Biology 252/254 **Exam 3, Fall 2011**

Your Name _____

Answer all questions. You must show your work clearly to get full credit. Also, you may be eligible for partial credit even if your answer is wrong. It is probably better NOT to erase as sometimes there is work worth some credit in that scribbling.

1. (12 points) Describe all the enzymatic capabilities of **DNA polymerase I.** Explain when the enzyme would likely be performing these different activities (be clear and precise as usual)?

A) DNA pol 1 can synthesize (polymerize) DNA in a 5' to 3' direction using a DNA template. It does this during repair or to replace the gap created by the removal of the primer during DNA replication.

B) DNA pol 1 has 3' to 5' exonuclease activity. This is a proof reading capability that all DNA polymerases have and allows the enzyme to back up and remove incorrect bases if errors during replication are detected.

C) DNA pol 1 has 5' to 3' exonuclease activity. This is unique to this polymerase and is used during replication to remove the RNA primer.

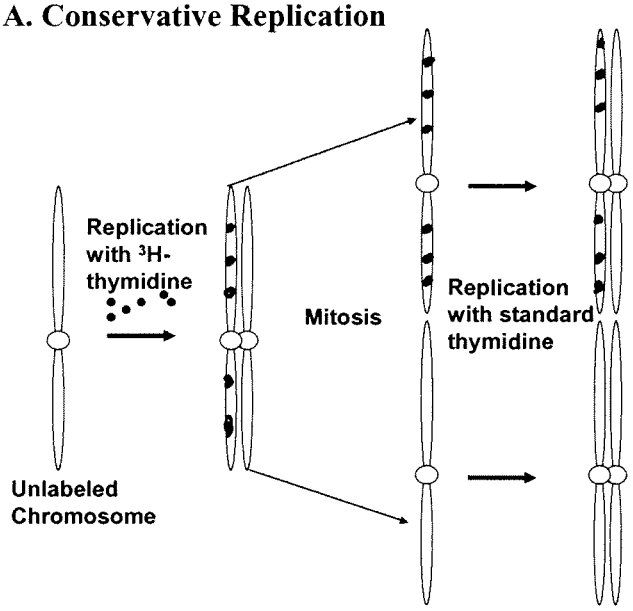
2. (12 points) DNA is extracted from the blood of a frog. The DNA is analyzed and 22% of the nitrogenous bases are guanine. **A)** How much of the other bases would be found in the DNA from blood? **B)** What would be the proportions of these bases found in the unfertilized eggs of the frog?

A) If there is 22% guanine, there must be 22% cytosine. The remaining 56% would be 28% Thymine and 28% Adenine.

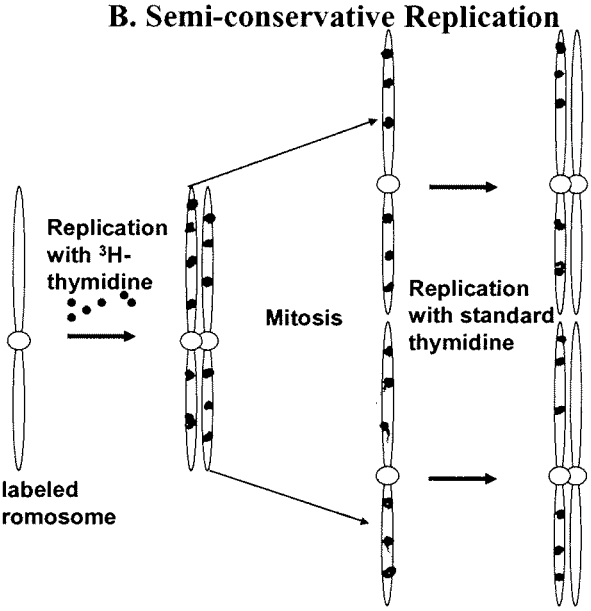
B) Same as above. Haploid genomes still have double stranded DNA with the same ratios of the bases. It does not matter if you look at one copy, two copies or 1000s of copies of the chromosomes (you might note that when you extract DNA to look at base ratios, you would be looking at 1000s of copies of chromosomes since you will be extracting from 1000s of cells whether you extract from 1000s of gametes or somatic cells.

3. (16 points) Taylor, Woods and Hughes (1957) designed an experiment to test the hypotheses regarding the mode of DNA replication in eukaryotes (they used the bean plant). Below are two identical flow charts that you are to use to diagram the predicted outcomes of their experiment if A) Conservative DNA replication or B) Semi-conservative DNA replication was the correct mode of replication. **A)** Use the **black dots** (as shown) to indicate the location of radioactive ^{3}H - thymidine in the chromosomes. **B)** What did they actually find when they ran the experiment?

A. Conservative Replication



B. Semi-conservative Replication



B) In this experiment they excluded Conservative Replication and dispersive (not shown), eventually concluding that DNA is replicated in a semi-conservative manner.

4. (16 points) Carefully explain what reverse transcriptase does. Give two different examples of where the genes that encode these molecules are found and describe their specific activities or functions in these two cases.

Reverse transcriptases (RT) use RNA as a template to synthesize a complementary strand of DNA.

Most eukaryote genomes with linear chromosomes encode telomerase which is a RT that uses an RNA template to extend the ends of chromosomes by adding additional copies of the short telomere repeat. This counteracts the losses that occur during replication.

There are RT viruses such as HIV that encode a RT. These RNA viruses use the RT to synthesize a DNA copy inorder to insert this into the host genome.

There are also RT encoded in retro transposable elements. SINEs and LINEs use this RT to copy and subsequently paste themselves into your genome.

5. (18 points) Seymour Benzer designed a **complementation test** for studying the array of rII mutants of T4 phage that he discovered. **A)** Explain the purpose of this test and how it works. Be clear. **B)** From the data below give the location of the mutants. Mutant 1 is in rIIA.

Test pair of mutants	Results (+ = lysis of K12)
1, 2	-
1, 3	+
1, 4	-
1, 5	-
2, 3	-
2, 4	-
2, 5	-
3, 4	+
3, 5	+

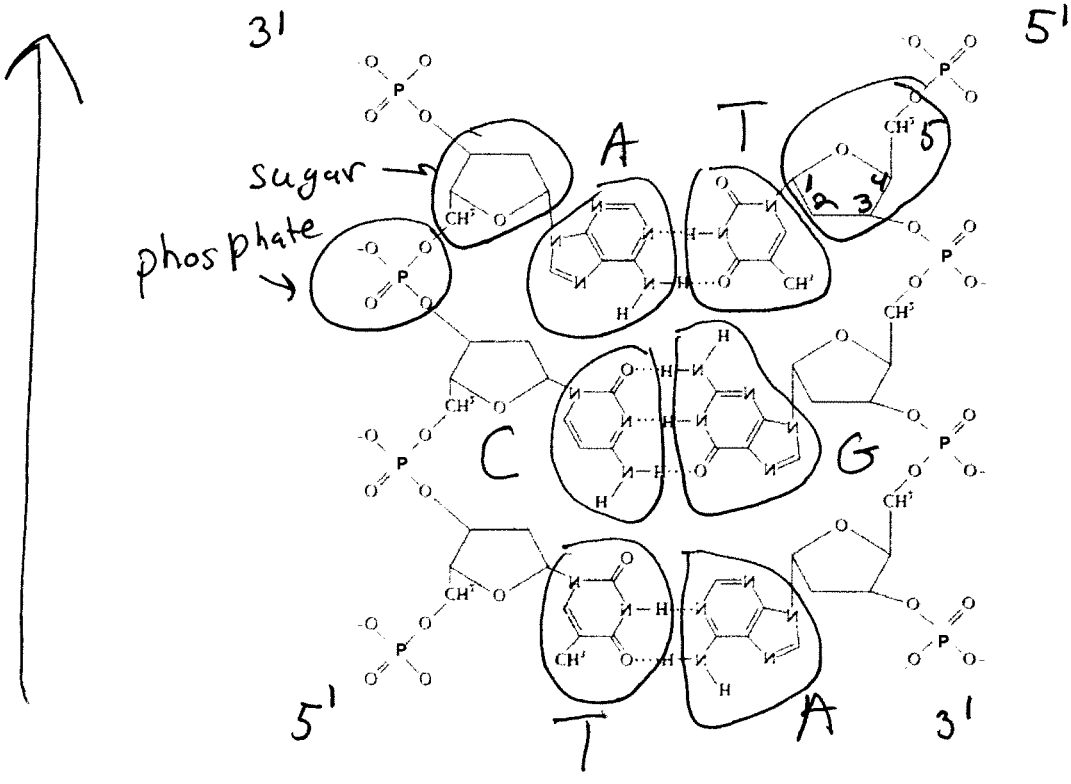
Complementation tests are used to determine if 2 mutations that give you the same phenotype (here lyse E.coli K12) are located in the same gene. In this study, two viruses at a time that both have a mutation that prevents them from lysing K12 are used to simultaneously infect K12. If the mutations are in the same gene, then they are both missing the same component needed to lyse K12. If the mutations are in different genes, each is missing alternative components but together they would have all the components needed to lyse the E. coli K12.

Mutants 1,4,5 are in gene A (can not complement each other).

Mutants 3 is in gene B (it complements 1, 4, 5).

Mutant 2 can not complement any other mutant so it must have mutations in both A and B (possibly a deletion covering both).

6. (20 points) Shown below is a piece of DNA from the middle of a much longer piece. **CIRCLE** (be precise, sloppy circles including incorrect or partial molecules will be marked wrong) and identify one phosphate molecule, one deoxyribose sugar, and **circle each of** the nitrogeneous bases in the figure. These bases must also be labeled with the specific name of the base. Also, **number** the carbons of one sugar and **label the polarity** of the molecule (the 3' and 5' orientation). **Finally, if the right side of the molecule was the template strand, in what direction would synthesis be proceeding (draw an arrow)?**



7. (16 points) There are three steps to both the Davis experiment (left figure) and the Lederberg & Zinder experiment (right figure) shown below. For Step 1 of both studies, the investigators plated each of the strains of E. coli (labeled for convenience in the pictures below as A, B, C, D) on minimal media and observed no colonies. In Step 2 of each experiment, the investigators mixed two strains (A & B in the first experiment and C & D in the second), in a common flask and then plated them on minimal media and colonies were observed. Finally, in Step 3 of the experiments, the investigators allowed the strains to mix in the U-tube and got the results shown below.

i) Why did they perform Step 1 of the experiments and what did it show?

This step is a control and shows that the strains are auxotrophs carrying one or more mutations that require supplemental compounds such as vitamins, amino acids etc for growth.

ii) What did Step 2 of the experiment demonstrate?

This step shows that prototropic recombinants are produced when combining the two strains. The frequency is high enough to discount mutation which would have appeared in step 1 at the same rate and suggests some form of gene transfer between strains.

iii) Why did the two experiments conducted in the same manner give such different final results in Step 3? Explain. Be sure to mention the major phenomenon discovered in each of the experiments.

In the first experiment the lack of prototrophs in this step, compared to the presence in step 2 indicates that the strains need to be in direct contact with each other. This led to the discovery of conjugation with one strain (F+) delivering genes to the other strain (F-).

In the second experiment, direct contact was not necessary (therefore not conjugation). Transfer was clearly unidirectional and they showed that transfer stopped if the pore size was reduced to prevent the passage of the vector (viruses) and this led to the discovery of transduction. In this experiment, one of the strains (D here) carried a virus in the lysogenic phase (quietly residing in the genome of the bacteria). A few popped out, traversed the barrier and attached the susceptible strain C. Some captured bacterial genes from the destroyed cells of strain C instead of viral DNA in the virus heads and subsequently transferred these genes back to strain D side of the tube and re-infected strain D to create recombinant prototrophs.