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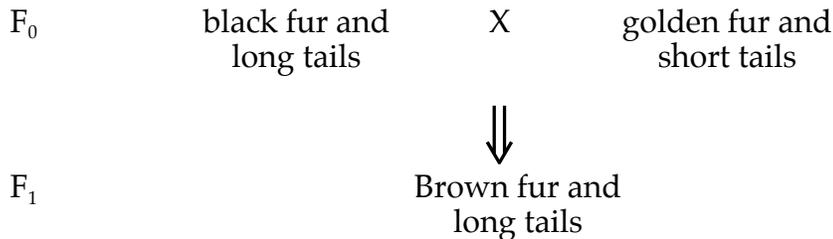
Section: _____

7.012 Problem Set 3

Please print out this problem set and record your answers on the printed copy. Answers to this problem set are to be turned in at the box outside by 4:10 Wednesday, October 1. Problem sets will not be accepted late. Solutions will be posted on the web October 2, 2003.

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.

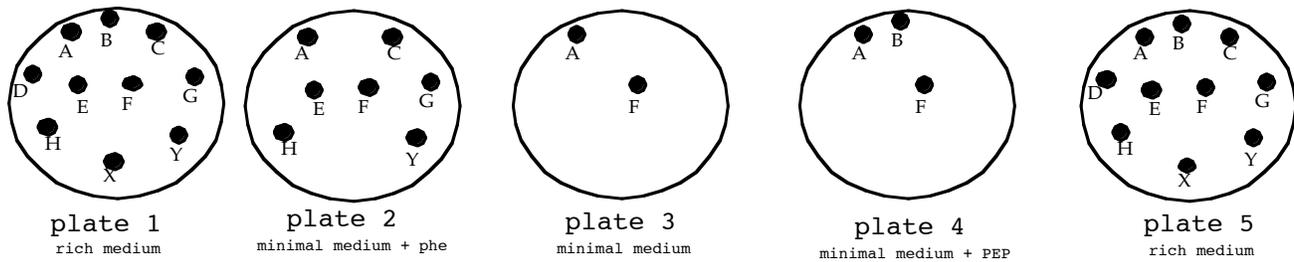


a) Predict genotypes for the mice shown in the cross above. Define your notation.

- i) black fur and long tails:
- ii) golden fur and short tails:
- iii) Brown fur and long tails:

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?

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?

b) You obtain some phe^- colonies in this manner. Identify the colonies from FIGURE 1 that are phe^-

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.

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

i) What is a complementation test?

ii) What is the purpose of a complementation test?

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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.

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 _____

ii) m2 has a mutation in gene _____

iii) m3 has a mutation in gene _____

iv) m4 has a mutation in gene _____

v) m5 has a mutation in gene _____

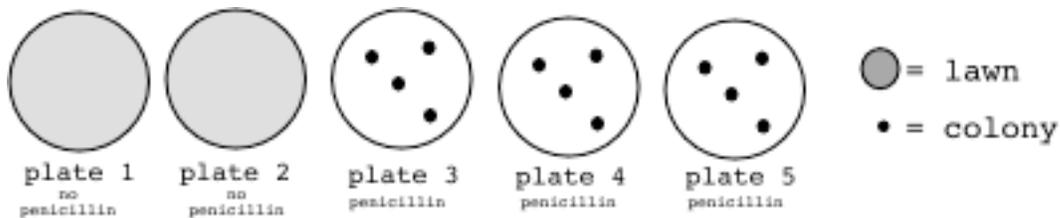
Question 3

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 (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, pen^R colonies had arisen from a 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 pen^R .

Model B: "Pre-existing Mutations" A second group of researchers argued that 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 5×10^6 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.

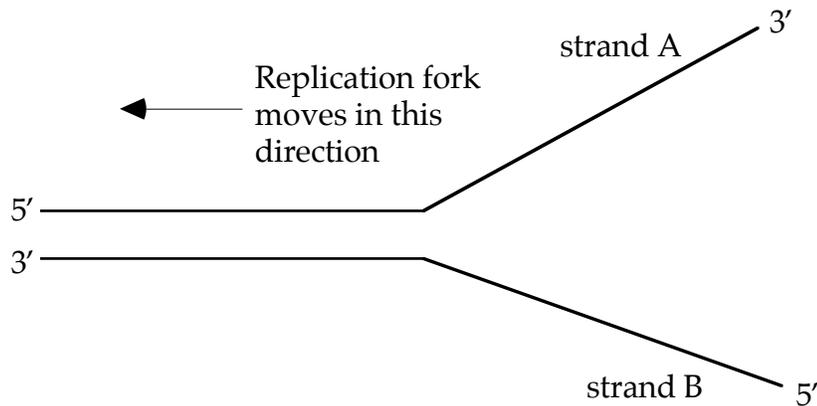


The distribution of colonies on plates 3, 4, and 5 is identical.

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

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.



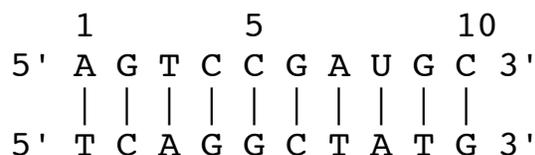
a)

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?

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?

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.

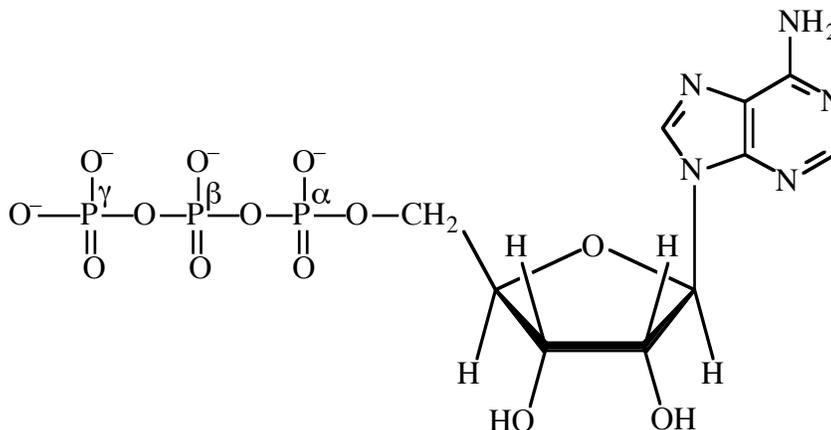
b) There are inaccuracies in the DNA molecule shown below.



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

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

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.

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.