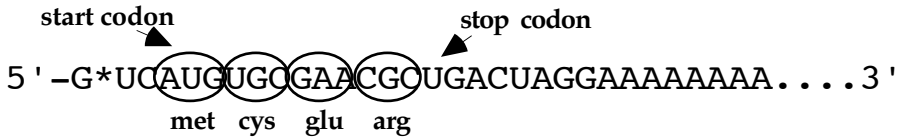
iv) FALSE. DNA synthesis occurs by addition of dNTPs to the 3' OH group of the nucleotide at the end of the growing strand.

v) FALSE. Transcription terminates at the transcription termination sites in the DNA. Translation terminates at stop codons in the mRNA.

b) genomic DNA sequence:

5'–AGCTCATGTGCGAGTCCTGACGCTGACTAGG–3'
3'–TCGAGTACACGCTCAGGACTGCGACTGATCC–5'

mature mRNA sequence (*G = *G* cap):**



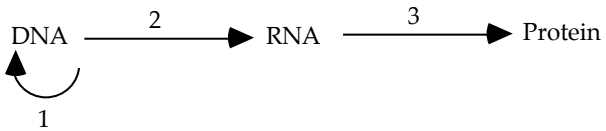
i) see DNA sequence above

ii) 1) 5' capping 2) 3' polyadenylation

iii) There are four amino acids in this viral peptide: NH_3^+-met-cys-glu-arg-COO^-

iv) In eukaryotic cells because the RNA processing and splicing machinery is only present in eukaryotes.

Question 2
The term "central dogma" refers to the flow of biological information from DNA to RNA to protein.



a) i) In the spaces below, indicate the process that corresponds to each arrow.

1. *replication* **2. *transcription,*** **3. *translation***

ii) Name the initiation site for each processes, and on which molecule this site exists.

1. ***Origin of replication, DNA***
2. ***Promoter, DNA***
3. ***Start codon (first AUG), mRNA***

iii) What cellular machinery carries out each process?

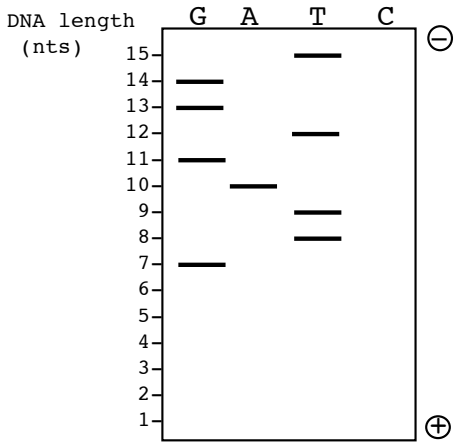
1. DNA polymerase 2. RNA polymerase 3. ribosome

b) What is a gene? Please answer in one sentence. The first sentence written will be considered as your answer.
A gene is a segment of DNA containing information for directing the synthesis of a protein (or RNA).

c) What two specific effects will streptomycin have on protein synthesis in prokaryotes?
Streptomycin will prevent the correct initiation of protein synthesis since it prevents association of the met-tRNA with the ribosome. Streptomycin will also lead to inaccurate translation (insertion of incorrect amino acids) in those proteins that were in the process of being translated.

Question 3

A. **primer:** **5'-ACTGAC-3'** **template DNA:** **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.

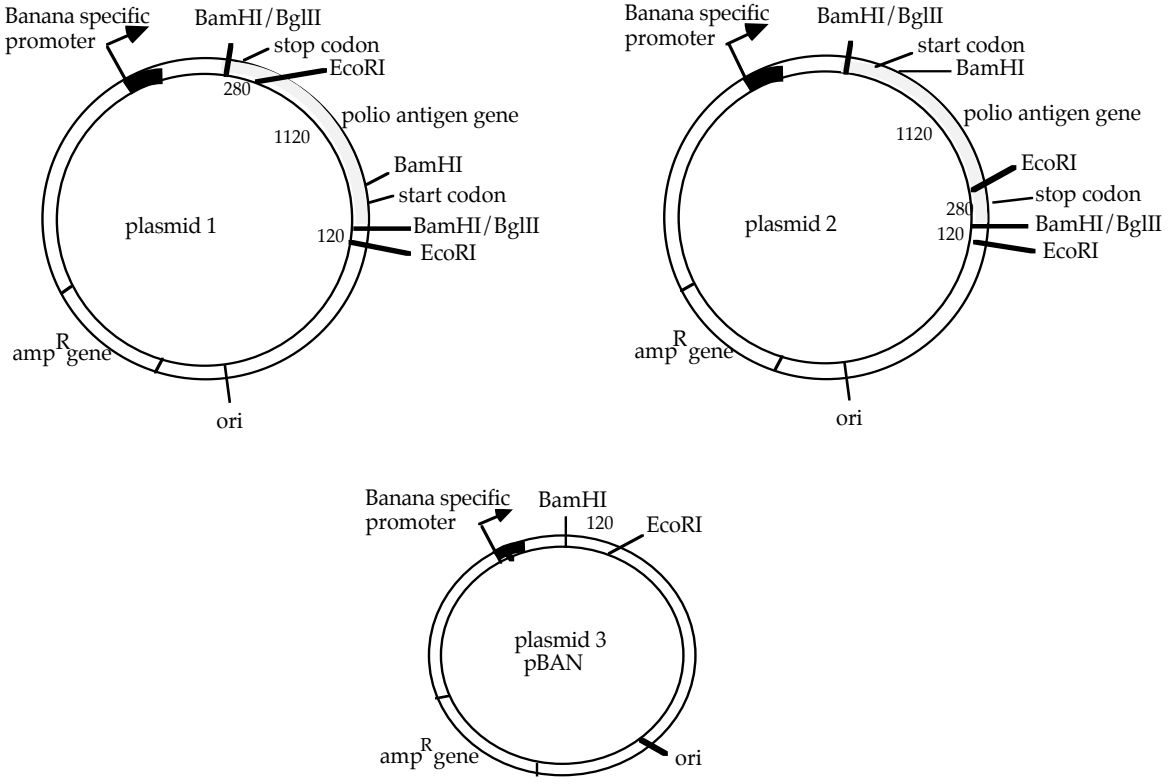
c) You next transform *E. coli* with the plasmids you have made. You grow the transformed cells on media containing (circle one):

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 yor reasoning.

Only plasmid two would allow synthesis of functional protein.

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

.Question 4

i)

Strain	X	P	S	A	-W	+W	Expression
Wild type	+	+	+	+	**0**	**200**	**inducible**
M1	-	+	+	+	**200**	**200**	**constitutive**
M2	+	-	+	+	**0**	**0**	**uninducible**
M3	+	+	-	+	**200**	**200**	**constitutive**
M4	+	+	+	-	**0**	**0**	**uninducible**

ii) Protein X acts as a repressor. In mutants 1 and 3 that lack either the repressor or the repressor binding site, expression is constitutive (high with or without W).

b) Put the total number of molecules of enzyme A on the lines provided.

X^+ P^+ S^+ A^+	0	400
X^- P^+ S^+ A^+		
X^+ P^+ S^- A^+	200	400
X^+ P^+ S^+ A^+		
X^- P^+ S^+ A^+	0	200
X^+ P^+ S^+ A^-		
X^+ P^+ S^- A^+	200	200
X^+ P^+ S^+ A^-		
X^+ P^+ S^- A^-	0	200
X^+ P^+ S^+ A^+		

Question 5

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 6

a) i) Where would this receptor protein be localized if the cell was lacking SRP?

The receptor protein would be found in the cytosol. It would be non-functional.

ii) Where would this receptor protein be localized if its signaling domain were deleted?

It would be inserted into the plasma membrane, but would not be functional without a signaling domain.

iii) What common characteristic do the side chains of the amino acids found in a transmembrane domain share, and why?

The transmembrane domain, is composed of amino acids with hydrophobic side chains because the interior of the lipid bilayer is hydrophobic.

iv) Which domain (ligand-binding, transmembrane, or signaling**) of the wild-type receptor protein is inserted into the ER lumen during translation? Why must the receptor protein be inserted in this fashion to become functional?**

The ligand-binding domain is inserted into the ER lumen. Upon secretory vesicle fusion with the plasma membrane, the interior of the ER becomes the outside of the cell where the ligand-binding domain must be in order to bind ligand.

b) i) How can you account for this size difference?

A signal sequence of 22 amino acids is cleaved during translation into the ER.

ii) Would a <u>transmembrane</u> protein also show this disparity? Briefly explain.

Yes. A transmembrane protein also has a signal sequence, which is also cleaved during translation into the ER.