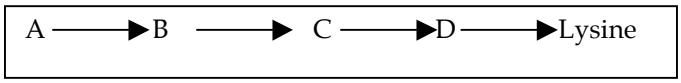
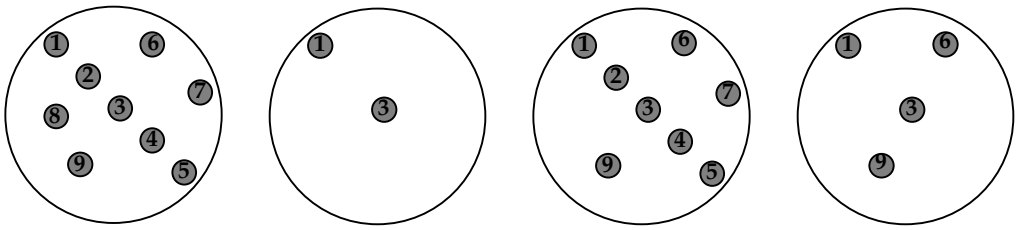
Solution key - 2010 7.012 Problem Set 3

Question 1

The following schematic represents the only pathway for the synthesis of the amino acid lysine in yeast cells. Each step in this pathway is catalyzed by a specific enzyme, which is encoded by a specific gene. This pathway begins with a hypothetical compound A and proceeds via three intermediates B, C and D to produce lysine.



A yeast cell that is missing any of genes encoding these four enzymes cannot synthesize lysine. A cell that cannot synthesize lysine cannot grow and form a colony on minimal media (media that does not have any added supplements) but can grow and form a colony on media that contains lysine. You do a mutant hunt to identify the enzymes that are involved in lysine biosynthesis. You start with a population of wild type yeast cells, mutagenize them using UV and allow the UV irradiated cells to grow and divide on a master plate containing rich medium (i.e. medium containing all essential compounds and added supplements). You then replica plate the cells from each colony on the master plate to the following plates containing minimal media or minimal media and the specified nutrients.



a) From these plates…

i) Identify the colony or colonies of wild-type cells. **Explain** why you selected these colonies.

Colonies #1 and 3 are composed of the wild-type cells since they can grow on minimal medium plates.

ii) Assume that each colony is composed of cells that carry a single mutation. Identify the colony or colonies of cells that cannot synthesize lysine. **Explain** why you selected these colonies.

Colonies #2, #4, #5, #6, #7 and #9 are the colonies of the cells that do not grow on minimal media, but can grow on minimal media with added lysine.

iii) Identify the colony or colonies of cells that carry a mutation in a gene that codes for a protein involved in biosynthetic pathway different from the lysine biosynthetic pathway. **Explain** why you selected this colony or colonies.

Colony #8. These cells are auxotrophs since they cannot grow on minimal. Since they also cannot grow on minimal with lysine, they must be missing a gene important for some other pathway,

iv) Identify the colony or colonies of cells that carry a mutation in either the gene that encodes the enzyme that converts A → B or the gene that encodes the enzyme that converts B → C. **Explain** why you selected this colony or colonies.

Colonies #6 and #9. These cells cannot make lysine (compare plate 1 and 2), so they are clearly carrying a mutation in one of the enzymes needed to make lysine. Because they can grow on plates with minimal + intermediate C, we know that these cell can take intermediate C and make lysine. This indicates that the final two steps in the pathway are intact, which means that either the enzyme that converts A → B or the enzyme that converts B → C is missing.

Question 1, continued
By repeating the mutagenesis experiment you isolate 10 mutants that cannot synthesize lysine. You then perform a complementation test on these mutants.

b) What type of yeast cells would you begin with (haploid / diploid) for the complementation test? **Explain** why you selected this option.
You would choose haploid cells so when a cell obtains a mutation resulting in a recessive phenotype, the phenotype can be easily recognized, i.e., the phenotype can not be masked by the wild-type allele. When two haploid cells that each have a mutation in the same gene are fused to form a diploid, the resulting diploid will show the phenotype (failure to grow without added lysine). Fusion of two haploid cells that have a mutation in different genes, will result in diploid that is normal.

Your complementation test gives you four complementation groups: group 1 contains mutants m1, m6, m7 and m9, group 2 contains mutants m3, m5 and m8, group 3 contains mutants m4 and m10 and group 4 contains mutant m2.

c) Based on this observation fill in the following complementation table for mutants m1-m10.
Note: Use"+" to indicate growth of the yeast cell in minimal medium and "-" to indicate that yeast cell fails to grow on minimal medium.

	m1	m2	m3	m4	m5	m6	m7	m8	m9	m10
m1	-	+	+	+	+	-	-	+	-	+
m2	+	-	+	+	+	+	+	+	+	+
m3	+	+	-	+	-	+	+	-	+	+
m4	+	+	+	-	+	+	+	+	+	-
m5	+	+	-	+	-	+	+	-	+	+
m6	-	+	+	+	+	-	-	+	-	+
m7	-	+	-	+	+	-	-	+	-	+
m8	+	+	+	+	-	+	+	-	+	+
m9	-	+	+	+	+	-	-	+	-	+
m10	+	+	+	-	+	+	+	+	+	-

d) You next characterize the following mutants based upon the type of intermediates it accumulates.
- Mutant m1 accumulates compound A
- Mutant m4 accumulates intermediate C
- Mutant m3 accumulates intermediate B
- Mutant m2 accumulates intermediate D

Based on the above data, which step in the pathway is affected in each of the mutants? Choose from: A → B, B → C, C → D, or D → lysine
- Step affected in m1: A → B
- Step affected in m4: C → D
- Step affected in m3: B → C
- Step affected in m2: D → Lysine

Having identified the genes whose expression is important for the synthesis of lysine you decide to do an epistasis test.

e) Describe how an epistasis test differs from a complementation test. Also state the information that is exclusively provided by the epistasis test and **NOT** by the complementation test.
In an epistasis test, a double mutant cell (it could be a haploid cell with the genotype ab, or a diploid cell with the genotype aabb) is created and the phenotype of that double mutant is evaluated. This can help to determine the order the genes in a pathway. In a complementation test, a heterozygote cell is formed from two mutants and evaluated to determine if the recessive phenotype persists. If it does, the two mutations are in the same gene. If it does not, then the two mutations are in different genes.

Question 1, continued

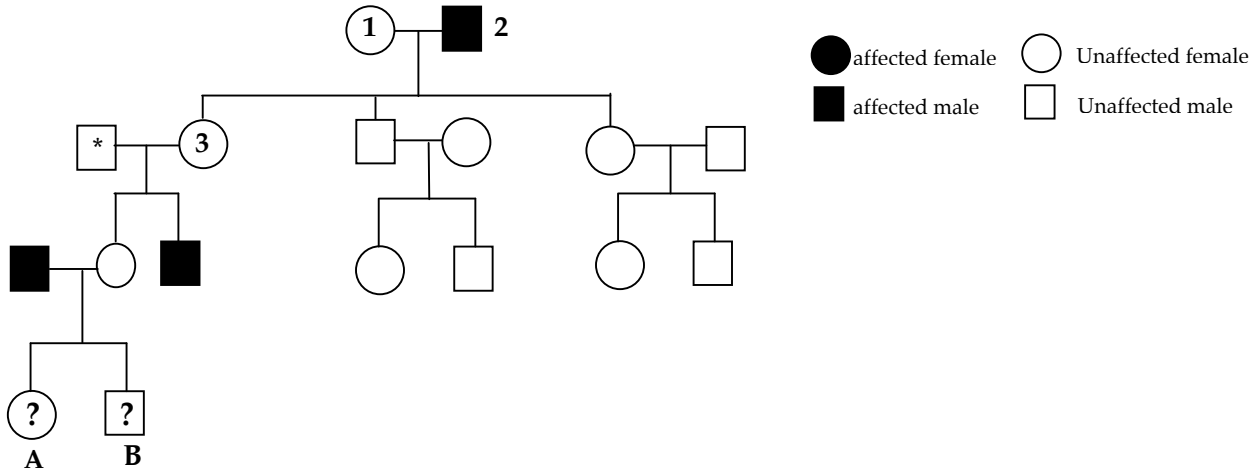
f) Predict the phenotype of each of the following:

- A haploid cell that carries both m1 and m4 accumulates __A__ and can grow on media supplemented with __D and lysine__.
  For each blank choose from: compound A, intermediate B, intermediate C, intermediate D, or lysine

- A haploid cell that carries both m2 and m4 accumulates __C__ and can grow on media supplemented with __lysine.__
  For each blank choose from: compound A, intermediate B, intermediate C, intermediate D, or lysine

Question 2

You are analyzing the following human pedigree.
Assume that the individual marked with an asterisk (*) does not carry any allele associated with the affected phenotype and that no other mutation spontaneously occurs. Also assume complete penetrance. Use the letter "R" for the allele associated with the dominant phenotype, "r" for the allele associated with the recessive phenotype.



i) What is the **most likely** mode of inheritance of this disease? Choose from:
autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive.

X linked recessive

ii) List **all possible** genotypes of the following individuals in the pedigree.

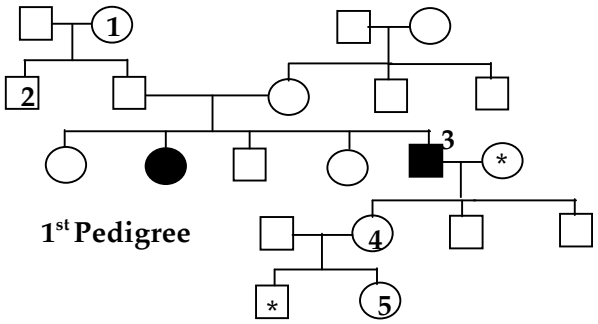
Individuals	Genotypes
#1	$X^R X^R$
#3	$X^R X^r$

iii) What is the probability of **Individual A** being **affected**? **Show** your work.
The father of Individual A has the genotype X^rY. Individual A is a female so she will inherit the X^r from her father. The probability that Individual A's mother is a carrier (X^RX^r) is ½ since female #3 is a carrier (#3 has an affected son). If Individual A's mother is a carrier (X^RX^r) then the probability that Individual A will inherit a X^r from her mother is ½. The combined probability that that Individual A will inherit a X^r i is ½ x ½, or ¼ .

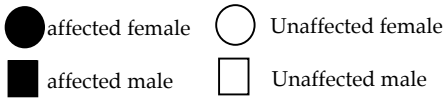
iv) What is the probability of **Individual B** being **affected**? **Show** your work.
Because individual B is male, he will inherit the Y chromosome form his dad. Thus the probability of Individual B being affected is the same as the probability of Individual B inheriting the X^r from his mother. Given the explanation above, this is ¼.

Question 3

The following human pedigree shows a family affected by a specific disease.
Assume that the individuals marked with an asterisk (*) do not carry any allele associated with the affected phenotype and that no other mutation spontaneously occurs. Also assume complete penetrance



1st Pedigree



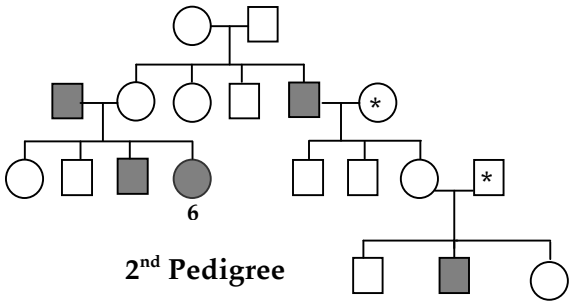
a) State the **most likely** mode of inheritance for this disease. Choose from: autosomal dominant, autosomal recessive, X-linked dominant, X-linked recessive. *autosomal recessive*

b) Write **all possible** genotypes of the following individuals in the pedigree. *Use the uppercase "A" for the allele associated with the dominant phenotype and lowercase "a" for the allele associated with the recessive phenotype.*

Individuals	All possible Genotypes
#1	*AA or Aa*
#2	*AA or Aa*
#4	*Aa*

c) What is the probability that Individual 5 will be a carrier?
Individual 4 is a carrier since she gets a disease allele from her father. Individual 4 marries a person who is AA (this was given in the prompt). Therefore the chance that Individual 5 is a carrier is ½.

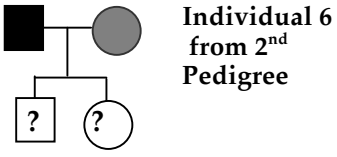
d) The following human pedigree shows a family affected by **a different disease**. *Assume that the individuals marked with an asterisk (*) do not carry any allele associated with the affected phenotype and that no other mutation spontaneously occurs. Also assume complete penetrance*



2nd Pedigree

Note: Use the notation such as "R or X^R" for the allele associated with the dominant phenotype and "r or X^R for the allele associated with the recessive phenotype.

Individual 3 from the **1st pedigree** has a second marriage with Individual 6 from the **2nd pedigree**. They have a son and a daughter as shown below.



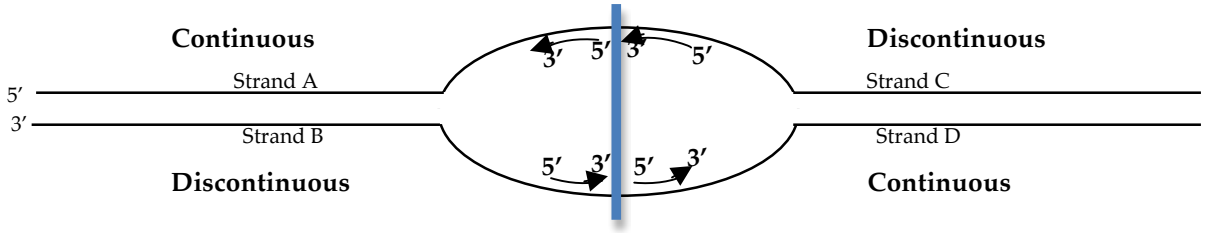
i) What would be the **genotype** of their **son** for the two disease genes? *Aa X^rY*

ii) What would be the **genotype** of their **daughter** for the two disease genes? *Aa X^RX^r*

Question 4

a) In the space below is drawn a schematic of an origin of replication.

- Label the 5′ and the 3′ ends of the primers.
- Extend each primer in the direction of replication.
- Indicate how each of the four strands (strand A, B C, and D) is copied, *i.e.*, in a continuous or discontinuous manner.



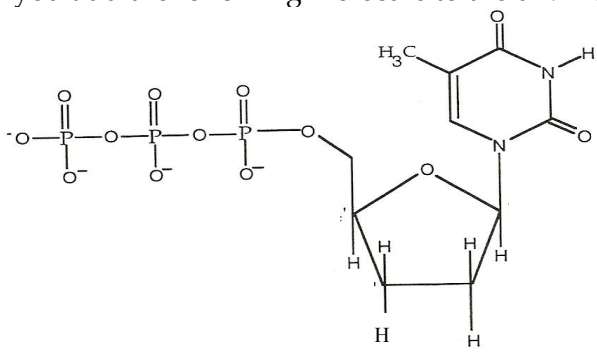
b) Below is the partial sequence of a bacterial gene.ORI

5′AATCGACC3′

i. Using this strand as the template, write the sequence of the complementary strand and label its 5′ and 3′ ends.

3′ TTAGCTGG 5′

ii. Assume that you have an *in vitro* DNA replication system. During one of your experiments, you add the following molecule to the dNTPs mixture.



- Could this molecule be added to the **growing end** of the replicating DNA strand (**Yes/No**)? **Explain** why you selected this option.

Yes, since it has the phosphate group at the 5′C of the ribose that it needs to form a covalent phosphodiester bond with the growing 3′OH end of a DNA strand.

- Could this molecule form a **covalent bond** with an **incoming dNTP** (**Yes/No**)? **Explain** why you selected this option.

No, since it lacks the -OH group at the 3′C of the ribose that it needs to form a covalent phosphodiester bond with the growing phosphate group of the incoming base.

d) You are studying replication in two different bacterial cells- Type I and Type II. All the growth conditions for replication in the Type I and Type II cells are the same. However, the replication error rate in Type I is 1000 fold higher than Type II. List what activity of DNA polymerase could be altered to explain this difference and briefly explain how this activity normally functions to increase fidelity.

Type II bacterial cell, unlike Type I, has a DNA polymerase that lacks the 3′→5′ exonuclease activity that results in decreased replication fidelity and increased error rate. The 3′→5′ exonuclease activity removes the incorrectly matched base which allows the polymerase to insert the correct one.