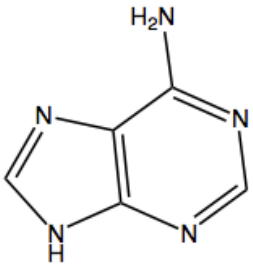
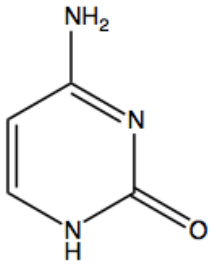
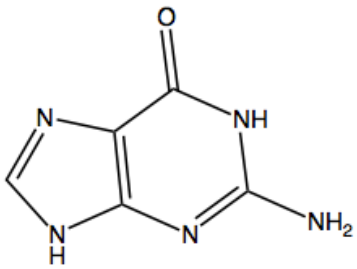
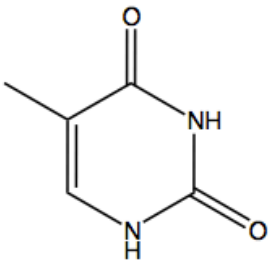
Final Exam Name:_____

1.)	4 questions	@ 3 pts/Q	(12 pts)
2.)	12 questions	@ 3 pts/Q	(36 pts)
3.)	5 questions	@ 4 pts/Q	(20 pts)
4.)	3 question	@ 5 pts/Q	(15 pts)
5.)	1 question	@ 10 pts	(10 pts)
6.)	23 questions	@ 3 pts/Q	(69 pts)
		Total:	162 pts out 150 pts possible









1.) Imagine that you want to study the protein *RNase III* from *Enterococcus faecalis*. You would like to engineer *E. coli* to produce the RNase III for you. First, you need to obtain DNA. Just as in class, the Qiagen corporation has a kit that allows you to purify the *Enterococcus faecalis* chromosomal DNA from a culture.

A.) *Enterococcus faecalis* can be grown in bile esculin azide media, which has the following components:

25 g Enzymatic digest of Casein
9.5 g Yeast enriched meat peptone
1.0 g Oxbile
5.0 g Sodium chloride (58.44 g $mole^{-1}$)
1.0 g Sodium citrate (258 g $mole^{-1}$)
0.5 g Ferric ammonium citrate (279 g $mole^{-1}$)
1.0 g esculin
0.25 g sodium azide (65.0 g $mole^{-1}$)

(Dissolve in 1.0 L of H_2O and sterilize)

While the media is being sterilized in an autoclave, you wonder what the total concentration of citrate in the solution is. Citrate (189.0 g $mole^{-1}$) can be a source of energy for a cell. **What is the <u>total</u> final molar concentration of citrate in the media?**

B.) Your DNA purification yields 50 uL of concentrated product. You make a 1:35 dilution of this concentrated stock, which has an A_{260} of 0.23. (1 A_{260} = 50 ug mL^{-1} DNA). **What is the concentration of DNA in the original concentrated product?**

C.) The *Enterococcus faecalis* chromosome is closed (no nicks) and circular. It contains 3,218,031 base pairs. **In the relaxed state (no writhes), what is the linking number of the *Enterococcus faecalis* chromosome?**

To be able to molecularly manipulate the gene for RNase III, you will need to increase the number of copies of the specific DNA encoding the information for the gene using PCR. The following is the DNA sequence of the gene that contains 693 bases of which 142 are guanines and 123 are cytosines:

```
atggacaatc agttaacaac agagttaaaa gaacgttacg gcattgtttt
ccatgatgtc aatctattag agcaagcttt tactcattca tcctatgtga
atgagcatcg ctatttaaaa ttatccgata atgaacgtct tgaatttta
ggagatgcag ttttagaatt aattgtttca caatatttgt atttaaaatt
cccagaactt ccagaaggaa aattaacgaa gatgcgcgca gccatcgttc
gggaagatag tttagccaaa tttgcgaaag aatgtcactt cgacaactac
attttattag gtaaaggaga agaagcatcg ggcggacgaa cacgtgcatc
attattatgt gacttatttg aagccttttt aggtgccctc tacttagacc
aaaaagttgg cgcagccaag aaatttattg aagacgttat ttttccgaaa
attgatgccg gtgctttttc acatgagatg gatcacaaaa cacaattaca
agaagtttta caacgcaaag gcgatgtttc aattgaatat cgcttaatta
aagaagaagg ccctgctcat gaccgcacct ttttcactga agtttacatg
aatggtgaac tcattgggtt aggccaagga aaatcgaaga agttagctga
acaggatgcc gctgagcggg cactgaaaag tattcctcag taa
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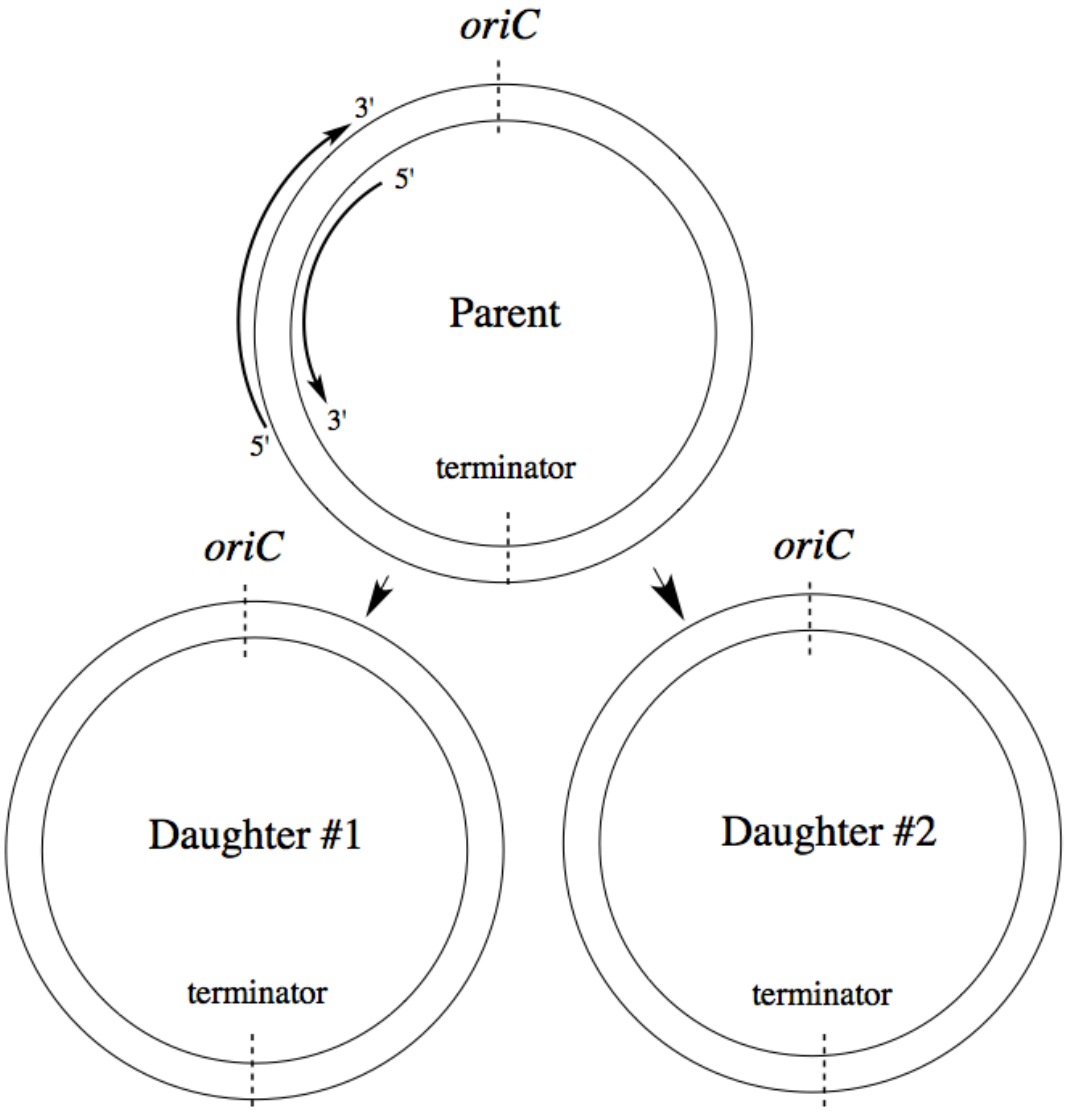
D.) List the sequences of primers that are 25 bases in length, which would amplify the above sequence.

E.) How many angstroms in length will each PCR DNA product of the RNase III gene be?

F.) How many thymidines will each of the <u>double-stranded</u> PCR DNA products of the RNase III gene contain?



2.) Answer the following on the above diagram. At the top you will find a cartoon of a parent *E. coli* chromosome. At the bottom you will find the result of DNA replication.

A.) Label one strand of the parent DNA "A" and one strand "B". Use the same labeling scheme to indicate the parent DNA stand(s) of the daughter (product) chromosomes.

B.) Indicate the directionality (5'→3') for each strand of the daughter chromosomes.

C.) For the newly synthesized portions of the daughter chromosomes, indicate whether each was the leading or lagging strand.

D.) Imagine that the cartoon of the daughter chromosomes represents the product of the replication process before DNA Pol I and DNA ligase act. Indicate the position of RNA primers by drawing shaded boxes along each strand where appropriate

3.)

A.) Draw the oxidative deamination product of cytosine (replace the primary amine at C4 of cytosine with a carbonyl group).

B.) Is the resulting product a pyrimidine or purine based structure?

C.) Label the hydrogen bond donors (D) and acceptors (A) on your drawing.

D.) If this mutated cytosine base had to pair with another base in the normal Watson-Crick face, with which base is it most likely to pair?

E.) This base mutation is corrected in the cell by the process of *base excision repair* (BER). During the first step of the BER process, the glycosidic bond (between the C1' of the deoxyribose and N1 of the base) is hydrolyzed; leaving a hydroxyl group attached to C1'. **Draw the chemical structure of the product of this first step. Be sure to include the chemical bonds attaching the mutated position to adjacent nucleotides.**

F.) In the second step of the BER process, AP endonuclease (a DNA endonuclease) acts on the phosphate immediately in the 5' direction of the mutated position. **Draw a schematic of the product of this second step.**

G.) What is the nucleophile and leaving group during the chemical reaction of the second step of the BER process (be very explicit)?

H.) Several adjacent residues are removed by a DNA exonuclease in the 5' to 3' direction. This creates a single-stranded gap that looks similar to the product DNA after the action of UvrD during nucleotide excision repair except that the gap contains ~5 missing residues instead of 14. **Draw a schematic of the product of this step.**

I.) The gap is filled in by the action of DNA polymerase I as in nucleotide excision repair. **What is the nucleophile and leaving group during the chemical reaction of this penultimate step of the BER process (be very explicit)?**

J.) The resulting DNA nick is sealed by DNA ligase. **What are the nucleophiles and leaving groups during the chemical reaction of the DNA ligation step of the BER process (be very explicit) and group in pairs?**

4.) During a sequencing reaction to determine the position of "A"s, the PCR mixture is spiked with a few molecules of dideoxyadenosine triphosphate. Unlike deoxyadenosine triphosphate which is reduced at the C2' position, dideoxyadenosine triphosphate is reduced at both the C2' and C3' positions.

A.) Draw dideoxyadenosine triphosphate.

During the PCR elongation phase, the DNA polymerase will extend the primers using the deoxynucleotide triphosphates, which make up most of the total population. Occasionally, the polymerase will utilize the dideoxyadenosine triphosphate for the reaction.

B.) Draw the electron-pushing mechanism for the addition of dideoxyadenosine triphosphate onto the 3' end of a growing DNA strand.

C.) What happens when DNA polymerase tries to incorporate the next deoxynucleotide triphosphate into the growing DNA chain after it incorporates the dideoxyadenosine?

5.) The following three DNA fragments (A, B, and C; dashes represent any base) were used as promoters for the RNase III gene. Imagine that you mixed each template with RNA polymerase, sigma-70, ATP, UTP, GTP, and CTP.

```
                          -35                  -10          +1
      (A) 5'----------TTGACA----------TATAAT------A----------3'
      (B) 5'----------TACTCA----------GATAAT------A----------3'
      (C) 5'----------CCTGTT----------GTACGA------A----------3'
consensus 5'----------TTGACA----------TATAAT------A----------3'
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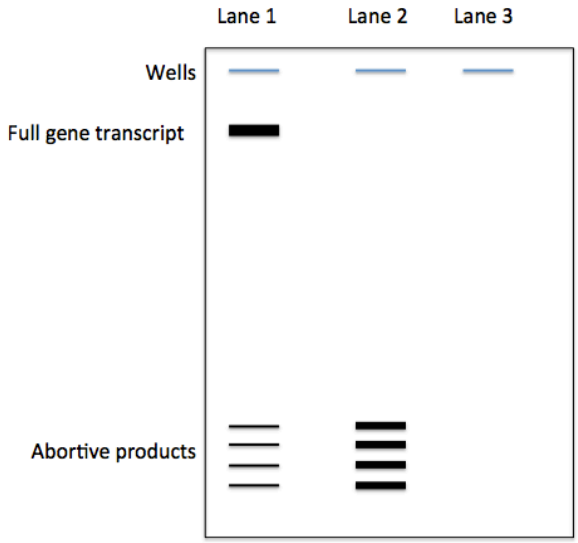
The resulting RNA transcription products were resolved on a gel.



A.) Indicate the charge of the electric field beside the gel.
B.) Predict which fragment (A, B, or C) was utilized in the transcription reaction resolved in Lane 1. Explain the basis for your decision.

C.) Predict which fragment (A, B, or C) was utilized in the transcription reaction resolved in Lane 2. Explain the basis for your decision.

D.) Predict which fragment (A, B, or C) was utilized in the transcription reaction resolved in Lane 3. Explain the basis for your decision.