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7.016 Problem Set 4- 2018

Question 1
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:** _____
ii. **P2:** _____
iii. **F1:** _____

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:** _____

ii. **Phenotypes** and **corresponding ratios:** _____

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.

Genotypes?	Corresponding phenotype?	Corresponding number?

ii. In the table above, circle the **recombinant (non-parental) F2 classes**:

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Question 2
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 tri-hybrid crosses would help you determine the chromosomal map? **Explain why** you selected this option as opposed to the others.

i. AABBDD x AABBDD

ii. aaBBDD x AABBdd

iii. aaBBdd X AAbbDD

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...

P1 fly: _____ **P2 fly: _____**

F1 fly: _____ **Fly that mated with F1 fly: _____**

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**.

  _____

- Which gametes in the first bullet point of part (ii) are produced by a **SINGLE** crossover event (SCO)? _____
- Which gametes in the first bullet point of part (ii) are produced by **NO** crossover event (NCO)? _____
- Which gametes in the first bullet point of part (ii) are produced by a **DOUBLE** crossover event (DCO)? _____

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 **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.*

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Question 3

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?

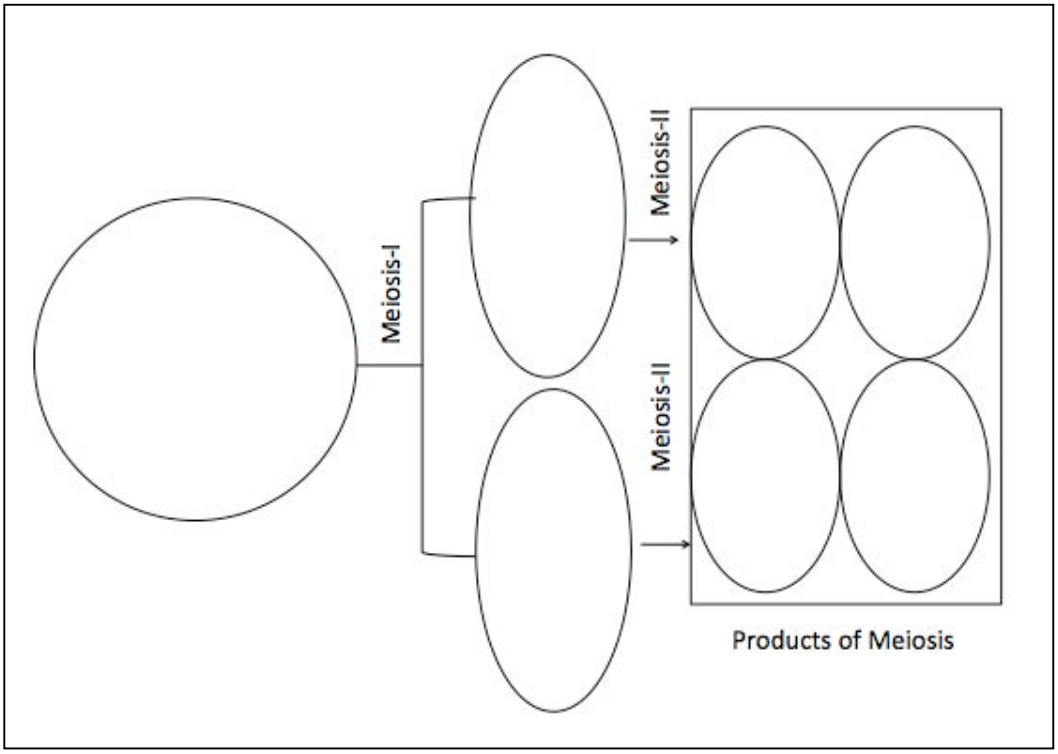
b) Based on what we have learned about yeast genetics….

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.

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

ii. **Explain** whether the two genes in part (i) above are linked or unlinked.

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.



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Question 4
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.

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?

ii. How does an (X^X)Y female differ from an XX female fly with respect to the X chromosome?

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).

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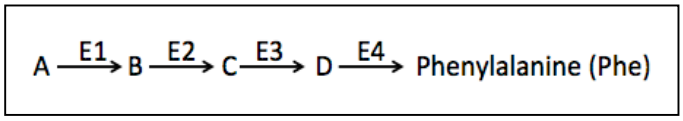
Question 5

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.

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. List **ALL** that apply and **explain** why you selected these yeast strains?

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

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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′

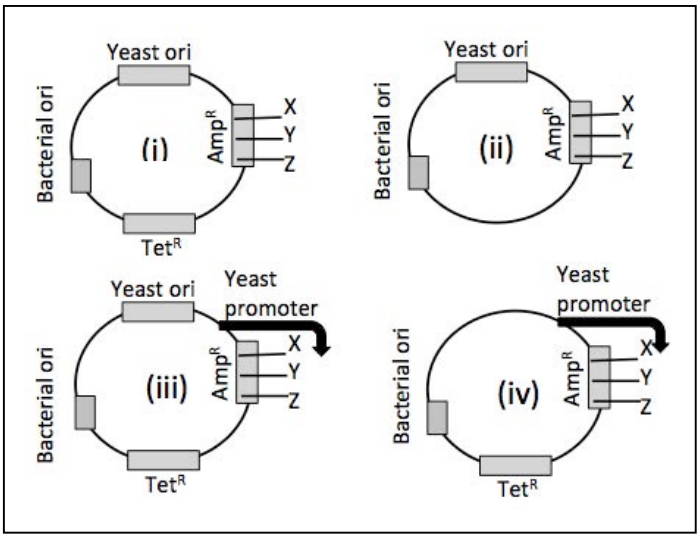
Enzyme Y
5′T AGCT/A3′
3′A/TCGA T5′

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'_____3' 3'_____5'	**Yeast genomic DNA fragment**	5'_____3' 3'_____5'

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)?**

d) Explain how you would identify bacterial cells that have been transformed with **recombinant plasmids** containing 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 a homologous gene from another yeast species.*

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Question 5 continued

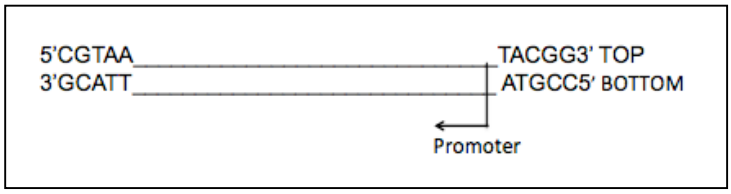
f) You grow the bacterial clone that you have successfully identified and isolate the recombinant plasmid containing yeast Gene 1 from them. As a final step you want to transform the $E1^{m}E2^{WT}E3^{WT}E4^{WT}$ yeast auxotroph with this recombinant plasmid. **Explain** how you can identify the $E1^{m}E2^{WT}E3^{WT}E4^{WT}$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

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

Question 6

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**?

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'_____3'**

II. **Reverse Primer that extends to make the bottom strand: 5'_____3'**

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?

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

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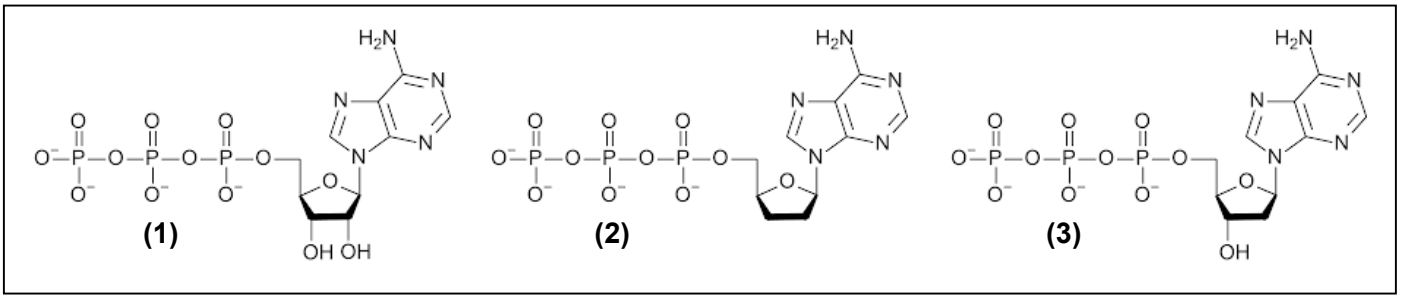
Question 6 continued

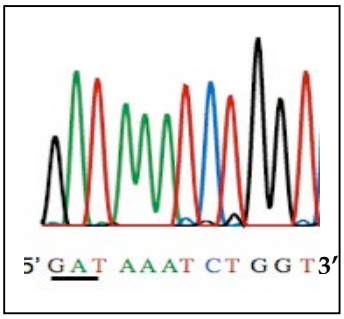
d) You decide to determine the complete nucleotide sequence of the mutant allele of *PAH* gene that accounts for a form of PKU.



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

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

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

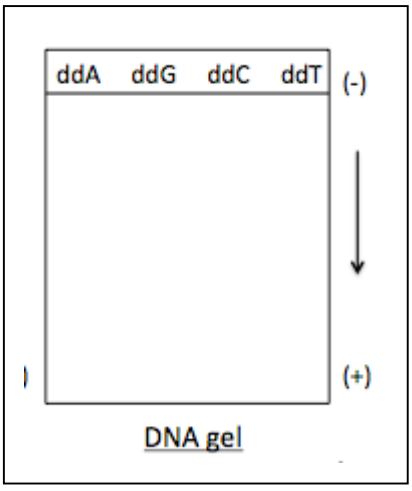


i. Write the corresponding mRNA sequence:
5'- _____ **-3'**

ii. Write the sequence of the template DNA strand:
5'- _____ **-3'**

iii. Write the sequence of the amino acids 40- 43: **N-**_____**-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.

g) What is the type of mutation in this mutant allele of *PAH* gene?

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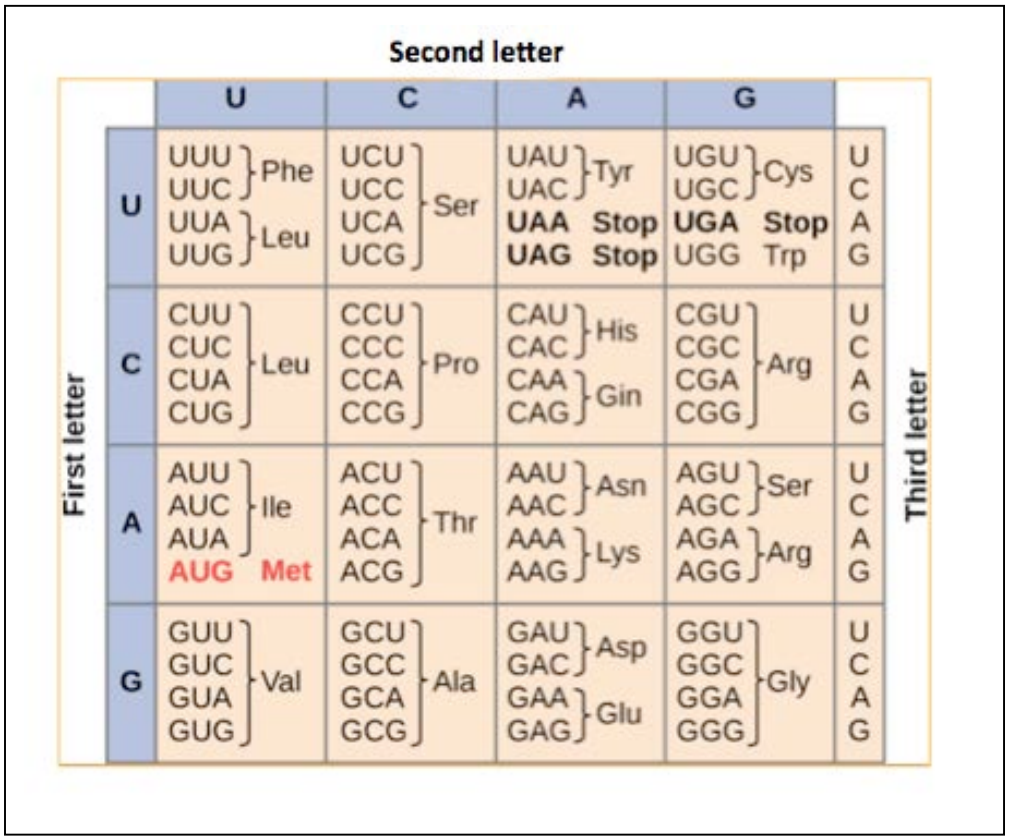
CODON CHART



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