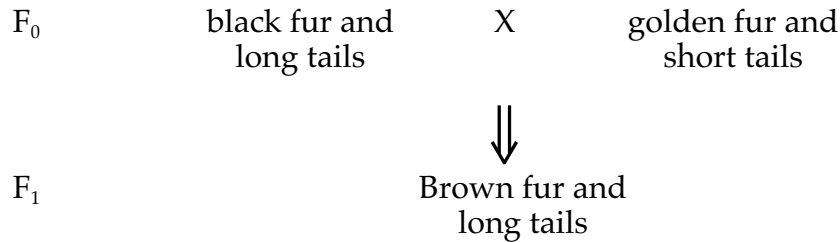


# Solutions to 7.012 Problem Set 3

## Question 1

You have two pure-breeding colony of mice. Colony I mice have black fur and long tails, Colony II mice have golden fur and short tails. You cross mice from each of these colonies.



a) Predict genotypes for the mice shown in the cross above. Define your notation.

- i) black fur and long tails:  $BB LL$
- ii) golden fur and short tails:  $B'B' ll$
- iii) Brown fur and long tails:  $BB' Ll$

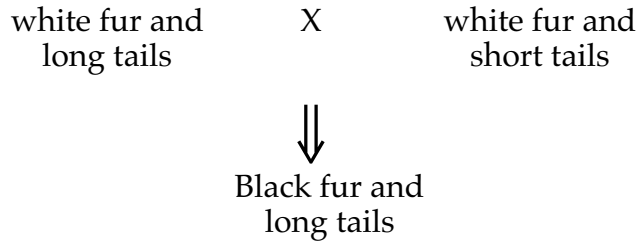
b) You mate a male and a female from the  $F_1$  generation. Given your prediction in (a), what genotypes and phenotypes do you see in the  $F_2$  generation, and in what ratios?

$BB' Ll \times BB' Ll$

	BL	Bl	B'L	B'l
BL	$BBLL$ <i>black fur and long tails</i>	$BBLl$ <i>black fur and long tails</i>	$BB'LL$ <i>brown fur and long tails</i>	$BB'Ll$ <i>brown fur and long tails</i>
Bl	$BBLl$ <i>black fur and long tails</i>	$BBll$ <i>black fur and short tails</i>	$BB'Ll$ <i>brown fur and long tails</i>	$BB' ll$ <i>brown fur and short tails</i>
B'L	$BB'LL$ <i>brown fur and long tails</i>	$BB'Ll$ <i>brown fur and long tails</i>	$B'B'LL$ <i>golden fur and long tails</i>	$B'B'Ll$ <i>golden fur and long tails</i>
B'l	$BB'Ll$ <i>brown fur and long tails</i>	$BB' ll$ <i>brown fur and short tails</i>	$B'B'Ll$ <i>golden fur and long tails</i>	$B'B' ll$ <i>golden fur and short tails</i>

## Question 1, continued

Your colleague has two other colonies of pure-breeding mice. Colony III mice have white fur and long tails, Colony IV mice have white fur and short tails. You cross mice from each of these colonies.



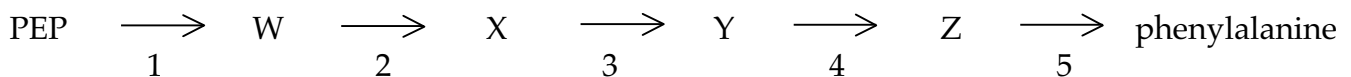
c) How can you explain this result?

*There are mutations in two different genes, F and W that result in the same recessive phenotype, white fur. The cross could be:*

*ffWWLL (white fur with long tail) X FFwwll (white fur with short tail) to give:*  
*fFWwLl = black fur with long tails*

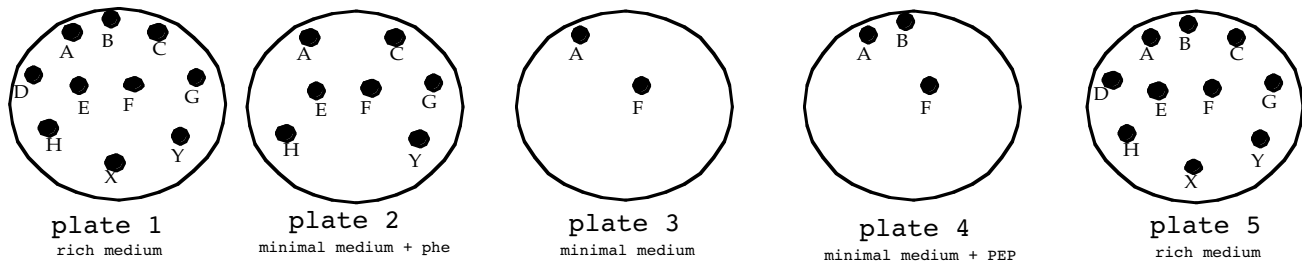
## Question 2

In a hypothetical type of yeast, the biosynthetic pathway for phenylalanine (shown below) has five reaction steps each catalyzed by a different enzyme (1 – 5). The pathway is known to begin with phosphoenolpyruvate (PEP) and proceed via 4 intermediates W, X, Y, and Z to phenylalanine.



You want to identify the enzymes (five total) involved in this pathway by isolating yeast that fail to synthesize phenylalanine (these yeast are referred to as “phe<sup>-</sup>”). You know that mutant yeast that fail to synthesize phenylalanine (and thus can not grow without addition of phenylalanine) are likely to be defective in one of the enzymes involved in the phenylalanine synthesis pathway. You start with a population of wild-type (“phe<sup>+</sup>”) yeast, mutagenize it with UV light, and allow the treated cells to grow into isolated colonies on plate 1 (see FIGURE 1). You then use the replica plating technique to transfer some yeast from each colony onto plates 2, 3, 4, and 5. The contents of the growth medium are listed below each plate. Rich medium contains all nutrients and allows growth of both wild type, and mutant cells. Minimal medium contains nutrients sufficient to allow only wild-type, prototrophic yeast to grow. It will not support the growth of auxotrophic cells.

FIGURE 1:



a) Plate 1 is the original rich medium plate that the mutagenized cells were grown on. It is standard practice for the last plate in a series of replica plates to have the same growth medium as the original plate. What purpose might this serve?

*It is a control. You want to make sure that cells from each colony were transferred to all previous plates so you can assess growth versus no growth.*

b) You obtain some  $\text{phe}^-$  colonies in this manner. Identify the colonies from FIGURE 1 that are  $\text{phe}^-$   
*C, G, E, H, Y*

c) You notice that some colonies do not grow on plates containing minimal medium + phenylalanine (plate 2). Identify these colonies and give one possible explanation for the growth behavior of these colonies.

*Cells from colonies B, D, and X may have a mutation in a gene that is important for the synthesis of any compound other than phenylalanine.*

d) This strategy for isolating  $\text{phe}^-$  mutants works well so you repeat the mutagenesis and replica plating experiment to isolate more  $\text{phe}^-$  mutants. You then perform a complementation test on these  $\text{phe}^-$  mutants.

i) What is a complementation test?

*A complementation test consists of creating a diploid that carries two independently isolated mutations and examining if the diploid has a mutant or a wildtype phenotype.*

*If the diploid has a wildtype phenotype, then one assumes that the two mutations are in different genes. If the diploid has a mutant phenotype, then one assumes that the mutations are in the same gene.*

ii) What is the purpose of a complementation test?

*A complementation test can identify how many different genes are represented in a group of mutants.*

## Question 2, continued

In the table below, a (+) indicates that the diploid created grows on minimal media, a (-) indicates that the diploid fails to grow on minimal media.

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

ii) Assign the mutants 1-10 into complementation groups.

*m1, m2, m7*

*m3*

*m4, m10*

*m5, m9*

*m6, m8*

e) You also characterize your mutants based upon which of the intermediates accumulates

m2: accumulates X

m3: accumulates Z

m4: accumulates PEP

m5: accumulates Y

m6: accumulates W

Based on the above data, you predict that

i) m1 has a mutation in gene   3  

ii) m2 has a mutation in gene   3  

iii) m3 has a mutation in gene   5  

iv) m4 has a mutation in gene   1  

v) m5 has a mutation in gene   4

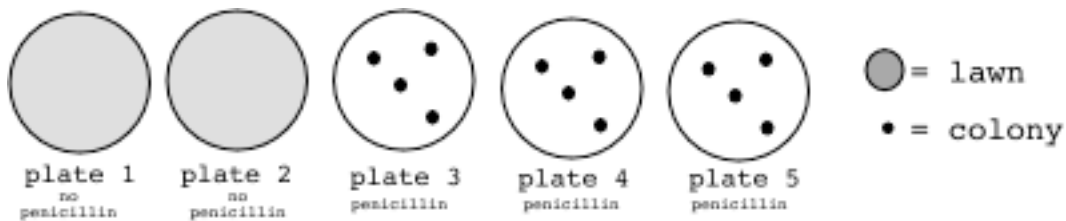
### Question 3

Replica plating has been used to address profoundly important questions in bacterial genetics. For example, in the 1940's there was much debate regarding the issue of whether or not mutants pre-exist in a population of bacteria. Researchers observed that when they inoculated wild type ( $\text{pen}^S$ ) bacteria onto growth medium containing penicillin, and thus selected for bacteria that had mutated to become penicillin resistant, a small fraction ( $\sim 10^{-6}$ ) of cells would always grow. Thus,  $\text{pen}^R$  colonies had arisen from a  $\text{pen}^S$  population. There were two models for this:

**Model A: "Directed Mutations"** One group of researchers argued that these mutants originated as a result of the selective pressure. Their line of reasoning was that the bacteria can sense the need to grow on penicillin and that a small fraction of them successfully mutate in a directed manner so that they become  $\text{pen}^R$ .

**Model B: "Pre-existing Mutations"** A second group of researchers argued that  $\text{pen}^R$  mutants pre-existed within the wild type population before ever coming into contact with penicillin; thus, (they argued) penicillin doesn't direct mutations, it simply reveals mutants.

Replica plating provided a rapid means for testing these two hypotheses. The following is a simplified version of the experiment. Plate 1 contains a "lawn" of cells (a solid layer of cells packed together), all of which are the offspring of a single, wild type cell. About  $5 \times 10^6$  cells were spread on a plate, and after a day of growth, they formed a lawn containing about  $10^9$  cells. Plate 1 was used as the master plate that was replicated onto plates 2, 3, 4, and 5.



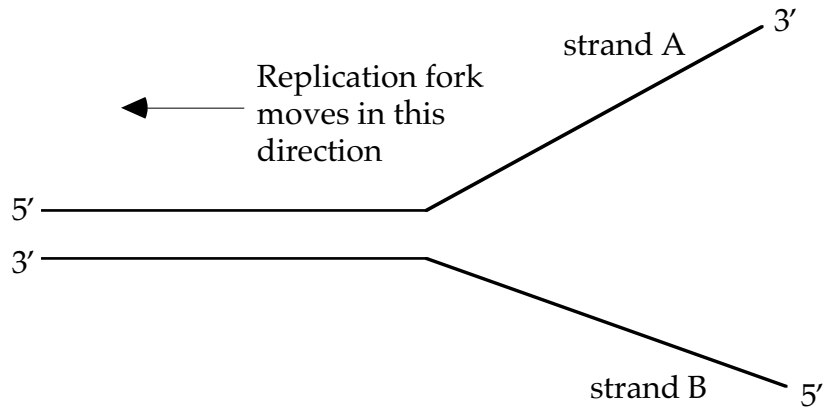
The distribution of colonies on plates 3, 4, and 5 is identical.

a) Which of the two hypotheses (directed mutations or pre-existing mutations) does this result more strongly support. Explain your reasoning.

*These data support Model B, the pre-existing mutations model. Each  $\text{pen}^R$  colony on plate 3 is also represented on plates 4 and 5, suggesting that these  $\text{pen}^R$  colonies already existed on plate 1. If the mutations were arising anew upon exposure to penicillin, you would expect different patterns of  $\text{pen}^R$  colonies on plates 3, 4, and 5.*

## Question 4

Shown below is a diagram of a replication fork in a double-stranded (ds) DNA molecule found in a prokaryotic cell. Each DNA strand (A and B) serves as a template for polymerization by DNA polymerase, resulting in the formation of a newly synthesized "daughter" DNA strand.



a)

i) On which template strand (A or B) would there be **continuous** replication by DNA polymerase? What is this newly synthesized daughter strand called during DNA replication?

*Strand A will be copied in a continuous fashion. The daughter strand will be the leading strand.*

ii) On which template strand (A or B) would there be **discontinuous** replication by DNA polymerase? What is this newly synthesized daughter strand called during DNA replication?

*Strand B will be copied in a discontinuous fashion. The daughter strand will be the lagging strand.*

iii) Chemicals that inhibit the enzyme DNA ligase will primarily affect synthesis on one of the two template strands (A or B). Explain on which template strand (A or B) polymerization will be primarily affected and why this occurs.

*Synthesis of the lagging strand using strand B as a template will be affected most by inhibition of DNA ligase. Without DNA ligase, the Okazaki fragment can not be joined together to form a contiguous length of DNA.*

b) There are inaccuracies in the DNA molecule shown below.



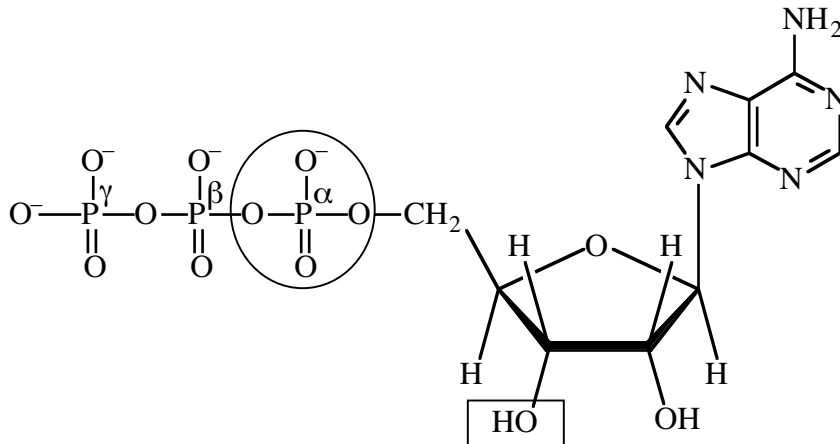
i) Name three things that are wrong in the above DNA sequence.

- The strands are not anti-parallel
- In DNA there should not be any U's
- G would not base-pair with T

ii) What type of chemical interaction is indicated by a " | " in the above diagram? What happens to these interactions during DNA replication?

*This line indicates hydrogen bonding between the two complementary bases. It would represent 2 hydrogen bonds between A and T or 3 hydrogen bonds between G and C. These bonds are disrupted to allow replication of the DNA and new bonds form with the newly synthesized strands.*

c) Shown below is the structure of a monomer used in nucleic acid synthesis.



i) Would this monomer be used to form part of an RNA strand or a DNA strand?

Briefly explain your answer.

*RNA because it has a 2' OH*

ii) In nucleic acid synthesis, one of the phosphate groups of this monomer is covalently linked to a new strand that is being elongated. In the figure above, draw a circle around the phosphate group that would be linked.

iii) When this monomer is already part of the newly synthesized strand, one of the hydroxyl groups of this monomer will be linked to the phosphate group of the next monomer added. In the figure above, draw a box around the hydroxyl group that will be linked.