Bio 213 GENETICS (Fall 2000)
Problem Set 3

DNA Replication

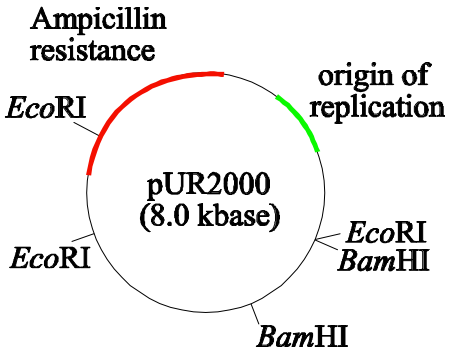
3.1. Examine Fig. 11-9. How would life change if evolution had given us nucleotides phosphorylated on their 3' ends rather their 5' ends?

*3.2. A linear piece of DNA has just finished replicating bidirectionally from an origin more or less in the center of the molecule. Draw a picture of the final result of replication that proceeded in a mutant cell that does not have the enzyme DNA ligase. Be sure to label the ends of all strands (both new and old).

Plasmids and Cloning

3.3. *The State of Virginia vs. T.J. (Tomato Juice) Thompson*

You, a University of Richmond security officer, spot a student parking in a faculty / staff only zone. Seeing you, he jumps back into his car and tears out onto Westhampton Way. You give chase, apprehending the suspect just outside King's Dominion. The suspect denies any wrongdoing, and so you return to the scene of the crime to seek corroborating evidence. At the parking place you find the tip of an index finger, evidently sliced off when the suspect slammed the door in his haste to escape. The suspect is missing the tip of his index finger (he says he cut himself shaving).



You, not only a security officer but also an expert molecular geneticist, isolate DNA from the index finger and from the suspect's stump. Your first thought is to clone some DNA from each sample. Accordingly, you cut both DNA samples with the restriction enzyme *Bgl* II and ligate it to the plasmid shown to the right, cut with *Bam*HI. The two restriction enzymes cut as shown below.

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Bam HI ...G|GATCC...          ...G          GATCC...
       ...CCTAG|G...          ...CCTAG          G...

Bgl II ...A|GATCT...   ------>  ...A        GATCT...
       ...TCTAG|A...   ------>  ...TCTAG         A...
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3.3a. How many fragments were generated by the digestion of the plasmid with *Bam*HI?

3.3b. What is the approximate size of the DNA fragment of pUR2000 that you used for cloning?

3.3c. Why didn't you use *Eco*RI to clone the DNA?

You sequence a bit of a particular clone, taken from finger DNA and stump DNA. Note that the two sequences to the right are identical. You take this result to the suspect and demand a confession, but T.J., no mean geneticist himself, points out that you may have just sequenced pUR2000 DNA, not finger/stump DNA, so of course the two sequences are identical.

Sequenced DNA

Stump: GGTTATAGGAGCATTCTGGATCTTAATG
Finger: GGTTATAGGAGCATTCTGGATCTTAATG

3.3d. Is he right? Circle the part of the sequence that makes you think so or think not. Label the DNA as plamid-derived (**P**) or finger-derived (**F**) and explain your reasoning.

Gene-protein relationships

3.4. By analogy with alkaptonuria, what phenotype would you expect from phenylketonuria (PKU)? If a person with PKU can be identified shortly after birth, there is an effective treatment: a special diet with low levels of phenylalanine. Why does this diet prevent the effects of PKU?

3.5. Suppose that people heterozygous for alkaptonuria have half the amount of homogentisic acid oxidase. Would you predict that such a person would have gray urine? To answer this question, think about the capabilities of individual cells.

3.6. In the experiment performed by Beadle-Tatum and described in the text, what results would you have expected if the one-gene-many-enzyme hypothesis were true? What would have been the results if the many-gene-many-enzyme hypothesis were true?

3.7. Suppose that people heterozygous for alkaptonuria have half the amount of homogentisic acid oxidase. Would you predict that such a person would have gray urine?

*3.8. Before George Beadle teamed up with Edward Tatum, he worked with Boris Ephrusi on a remarkable experiment regarding the nature of genes and proteins. They worked with the fruit fly, *Drosophila melanogaster*, which normally have bright red eyes (you'll soon learn to recognize them better than your own). They took the embryonic eye (or imaginal disk) from a developing *Drosophila* larva and then -- this is going to sound weird -- put it in the abdomen of another larva. With the appropriate lighting strikes at midnight, the larva grew up into an adult fly that happened to have an extra eye in his belly.

To make it interesting, they tried imaginal disks from mutant flies that would be expected to develop eyes of mutant color -- cinnabar or vermillion. When transferred to a different fly, would the disks develop color according to their own genetic endowment or that of their adopted body?

The results are shown on the next page.

3.8a. Formulate a hypothesis to explain the first four results.

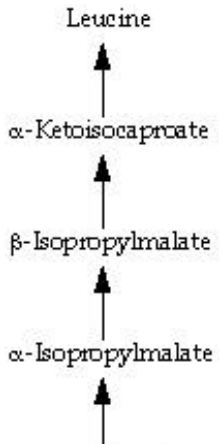
3.8b. Formulate a hypothesis to explain the first five results.

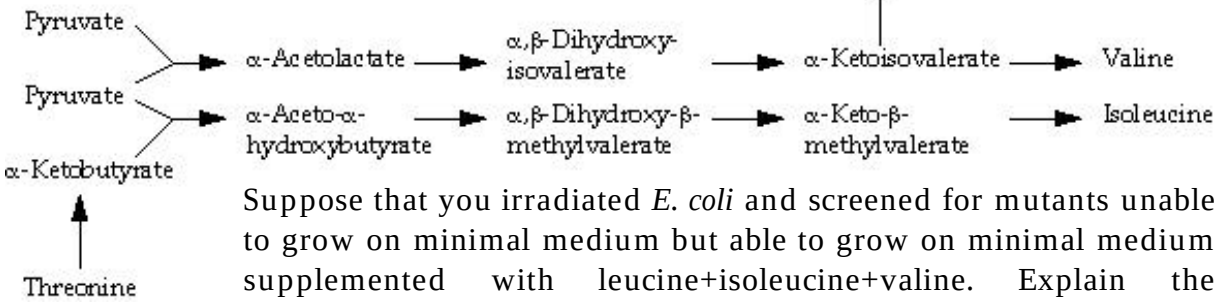
3.8c. Formulate a hypothesis to explain all of the results. This one is tough. A major hint is available on the web. Go to the Problem Sets page and click on *Hint for problem 3.8.*

Results of eye transplant experiments

Genotypes of flies		Ultimate color of transplanted eye
Eye donor	**Recipient**	
wt	vermillion	wt
vermillion	wt	wt
wt	cinnabar	wt
cinnabar	wt	wt
vermillion	cinnabar	wt
cinnabar	vermillion	cinnabar

*3.9. Look at the structures of leucine, isoleucine, and valine. They're awfully similar, no? You shouldn't be surprised to learn that the pathway cells use to synthesize the three branched chain amino acids are also very similar. The pathway is shown below and to the right. Note that leucine and valine follow the same pathway up until a-ketoisovalerate and that the biosynthesis of isoleucine is almost the same as leucine / valine, differing only by a single carbon.





Suppose that you irradiated *E. coli* and screened for mutants unable to grow on minimal medium but able to grow on minimal medium supplemented with leucine+isoleucine+valine. Explain the following observations by predicting which step in the pathway is affected.

3.9a. Mutants **A**, **B**, and **C** can grow on leucine alone or ß-isopropylmalate alone, but they cannot grow on a-isopropylmalate alone.

3.9b. Mutant **X** can grow on a-aceto-a-hydroxybutyrate alone or a-ketobutyrate alone, but cannot grow on threonine alone.

3.9c. Mutant **Y** can grow on a-ketoisovalerate + a-keto-ß-methylvalerate, but it cannot grow on isoleucine alone, valine alone, leucine alone, a-ketoisovalerate alone, or a, ß-dihydroxyisovalerate + a, ß-dihydroxy- ß-methylvalerate.

3.9d. Explain your last result to Beadle and Tatum.

3.10. You cut the *E. coli* chromosome with the restriction enzyme *Pac*I and ligate it to a plasmid cut with the same enzyme. This gives you lots of different plasmids (see

problem 2.7). Call this **Library #1.** You send Library #1 into *E. coli* mutant **A** (see problem 3.9) in such a way that no cell gets more than one plasmid, and then you plate the cells on minimal medium, i.e. no leucine, isoleucine, or valine. Many *E. coli* get some plasmid, but you are able to recover only one of them (call it **pUR213**) that is able to grow on minimal medium. You isolate pUR213 to characterize it further and find:

- When pUR213 is introduced into mutant **C**, that strain also is able to grow on minimal medium.
- When pUR213 is introduced into mutant **B**, that strain is not able to grow on minimal medium. It still requires leucine or ß-isopropylmalate.

3.10a. Why didn't all the plasmids of Library #1 rescue mutant **A**?

3.10b. How do you explain the ability of pUR213 to rescue mutant **C**?

3.10c. How do you explain the inability of pUR213 to rescue mutant **B**?

Somewhat confused by these findings you decide to investigate further. You repeat the experiment, this time using mutant **B** as the source of DNA cut by *Pac*I, creating a new collection of plasmids. Call this one **Library #2**. You use the original *Pac*I fragment cloned into pUR213 to pull out a plasmid from Library #2 that carries an identical or nearly identical fragment from the chromosome of mutant **B** (we'll discuss later in the semester how this can be done). Now you have a new plasmid, call it **pUR214**.

3.10d. You put pUR214 into mutant **B**. Would you expect the resulting strain to grow on minimal medium?

3.10e. You put pUR214 into mutant **A**. What would you conclude if the resulting strain is now able to grow on minimal medium?

3.10f. Alternatively, what would you conclude if the resulting strain is still NOT able to grow on minimal medium? If pUR214 is put into wild-type *E.coli* would you expect it to grow on minimal medium?

For your own quiet contemplation

How private is one's DNA? Should T.J. Thompson (see question 3.3) be compelled to give a biological sample for DNA analysis? Does it matter if the process is invasive (e.g. a blood or tissue sample) or noninvasive (e.g. a urine sample)? No permission is required to analyze tissue (e.g. hair or skin) that is no longer connected to the suspect. Does it matter whether we (society) deem the analyzer to have a compelling interest, or could insurance agents or *National Inquirer* reporters sift through your garbage for a stray hair, hoping to find out about your medical predispositions or perhaps intent on digging up dirt on a political candidate? Do you think I'm being too paranoid here? Do you realize that your skin cells are all over those dilution problems you turned in?