BIOL 367 (Midterm Exam #1)

STUDENT NAME _____ **STUDENT ID#** _____

COURSE Biology **NUMBER** 367

EXAMINATION Midterm Exam # 1 **DATE** January 31, 2018

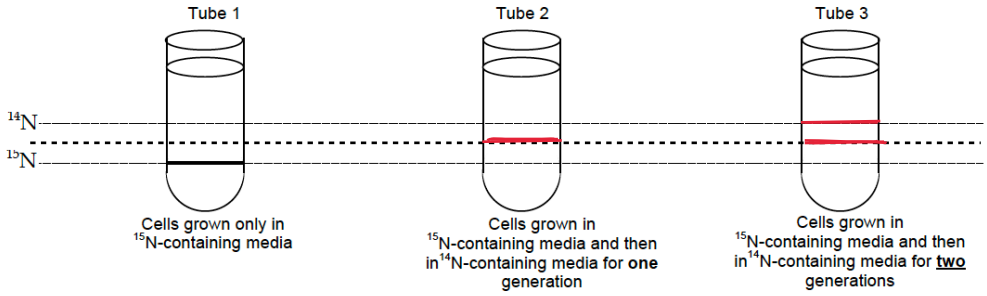
INSTRUCTOR Aashiq H Kachroo

MATERIALS ALLOWED Class notes and textbooks
MATERIALS NOT ALLOWED Laptops, tablets or phones
CALCULATORS ALLOWED Yes

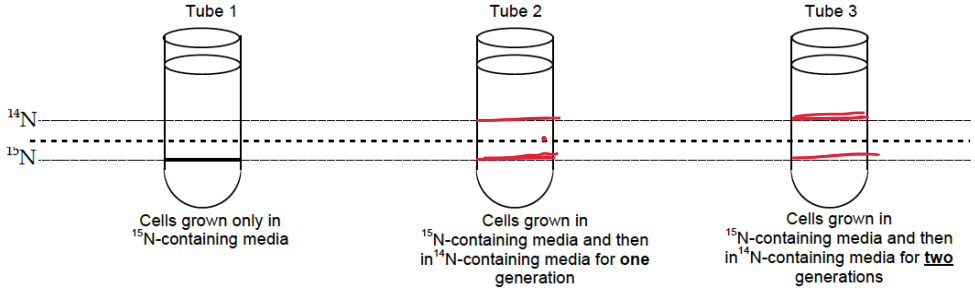
SPECIAL INSTRUCTIONS: Place all answers on the exam sheets. Write your names on all the sheets. **Maximum time = 60 minutes.**

1. The Meselson-Stahl experiment suggested that DNA replicates in a semi-conservative manner. *E. coli* cells were grown for several generations in a media with ^{15}N. These *E. coli* cells were then transferred into a ^{14}N media and allowed to replicate their DNA. DNA was extracted from the cells grown in ^{14}N-containing media and separated by cesium chloride density centrifugation. In tube 1 below, you see the band formed by the DNA isolated from cells grown exclusively in ^{15}N media.

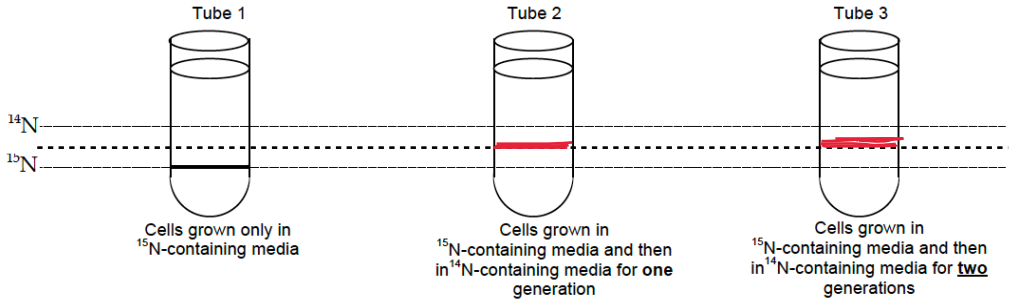
a) On the schematic below, draw the band(s) that you would expect in tubes 2 & 3 if the DNA duplex is copied by a semi-conservative mode of replication.



b) On the schematic below, draw the band(s) that you would expect in tubes 2 & 3 if the DNA duplex is copied by a conservative mode of replication.



c) On the schematic below, draw the band(s) that you would expect in tubes 2 & 3 if the DNA duplex is copied by a <u>dispersive mode</u> of replication.

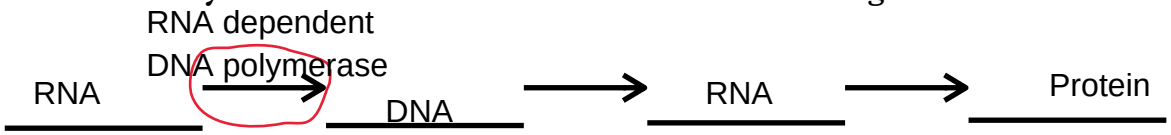


2. You have discovered a new virus that contains only RNA as its genetic material. Curious as to how this virus works, you infect host cells with this virus and discover that after infection, you find the host cell makes lots of viral RNA molecules and various viral proteins. Upon further examination, you discover that the viral genome integrated in the host genome now encodes viral proteins.

a. What type of virus have you discovered?

A Retrovirus

b. Outline the steps of the information flow for this virus by filling in the blanks below. Each arrow represents a process, for example, copying of DNA from a DNA template or from RNA to protein. Circle the arrow that represents a process not found naturally in the host cell and is encoded on the viral genome.



3. a. Given the sequence below, design primes (15 bases long) for PCR with clear 5′ and 3′ directions such that to amplify a PCR product that is 420bp long.

```
5'ATGAATCACGATCCTTTCAGTTGGGGCAGGCCCGCAGACTCTACTTATGGTGCTTACAAT
3'TACTTAGTGCTAGGAAAGTCAACCCCGTCCGGGCGTCTGAGATGAATACCACGAATGTTA

ACACAAATTGCGAACGCAGGCGCCTCTCCCATGGTTAATACACAGCAACCAATAGTAACA
TGTGTTTAACGCTTGCGTCCGCGGAGAGGGTACCAATTATGTGTCGTTGGTTATCATTGT

GGTACTTCCGTCATATCAATGAAATATGATAATGGGGTTATCATTGCAGCAGATAATTTA
CCATGAAGGCAGTATAGTTACTTTATACTATTACCCCAATAGTAACGTCGTCTATTAAAT

GGTTCATATGGCTCTCTTCTAAGATTCAATGGCGTGGAGAGGCTTATTCCCGTGGGTGAT
CCAAGTATACCGAGAGAAGATTCTAAGTTACCGCACCTCTCCGAATAAGGGCACCCACTA

AACACCGTTGTGGGCATTTCAGGTGATATTTCTGATATGCAACACATTGAGAGATTATTG
TTGTGGCAACACCCGTAAAGTCCACTATAAAGACTATACGTTGTGTAACTCTCTAATAAC

AAAGATCTAGTCACTGAAAATGCGTATGACAATCCTCTAGCGGATGCTGAAGAAGCGCTC
TTTCTAGATCAGTGACTTTTACGCATACTGTTAGGAGATCGCCTACGACTTCTTCGCGAG

GAACCCAGCTATATTTTTGAATATCTAGCTACCGTCATGTACCAGCGAAGATCAAAGATG3'
CTTGGGTCGATATAAAAACTTATAGATCGATGGCAGTACATGGTCGCTTCTAGTTTCTAC5'
```

Answer here:

Primer 1: 5' ATGAATCACGATCCT 3'

Primet 2: 5' CATCTTTGATCTTCG 3'

b. When using the Dideoxy Chain Termination method for sequencing DNA you include only a small amount of ddATP, ddCTP, ddGTP, and ddTTP while providing ample normal dNTPs.

- **Give two differences between ddNTP currently used in the sequencing reactions and the regular dNTP.**

1. No Hydroxyl at 2' and 3' carbon of the sugar.
2. Fluorescent probe modified ddNTP

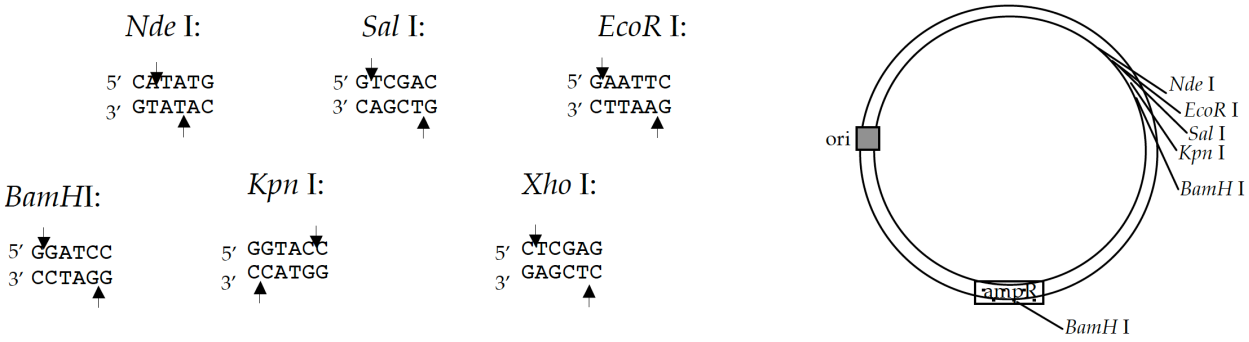
- **Explain why the Dideoxy Chain Termination method of sequencing would fail if you added too much of ddNTPS in the reaction.**

Too much ddNTPs will terminate most of the reactions earlier thus preventing the sequencing of the entire DNA.

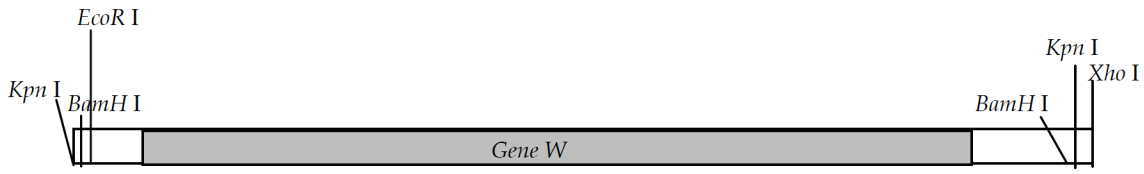
c. Recently scientists made artificial new bases X and Y that can be incorporated in DNA. How many hydrogen bonds stabilize X-Y base pair? Why? What is the take home message from this base pairing?

There were no hydrogen bonds instead the base pair was stabilized by hydrophobic interactions. This interaction showed that new bases can be added to DNA without the need for hydrogen bonding.

4. A schematic of the vector pYZ123 is shown. The restriction enzymes listed cut only where indicated; they do not cut anywhere else in the vector or insert.



a. A schematic of gene W is below. You want to clone all of gene W DNA into the pYZ123 vector. Give three different strategies that you could use to clone gene W into pYZ123, and obtain colonies that contain a recombinant plasmid.



- **Strategy 1 uses restriction enzyme(s) ……** Kpn1 **…… to cut the vector and the restriction enzyme(s) ……** Kpn1 **…… to cut the gene W**
- **Strategy 2 uses restriction enzyme(s) ……** EcoR1/Kpn1 **…… to cut the vector and the restriction enzyme(s) ..** EcoR1/Kpn1 **. to cut the gene W**
- **Strategy 3 uses restriction enzyme(s) …** EcoRI/SalI **….. to cut the vector and the restriction enzyme(s) ……** EcoRI/XhoI **…. to cut the gene W**

b. In which strategy (1, 2 or 3) would the Gene W be inserted into the vector in only one direction.

Strategy …2 & 3**….**

c. What determines whether the two DNAs digested by restriction enzymes can stick together?

The sequence at the overhangs.

5. In a hypothetical scenario you wake up one morning to your amazement that you have developed a superpower of X-ray vision. You have been supplementing your diet with a strange new fungus purchased at the local store. You take samples of the fungus to your lab and you find that this fungus does indeed make a protein (the Act1 protein) that stimulates brain function. You construct a fungal genomic DNA library in *E. coli* with the hope of cloning the Act1 gene. If you succeed you will be a billionaire! You obtain DNA from the fungus, digest it with a restriction enzyme, and clone it into a vector.

a. What feature(s) must be present on your plasmid that will allow you to use this as a cloning vector to make fungal genomic DNA library?

The vector must have an antibiotic selection marker. The vector must have an origin of replication to replicate in E. coli.

b. What is the most critical pre-requisite of the *E. coli* strain prior to transformation of your genomic library? How will that allow you to select for the *E. coli* that has the clones?

The E. coli strain must be sensitive to the antibiotic selection for which the resistant gene is present in the cloning vector.

c. What simple method will you use to check whether your plasmid does or doesn't have an insert?

We will restriction digest and run the digestion mix on an agarose gel to identify the size of an insert.

d. As you do these experiment, you noticed that the protein of interest Act1 is not made in *E.coli*. Explain why?

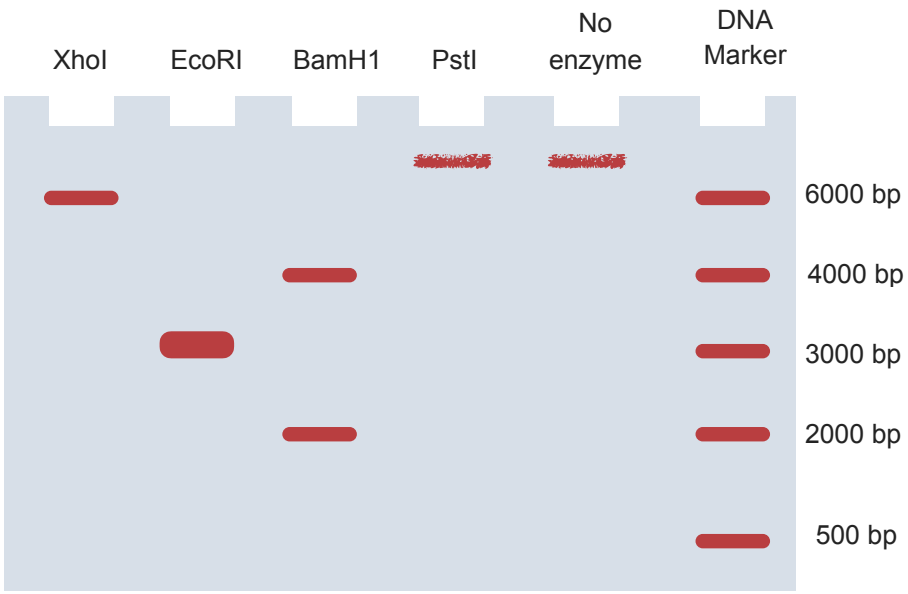
The genomic library has a fungal gene with introns and surrounding fungal promoter.

e. You decide to make a cDNA library of this fungus and use this to look for your protein Act1.

- **Why would a cDNA library help to make the protein?**

The cDNA library is made from mRNA using reverse transcriptase and thus does not have introns or promoters.

6. You are working with a plasmid of unknown sequence and size. You decide to first check the size of the plasmid using well known restriction enzymes. As a control you also use "no enzyme" and notice a band higher than 6000bp (<u>hint: circular plasmids do not appear true to size on agarose gel</u>). The image of the agarose gel electrophoresis of the experiment appears as follows:



a. What is approximate size of this plasmid in base pairs (bp)?

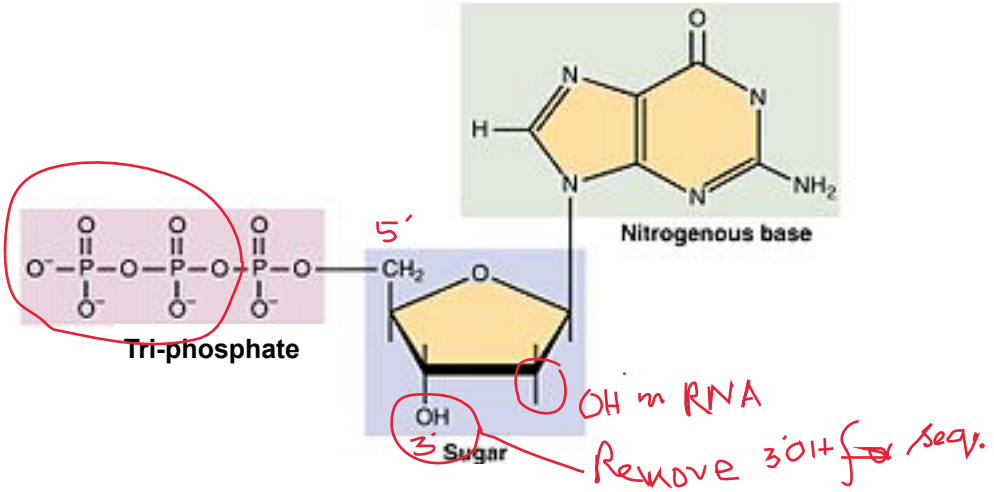
~6000bp

b. How many restriction enzyme sites are present in this plasmid for the following enzymes?

XhoI…1… EcoRI…2… BamHI…2… PstI…0…

7. Following is a chemical structure of a deoxy-nucleotide triphosphate.

- **Precisely circle the region that doesn't attach to the newly replicated DNA strand.**



- **Label 5′ end and the 3′ ends of the nucleotide by drawing the structure below:**

- **Explain the part of the nucleotide that is different in RNA. What is that modification?**

- **What would you modify in the above structure to terminate DNA replication?**

8. a. Which of the following statements are TRUE or FALSE (draw a circle) regarding Double Strand Break Repair pathway: covers HDR and SDSA

TRUE	**FALSE**	**Causes errors or deletions in DNA after repair.**
TRUE	**FALSE**	**Needs homologous DNA template to repair DNA.**
TRUE	**FALSE**	**Does not require DNA ligase.**
TRUE	**FALSE**	**Does not require DNA polymerase.**
TRUE	**FALSE**	**May result in gene conversion due to both the copies of DNA receiving the newly replicated DNA.**

a. Which of the following statements are TRUE or FALSE (draw a circle) regarding Non Homologous End Joining pathway:

TRUE	**FALSE**	**Causes errors or deletions in DNA after repair.**
TRUE	**FALSE**	**Needs homologous DNA template to repair DNA.**
TRUE	**FALSE**	**Does require DNA ligase.**
TRUE	**FALSE**	**May not require DNA polymerase.**
TRUE	**FALSE**	**Needs intact copy of DNA to repair the break.**

9. A DNA sequence below encodes a small polypeptide starting from the first ATG.

5′CCT TAC CGA TCG TGC AAT **ATG** CAA ATG TT**C** GCT ACT CTA ACC TGA TTC 3′

- **What is the sequence of the polypeptide? (The genetic code table is provided at the back)**

Met	Gln	Met	Phe	Ala	Thr	Leu	Thr	
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

- **If a spontaneous modification in the Cytosine (C) in the above sequence results in a C to T mutation. What is the likely cause of this mutation? why?**

  Cytosine deamination is the likely cause of this mutation. Cytosine demanination results in modification of Cytosine to Uracil and if its is not corrected, it will base pair with A in the next replication cycle. Thus changing the C-G bp to T-A bp.

- **What would be the result of this mutation on the composition of a polypeptide chain? Explain why?**

  There will be no change. These codons code for same amino acid due to redundancy of genetic code.

10. a. Describe a critical difference that distinguishes Base Excision Repair (BER) from Nucleotide Excision Repair (NER) pathway.

In BER only a single base is excised whereas in NER several nucleotides, along with a modified base, are removed.

b. Uracil in the DNA is repaired by which of these pathways?

BER

c. Thymine dimers are repaired by which of these pathways?

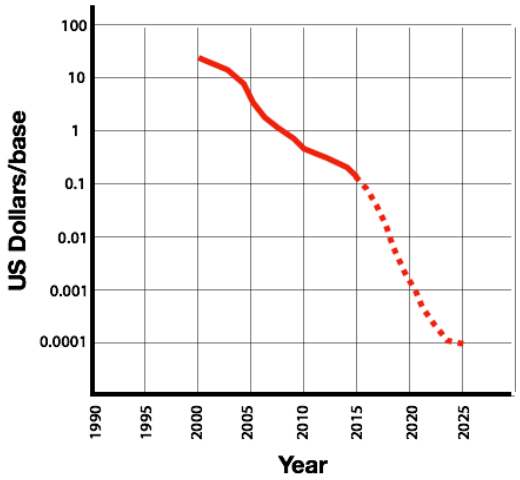
NER

11. Which of these statements are TRUE or FALSE (draw a circle).

TRUE	**FALSE**	**A mutations is an inheritable genetic change.**
TRUE	**FALSE**	**DNA glycosylase enzyme breaks a phospodiester bond**
TRUE	**FALSE**	**Both the sunlight and nucleotide excision repair pathway can repair Thymine dimers**
TRUE	**FALSE**	**Oxidative damage to Guanine does not result in a mutation**
TRUE	**FALSE**	**Thymine dimers are caused by visible light and repaired by UV light**

12. A truly bonus question!!!! It will be marked if answered but not penalized for the wrong answer.

The cost of DNA sequencing has been drastically reducing over years. On the other hand, cost of DNA synthesis has not seen such a drop. But scientists are beginning to build new synthetic genomes (using sophisticated genome foundries- one such foundry exists at Concordia genome center) from scratch which will reduce the cost over time. Here is the cost per base synthesis over years and future prediction.



Thus, in 2025 the cost/base would be ~100 fold lower than at present. In principle, we could synthesize the entire human genome (~3 x 10^9bp). Calculate the cost of synthesizing the entire human genome (in 2015 and in 2025).

Would you be willing to save up money to rather synthesize a genome for your future generation, getting rid of mutations that may cause disease or incorporating beneficial mutations, in principle creating a super human?

0.0001 x ~10^9 = ~10^5 x 2 = 6 x 10^5 US dollars = $60,000 in 2025 compared to now = $6,000,000 which is around 6 million.

This amount is significantly less than what parents will be saving for their kids to get a US college degree. Now let us check your creativity!!!!