Solution key-7.016 Problem Set 4- 2018

Question 1 (3pts)
Many plants are the source of traditional herbal medicines. For example, the roots of the Kava plant, *Piper methysticum,* are often used to treat insomnia (the inability to fall asleep). You cross a variety of Kava plant that is true breeding for **wide-green leaves (P1)** with another kava plant that is true breeding for **narrow-red leaves (P2)**. You obtain F1 plants all of which have **narrow-green** leaves.

a) Assume that **Gene A regulates leaf shape** (wide or narrow) and **Gene B regulates leaf color** (green or red). Give the genotypes of the following plants for both traits, using "A" and "B" for the alleles conferring the dominant phenotypes and "a" and "b" for the alleles conferring the recessive phenotypes.

i. **P1:** ***aaBB*** *(0.25)*
ii. **P2:** ***AAbb*** *(0.25)*
iii. **F1** ***aBAb*** *(0.25)*

b) Assuming that Gene A and Gene B were **closely linked (i.e. cannot be separated by recombination),** give the phenotypes, genotypes and the corresponding ratios of the F2 plants you would expect by **crossing two F1 plants**.

i. **Genotypes** and **corresponding ratios:** ***aaBB (1): AbaB (2):AAbb (1)***
(0.5, 0.25 for genotype and 0.25 for corresponding ratio)

i. **Phenotypes** and **corresponding ratios:** ***wide and green (1): narrow- green (2): narrow-red (1)***
(0.5, 0.25 for phenotype and 0.25 for corresponding ratio)

c) You cross an **F1 plant** with another plant that has the genotype **"aabb"**.

i. If Gene A and Gene B are **4cM apart**, complete the table below for each class of F2 plants. Assume there are 100 F2 plants in total. *(1pt, 0.25 for each row, all or none)*

Genotypes?	Corresponding phenotype?	Corresponding number?
aBab	*Wide- green*	*48*
Abab	*Narrow-red*	*48*
abab	*Wide-red*	*2*
ABab	*Narrow-green*	*2*

ii. In the table above, circle the **recombinant (non-parental) F2 classes**:
The last two rows in the table above represent the recombinants (0.25)

Question 2 (3pts)
You are studying three traits in a variety of fly: **body color** regulated by **Gene A** (alleles A and a), **eye color** regulated by **Gene B (alleles B and b) and the presence or absence of wings** regulated by **Gene D (**alleles D and d**)**. ***Note:*** *Genes A, B and D are located on the same chromosome.*

a) You want to determine the distance between Genes A, B and D. Which of the following trihybrid crosses would help you determine the chromosomal map? **Explain why** you selected this option as opposed to the others. *(0.5, with 0.25 for explanation)*

i. AABBDD x AABBDD
ii. aaBBDD x AABBdd
iii. aaBBdd X AAbbDD *(the flies being crossed are heterozygous at each of the three gene loci)*

b) You mate a true breeding **gray, red-eyed, winged fly** (**P1**) with a true breeding **yellow, white-eyed, wingless fly** (**P2**). All of the resulting **F1** flies are **gray,** have **red-eye** and are **winged**. You mate an F1 fly with a triple homozygous recessive fly and obtain 1000 F2 flies.

Note: *Assume each of the above genes is autosomal and there are only two alleles for each gene. Use the uppercase letter to represent the allele conferring the dominant phenotype and lowercase letter to represent the allele conferring the recessive phenotype.*

i. Give the genotypes of... *(1pt or 0.25 each)*

P1 fly: *AABBDD* **P2 fly: *aabbdd***

F1 fly: *AaBbDd* **Fly that mated with F1 fly: *aabbdd***

ii. Assume that Genes A, B and D are located on the same autosome in the order D-A-B.

- Give the genotypes of **ALL** possible gametes produced by the **F1 fly**.
  DAB, dab, Dab, dAB, DAb, daB, DaB, dAb (0.5 all should be correct here)
- Which gametes in the first bullet point of part (ii) are produced by a **SINGLE** crossover event (SCO)? *Dab, dAB, DAb, daB (0.25)*
- Which gametes in the first bullet point of part (ii) are produced by **NO** crossover event (NCO)? *DAB and dab (0.25)*
- Which gametes in the first bullet point of part (ii) are produced by a **DOUBLE** crossover event (DCO)? *DaB, dAb (0.25)*

c) The results of the triple hybrid cross show that **Gene A** is **25cM** apart both from **Genes D** and **B**. For each pairwise combination of the genes, are they are **linked or unlinked**: **D-A/ D-B/ A-B**? **Explain.**
Note: *You should remember that the order of the genes on the chromosomes is D-A-B.*
Gene D and Gene B are unlinked since they are far apart from each other on the chromosome (Map distance is 50cM). So these genes will assort independently as per the Mendel's law. Since the distance between Gene D and Gene A is 25cM (<50cM) they are linked. Similarly, since the distance between Gene A and Gene B is 25cM (<50cM) they are linked. (0.25)

Question 3 (4pts)

a) Yeast is a unicellular eukaryote that is often used as a model organism to identify the mutations in genes that are associated with a recessive phenotype without performing extensive crosses. What feature of yeast enables this?

A diploid cell that undergoes meiosis produces four haploid progeny as spores within one ascus/ tetrad. Since it can exist as a haploid, it can be used as an excellent experimental model to identify even the recessive mutations (genotype: a), which would have been otherwise missed in a diploid cells that had a heterozygous genotype (Aa). (0.5)

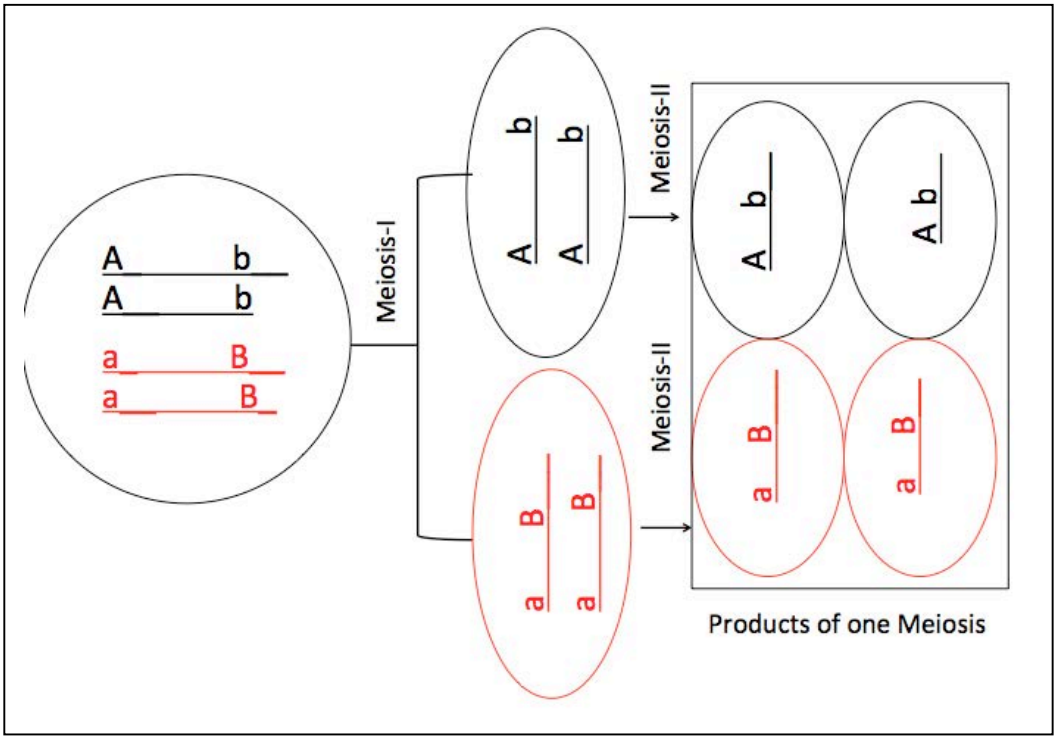
b) Based on what we have learned about yeast genetics….

i. In the table below, fill in the genotypes of Parental ditypes (PD), nonparental ditypes (NPD) and tetratypes (TT) by mating the following yeast haploids. *(1.5 or 0.5 for each column)*

Bd X ***bD***		
PD	**NPD**	**TT**
Bd	***BD***	***Bd***
Bd	***BD***	***bD***
bD	***bd***	***BD***
bD	***bd***	***bd***
Total: 100	**Total: 100**	**Total: 400**

ii. **Explain** whether the two genes in part (i) above are linked or unlinked.
They are unlinked since PD:NPD:TT ratio is 1: 1: 4. (0.5)

c) Draw out meiosis for the chromosomes. Include the position of "A" and "B" genes to produce the PD that you filled in the table above. *(1.5, 0.5 for each part, cell before replication, after M-I and after M-II)*



Question 4 (2pts)
Prof Martin introduced you to a few genetic screening methods used in model organisms (like flies and worms) to identify genes involved in multiple aspects of cellular and organismal physiology such as apoptosis and circadian rhythm.

a) Chemical mutagenesis is often performed using ethyl methane sulphonate (EMS). **Explain** how EMS randomly generates mutants. *(0.5pts)*
EMS is an alkylating agent that ethylates DNA bases and thus introduces errors in DNA replication with a consequent mutation in DNA. The mutations are typically single base pair (bp) changes, which can introduce missense or nonsense mutations thus changing gene expression.

b) In one example of chemical mutagenesis, the male flies (XY) were mutagenized and then crossed with (X^X)Y female flies. ***Note:*** *X^X means attached X chromosomes.*

i. Why are the male flies mutagenized as opposed to the female flies? *(0.5pts)*
Male flies are hemizygous for X sex chromosome i.e. they have only one X chromosome (Genotype: XY) unlike female flies who have two X sex chromosomes (Genotype: XX). So if the genes on the X chromosomes are mutated in males, it will result in an observable phenotype irrespective of the resulting phenotype being dominant or a recessive. In females you need to mutate both alleles of the gene to identify mutations that result in recessive phenotype.

ii. How does an (X^X)Y female differ from an XX female fly with respect to the X chromosome?
X^XY represents a female fly with two attached X chromosomes which always segregate together. In an XX female the two X chromosomes can assort independently. (0.5pts)

c) Outline a cross that can create a self-propagating fly stock in which all males carry a mutagenized X and all females carry a non-mutagenized (X^X). *(0.5pts)*

Non-mutagenized P1 female Fly (X^X)Y X Mutagenized P2 male fly (X^mY)

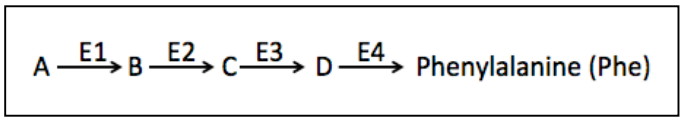
↓

F1 flies: *X^XY female flies and X^mY male flies.*
(The remaining progeny ($X^{\wedge}X\ X^m$ female and YY) are lethal)

↓

Starting with one male with a mutagenized X^m you produce a line where all males have the same mutation.

Question 5 (4pts)
The amino acid **Phenylalanine (Phe)** in **yeast** is synthesized through a **multi-step biochemical pathway** where each step is catalyzed by a specific enzyme (E1, E2, E3 and E4) as shown below. The enzymes E1, E2, E3 and E4 are encoded by Gene 1, Gene 2, Gene 3 and Gene 4 respectively.



You are working with a **haploid yeast auxotroph** that has a loss-of-function mutation in Gene 1, which encodes E1 but still has the wild-type alleles of Gene 2, Gene 3 and Gene 4 that encode the functional form of E2, E3 and E4 (Genotype: $E1^{m}E2^{WT}E3^{WT}E4^{WT}$). You want to introduce a wild- type copy of Gene 1 into the $E1^{m}E2^{WT}E3^{WT}E4^{WT}$ yeast auxotroph so that it becomes a prototroph.

a) Given below are five haploid yeast strains (#1- #5). ***Note:*** *"m" represents a loss-of-function mutation and "WT" represents the alleles associated with the wild-type/ functional enzymes .*

#1: $E1^{WT}E2^{m}E3^{WT}E4^{WT}$

#2: $E1^{m}E2^{m}E3^{WT}E4^{WT}$

#3: $E1^{WT}E2^{WT}E3^{m}E4^{WT}$

#4: $E1^{WT}E2^{WT}E3^{WT}E4^{m}$

#5: $E1^{WT}E2^{WT}E3^{WT}E4^{WT}$

i. Which of the above haploid yeast strains **require phenylalanine** in the growth medium for their survival and **why**? List **ALL** that apply.

Compared to Strain #5 (wild-type), all the remaining yeast strains (#1 - #4) have mutations in genes that encode the enzymes that catalyze a specific reaction step in the multi-step biochemical pathway for phenylalanine biosynthesis. So none of them (Strains #1 - #4) is able to synthesize phenylalanine. Hence all of them need phenylalanine in the growth medium for their survival. (0.5pts)

ii. From which of the above haploid yeast strains could you clone Gene 1 and subsequently use the cloned gene to transform an $E1^{m}E2^{WT}E3^{WT}E4^{WT}$ yeast auxotroph into a prototroph and **why**? List **ALL** that apply.

Any yeast strain that has the allele of gene 1 that encodes a functional, wild-type copy of Enzyme 1 can be used for this purpose. So you can use Strain 1, 3, 4 and 5 to transform strain #2 from an auxotroph to a prototroph that can synthesize phenylalanine. (0.5pts)

iii. Why is it not a good idea to use a diploid yeast for part **(ii)** above?

In a diploid yeast, the mutations that result in a recessive phenotype will NOT be identified since they will be masked by the wild-type allele that confers the dominant phenotype. (0.5pts)

Question 5 continued

You isolate genomic DNA from a yeast prototroph and digest it with HindIII restriction endonuclease. You create a **ligation mix** by cloning the HindIII digested yeast genomic DNA fragments into a plasmid vector that has the recognition sites for the restriction enzymes X, Y and Z. ***Note:*** *A slash (/) represents the cut site for the restriction enzyme.*

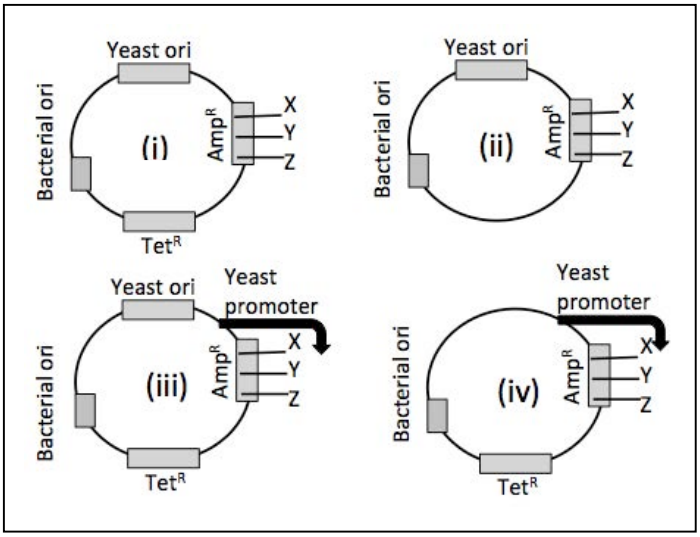
HindIII 5′A/AGCT T3′ 3′T TCGA/A5′
Enzyme X 5′C/AGCT A3′ 3′G TCGA/T5′
Enzyme Y 5′T AGCT/A3′ 3′A/TCGA T5′
Enzyme Z 5′A/AGTC T3′ 3′T TCAG/A5′

b) Which restriction enzyme (***Choose from X, Y and Z***) would you use to cut the plasmid to allow complementary 'sticky end' ligation with the HindIII digested genomic DNA fragments? Write the resulting **6-base pair sequences** at the **two points of ligation** of plasmid and the yeast genomic DNA fragments.

5' *CAGCTT* 3' 3' *GTCGAA* 5'	**Yeast genomic DNA fragment**	5' *AAGCTA* 3' 3' *TTCGAT* 5'

You would thus use enzyme X to cut the plasmid since it will generate ends that are complementary to the sequences at the two ends of HIndIII digested yeast genomic DNA fragments (0.25pts for enzyme and 0.25 for sequence)

You transform bacterial cells with the ligation mix to generate a yeast genomic library in bacteria. ***Note:*** *Amp^R and Tet^R represent the ampicillin and tetracyclin antibiotic resistance genes.*



c) Which plasmid(s) is best to use in order to make the yeast genomic library in bacteria and **why: (i)/ (ii)/ (iii)/ (iv)?**
(0.5pts) You can use plasmids (iii) or (i). Both have the bacterial and yeast ori and can therefore replicate in bacteria and yeast. They also has the Amp and Tet as the selection markers to identify the transformed clones and both have the cloning site for yeast genomic fragments. Although Plasmid (i) unlike (iii) does not have the yeast promoter,if you make the assumption that the cloned DNA sequence has its own inherent promoter it should be able to express the cloned gene just like (iii).

d) Explain how you would identify bacterial cells that have been transformed with **recombinant plasmids** containing yeast genomic DNA fragments. *(0.5pts)*
- Plate the ligation mix on a master plate that contains growth medium with NO antibiotics (Plate 1)
- Replica-plate the colonies from Plate 1 on Plate 2 that has Tetracyclins.
- Replica-plate the colonies from Plate 2 on Plate 3 that has ampicillin.
-Colonies that grow on Plate 2 but not Plate 3 are $Amp^S Tet^R$ and contain the yeast genomic DNA fragments

e) You successfully generate the yeast genomic library in bacteria. As a next step, you want to identify transformed bacterial clones that have a recombinant plasmid containing yeast Gene 1. **Briefly describe** an experiment that uses **hybridization** to identify this bacterial clone. ***Note:*** *You may assume that you already know the sequence of homologous gene from another yeast species. You will generate a DNA primer and radiolabel it with P^{32}. You will take an impression of all colonies on a nitrocellulose paper, break the cells open and incubate the paper with the P^{32}-labeled primer. You will incubate the paper with X ray film and develop it. The colony that lights up has the gene of your interest. (0.5pts)*

Question 5 continued

f) You grow the bacterial clone that you have successfully identified and isolate the recombinant plasmid containing yeast Gene 1 from them. As a final step you want to transform the $E1^{m}E2^{WT}E3^{WT}E4^{WT}$ yeast auxotroph with this recombinant plasmid. **Explain** how you can identify the $E1^{m}E2^{WT}E3^{WT}E4^{WT}$ yeast auxotrophs that have now been transformed to a yeast prototroph.

You would plate the transformed $E1^{m}E2^{WT}E3^{WT}E4^{WT}$ yeast auxotrophs on a plate that lacks phenylalanine. Any yeast cell that grows and forms colony on this plate is now a transformed prototroph that is capable of synthesizing phenylalanine and is therefore can survive and grow even in a growth medium that lacks phenylalanine. (0.25pts)

h) Would you be able to express a yeast gene in bacteria? **Why or why not?** ***Note:*** *Your explanations may vary. (0.25pts)*

Likely not.

-Yeast a eukaryote whereas bacterium is a prokaryote

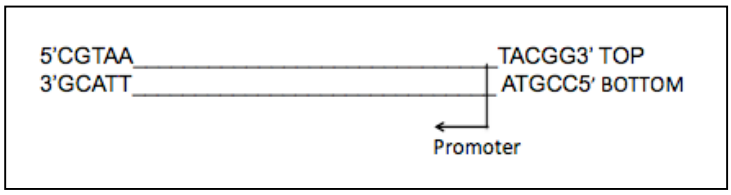
-Bacteria lack the splicing machinery. So the introns in yeast mRNA transcripts will not be spliced out resulting in non-functional proteins

-If the yeast protein is produced in bacterial cell, it is possible that will not undergo a proper post-translational modification in bacteria.

Question 6 ***(4pts)***

Phenylketonuria (PKU) is an inborn error of metabolism that is caused by the buildup of the amino acid phenylalanine in the body. It is often associated with the mutation in the *PAH* gene that encodes phenylalanine hydroxylase (PAH).

The following is the schematic of an allele of the *PAH* gene that is associated with PKU. The DNA sequence that flanks (at the two ends) this allele of the *PAH* gene is shown.



a) Based on the schematic of the *PAH* gene to the left, which strand is the template strand for transcription: **Top or Bottom**?

Top strand read 3'→5' with respect to the location of the gene promoter. (0.5pts)

b) You use the polymerase chain reaction (PCR) to amplify the allele of *PAH* gene that is associated with PKU. Design the primers (each 5 nucleotides long) that you would use to amplify both strands ***Note:*** *In real life primers are 20-25 bases long.*

I. **Forward Primer that extends to make the top strand: 5'*CGTAA*3'** *(0.25pts)*

II. **Reverse Primer that extends to make the bottom strand: 5'*CCGTA*3'** *(0.25pts)*

c) PCR is based on the principle of DNA replication. However, not all the enzymes and proteins in DNA replication are needed for PCR.

i. What special enzyme enables automated DNA replication of PCR?

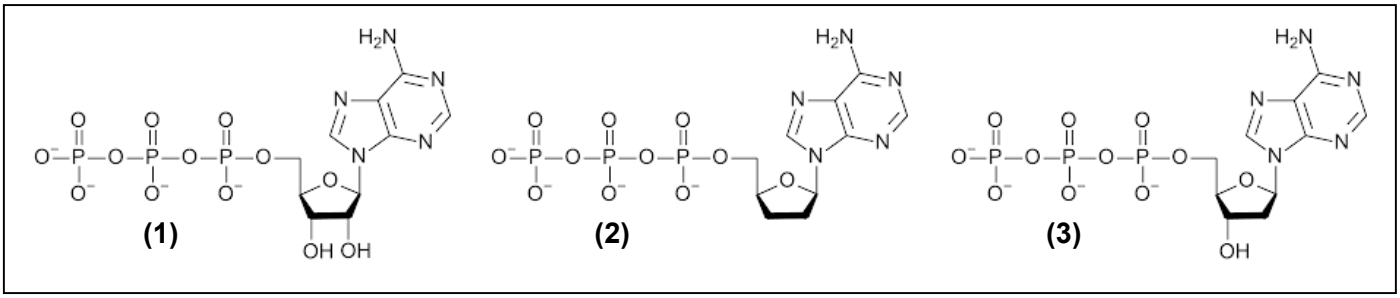
Taq DNA polymerase (0.25pts) since this is thermo stable

ii. What property of this enzyme is special and how does this facilitate the PCR?

The above enzyme is thermo stable at different temperature cycles of PCR i.e. the temperature at which the double stranded DNA denatures, temperature for primer annealing and temperature for elongating the 3' OH ends of the primers based on the sequence of the DNA templates. (0.25pts)

Question 6 continued

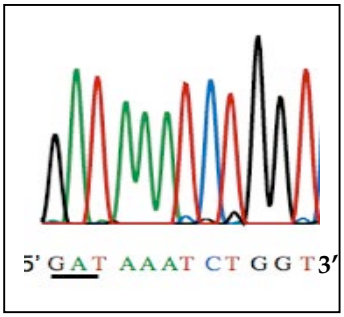
d) You decide to determine the complete nucleotide sequence of the mutant allele of *PAH* gene that accounts for a form of PKU.



i. Which of the above nucleotides are used in a DNA sequencing reaction: **1/** ***2/ 3***? *(0.25pts)*

ii. Which of the above nucleotide would be used to fluorescently tag DNA: **1/** ***2/*** **3**? *(0.25pts)*

e) Using fluorescent dideoxy sequencing, you derive the sequence of the **non-template DNA strand** of the wild-type allele of the *PAH* gene and get the following profile. ***Note****. A codon chart is provided on the last page and codon #40 is underlined.*

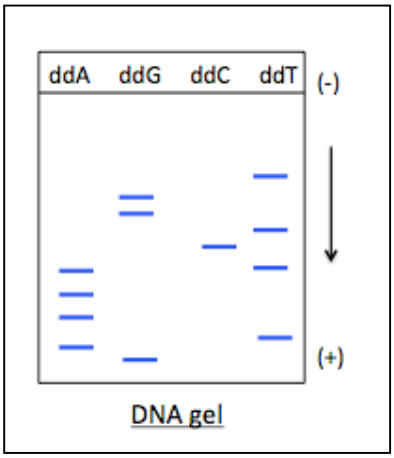


i. Write the corresponding mRNA sequence.
5'- *GAUAAAUCUGGU* -3' *(0.5pts)*

ii. Write the sequence of the template DNA strand.
5'- *ACCAGATTTATC* -3' *(0.5pts)*

iii. Write the sequence of the amino acids 40- 43: **N-*_Asp-Lys-Ser-Gly*-C**

Your friend sequences a completely different mutant allele of the *PAH* gene that also accounts for PKU by dideoxy-sequencing using radiolabeled nucleotides. She runs the products of each reaction on the following polyacrylamide gel. She finds that the mutant allele of *PAH* gene has an **A" base insertion after the codon** that is underlined in the schematic in **part (j).**



f) Draw the pattern of the bands she would obtain for the **non-template strand** for the mutant allele of *PAH* gene from the sequencing reaction.
Drawn to the left. (0.5pts)

g) What is the type of mutation in this mutant allele of *PAH* gene?
It is a point mutation that shifts the entire frame of the mRNA transcript (Frame shift mutation) (0.5pts)

7.016 Introductory Biology
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